Interferon Alpha as Monotherapy or Combination Therapy for Chronic Hepatitis B

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Interferon alpha als monotherapie of combinatie therapie voor chronische hepatitis B

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus
Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 2 februari 2005 om 15:45 uur

door

Monika van Zonneveld

geboren te Giessen, Duitsland

Promotiecommissie

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Aan mijn ouders

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Chapter 1

Introduction

The hepatitis B virus

The hepatitis B virus (HBV) is a DNA virus that belongs to the group of hepadnaviridae. The partially double-stranded DNA contains 4 open reading frames, encoding for the envelope (pre-S/ S), core (precore/ core), polymerase and X proteins (1). The pre-S/S open reading frame encodes for the large, middle and small surface glycoproteins. The precore/ core region encodes for both the nucleocapsid protein (hepatitis B core antigen) and a soluble protein, the hepatitis B e antigen (HBeAg). After attachment of HBV to the hepatocyte, the viral genome is converted into covalently closed circular DNA (cccDNA) within the hepatocyte nucleus (Figure 1). This cccDNA serves as template for the pregenomic mRNA. In the hepatocyte cytoplasm viral particles are assembled from HBV core protein, HBV DNA polymerase and the pregenomic mRNA. Within these particles the pregenomic mRNA is reverse transcribed by the HBV DNA polymerase into the minus strand HBV DNA. Finally the plus strand DNA is synthesized by the HBV DNA polymerase (2). There are seven major HBV genotypes (A to G), prevailing in different parts of the world (3-6). Recently another genotype, genotype H, was found in central America (7).

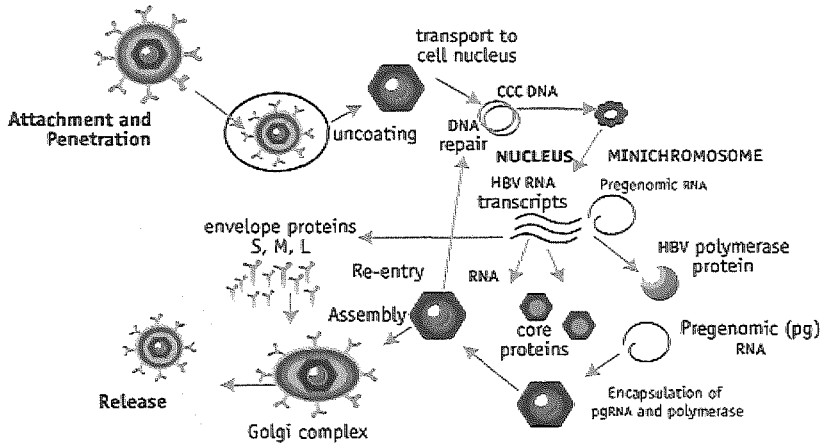

Fig 1 The replication cycle of the hepatitis B virus

Chronic hepatitis B

An estimated 400 million people worldwide are chronically infected with HBV (8). Chronic hepatitis B can lead to serious morbidity and mortality and is a major cause of liver cirrhosis and liver cancer

(8-12). HBV is transmitted by perinatal, sexual and percutaneous exposure and also by close household contact, especially among children in endemic areas. In countries with high prevalence of the disease (>8%), mainly Southeast Asia, China and sub-Saharan Africa, the disease is transmitted perinatally or during early childhood. In Northwestern Europe, North America and Australia, where the prevalence of chronic HBV infection is low (<1%), the main source of infection is sexual contact and through intravenous drug use. In the Mediterranean countries, the Middle East and India, areas with intermediate endemicity (1-8%) perinatal, household and sexual transmission were the major sources of infection in the past. These days intravenous drug use, nosocomial transmission, tattooing and body piercing are becoming important modes of transmission (13). After infection with HBV, the risk of chronicity varies with the age at which the infection is acquired. The risk of the infection becoming chronic is 90% for neonates and children younger than 1 year, 30 % for children aged 1-5 years, and 2% for children older than 5 years and adults (14). Three phases of chronic hepatitis B have been described (13, 15, 16). Patients who acquire the infection during childhood start with a long immune tolerance phase with detectable serum HBeAg, high levels of serum HBV DNA, normal or minimally elevated serum aminotransferases and near normal liver histology. This phase is followed by an immunoclearance phase, which is the first phase for patients who acquire the infection during adolescence or as adults. In this phase the host's immune system is activated in an attempt to eliminate the virus. As a result of this immune activation serum aminotransferases are elevated, serum HBV DNA levels decrease and liver histology shows active inflammation and fibrosis. This phase ends when HBeAg seroconversion and a dramatic reduction of HBV DNA occurs. Finally, there is an inactive phase (also called inactive carrier state) with undetectable serum HBeAg, normal serum transaminases, low or undetectable serum HBV DNA and resolution of necroinflammion. This may lead to resolution of the infection with HBsAg seroconversion. However, patients may show persistence or recurrence of disease activity and continue to HBeAg-negative chronic hepatitis B, particularly patients who acquired the disease during childhood. This thesis focuses on patients with HBeAg-positive chronic hepatitis B, who are in the immune clearance phase of the infection.

Management of chronic hepatitis B

1. Nucleoside/nucleotide analogues

Nucleos(t)ide analogues are able to suppress viral replication by inhibiting the HBV polymerase. Two drugs in this group have been approved for the treatment of chronic hepatitis B: lamivudine and adefovir dipivoxil.

Lamivudine is a nucleoside analogue of deoxycytidine, the (negative) enantiomer of 2'-deoxy-3'-thiacytidine. One year of lamivudine treatment results in HBeAg seroconversion in 16-18% of patients, suppression of HBV DNA to below detection level of hybridisation assays in almost all

patients, and improved liver histology in 49-56% of patients (17-19). Prolonged treatment leads to a sustained improvement of liver necroinflammation and has been shown to substantially improve fibrosis and even reverse cirrhosis in some patients (20, 21). However the efficacy of lamivudine treatment is limited by the emergence of lamivudine resistant strains that harbor mutations that affect the YMDD region of the HBV DNA polymerase. These lamivudine resistant mutants are found in an increasing frequency over time, which rises to 70-71% of patients after 4 years of lamivudine treatment (22, 23). Irrespective of the YMDD mutation relapse occurs after discontinuation of treatment in a proportion of patients, with reported relapse rates varying from 31-42% at 1 year and 46-64% at 3 years posttreatment (24-26). The optimal timing of stopping treatment is unknown, but the risk of relapse is lower if treatment is continued for 3-4 months after HBeAg-seroconversion (24).
Adefovir dipivoxil, an acyclic analogue of adenosine monophosphate, has recently been approved for the treatment of CHB. In a large international study, one year of adefovir therapy resulted in a strong suppression of HBV DNA (median reduction of HBV DNA 3.5 log, HBV DNA undetectable by PCR in 21% of patients), improved liver histology in 53% of patients and HBeAg seroconversion in 12% of patients (27). Adding adefovir to lamivudine in patients with lamivudine resistance resulted in virological and biochemical improvement in two studies involving patients with compensated liver disease, decompensated cirrhosis or recurrent CHB after liver transplantation (28, 29). In a pilot study of 58 patients with YMDD mutant hepatitis B virus, adefovir alone was as effective in suppressing HBV DNA as combination therapy of lamivudine and adefovir.

2. Interferon alpha (IFN)

In chronic hepatitis B IFN has a dual mode of action with direct antiviral and immunomodulatory effects. A meta-analysis (30) of 498 patients, treated with IFN for 3-6 months and followed up for 6-12 months, showed that IFN induced loss of HBeAg, HBV DNA (measured by hybridisation assays) and HBsAg in 33%, 37% and 8% of patients, respectively. The corresponding figures for untreated controls were 12%, 17% and 2%, respectively ($p<0.01$). The major pre-treatment factors that have been found to be associated with response are high ALT levels, low HBV DNA levels and a higher degree of inflammation and fibrosis on liver biopsy. Studies in Asian patients show less favourable results than in Caucasians (30, 31). This might be explained by the fact that most Asian patients acquire the HBV infection at birth and are immune tolerant to HBV. In a study with 411 Chinese patients, the response rate 6 months posttreatment was only 11.5%, but 21% for patients with elevated (>1.5 ULN) ALT levels (32).
The optimal duration of IFN therapy has not been established. A European multicenter trial with 162 patients showed that prolongation of therapy from 16 to 32 weeks had additional benefit in patients who had not cleared HBeAg after 16 weeks of therapy. A low HBV DNA level after 16 weeks of therapy predicted response during prolonged therapy (33).

Long-term follow-up studies with Caucasian patients showed that 91%-100% of responders remained HBeAg negative during 5-10 years of follow-up, and 25%-86% of responders ultimately lost HBsAg (34-37). In a study with 411 Chinese patients the rate of HBeAg relapse was higher and after long-term follow-up the proportion of patients with HBeAg loss was not different between IFN-treated patients and untreated controls (32). In a study with Taiwanese patients, who had high ALT levels (mean ALT 227 U/L), similar results were found as in Caucasian patients (38). The response rate was 42%, and response was sustained in 89% of patients during long-term follow-up.

With the current established therapies, IFN and lamivudine, only a minority of patients respond. Possible ways to increase efficacy are:

- Prolongation of therapy
  Prolonging IFN therapy to 32 weeks has been shown to improve response rates in patients who are still HBeAg positive after 16 weeks of therapy (33). Prolonging the duration of IFN therapy to 52 weeks may further increase the efficacy of treatment.
- Combination therapy of IFN and lamivudine
  It seems logical to combine the immunestimulatory effects of IFN with the virussuppressive action of lamivudine. The combination of 16 weeks of IFN and lamivudine appears to be superior to IFN alone (19). Also the sustained response after 24 weeks of treatment with IFN and lamivudine was higher than after one year of lamivudine alone (39).
- Using pegylated forms of IFN with an improved pharmacokinetical profile
  At the start of this study pegylated forms of IFN were being investigated in patients with chronic hepatitis B, and the first study showed promising results. Now, pegylated interferons have shown to be more effective than conventional IFN and have become the standard therapy in chronic hepatitis C (40-44). In a first study in patients with chronic hepatitis B, pegylated IFN-2a was more effective than conventional IFN based on combined HBeAg loss, HBV DNA suppression below 50000 copies/ml and ALT normalization (45).

The aims of this study are:

1. To evaluate the safety and efficacy of pegylated interferon alpha-2b alone or in combination with lamivudine for the treatment of chronic hepatitis B (chapters 2-5).
2. To study the long-term clinical outcome and survival after treatment with standard IFN for chronic hepatitis B (chapter 6).

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Chapter 2

Pegylated interferon α-2b alone or in combination with lamivudine as treatment for HBeAg-positive chronic hepatitis B

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Lancet, January 2005, in press

Abstract

Background: Current treatment of HBeAg-positive chronic hepatitis B (CHB) patients is effective in few patients. A combination of immunomodulatory peginterferon α2b and antiviral lamivudine may enhance the sustained response rate.

Methods: 266 interferon-naïve and -cxperienced HBeAg-positive CHB patients were randomized to receive combination therapy with 100 μg/week peginterferon α-2b and 100 mg/day lamivudine or monotherapy with 100 μg/week peginterferon α-2b and placebo. Total treatment duration was 52 weeks. During Weeks 32–52 the peginterferon dose was reduced to 50 μg/week in both treatment arms. All patients were followed up for 26 weeks after treatment.

***Results*:** 36% of patients receiving monotherapy and 35% receiving combination therapy lost HBeAg after the end of follow up and there was no difference between treatment groups (P=0.91). More patients on combination therapy initially cleared HBeAg (44% of patients, compared with 29% on monotherapy; P=0.01) at the end of treatment but relapsed during follow-up. Similar response patterns were seen when response was assessed by serum HBV DNA suppression and change in ALT levels. Response rates (HBeAg loss) varied by genotype (P=0.01): genotype A, 47%; genotype B, 44%; genotype C, 28%; and genotype D, 25%.

Conclusions: Peginterferon α-2b treatment is effective for HBeAg-positive CHB and combination therapy with peginterferon α-2b and lamivudine is not superior to monotherapy. HBV genotype is an important predictor of response to treatment.

Introduction

Patients successfully treated for chronic hepatitis B (CHB) are less likely to develop cirrhosis, liver failure and hepatocellular carcinoma (1,2), however, to date, treatment for CHB has been unsuccessful in the majority of patients. Of the few currently approved agents for treatment of chronic hepatitis B virus (HBV) infection, the most commonly used include interferon-α and nucleos(t)ide analogues, such as lamivudine and adefovir dipivoxil.

In early studies, standard (non-pegylated) interferon-α treatment reportedly cleared HBeAg and HBV DNA in approximately one-third of treated patients (1,2). More recently, response rates of only 19% have been noted (3). Interferon-α-induced HBeAg clearance has been reported to be durable in 80%–90% of patients and the clearance is associated with a reduction of hepatocellular carcinoma and death (4-7).

DNA polymerase inhibitors, of which lamivudine and adefovir dipivoxil are currently the most effective and studied, achieve profound virus load reduction (8,9). However, sustained response after treatment discontinuation is as yet unknown for adefovir dipivoxil and occurs in only 10–15% of patients treated with lamivudine (10). Durability of lamivudine response has been found to be significantly lower than that following interferon-α therapy (11). Furthermore, the long-term efficacy of nucleoside analogues is compromised by the almost inevitable emergence of drug-resistant HBV mutants, a scenario analogous to the antiretroviral experience (12). Lamivudine resistance has been reported in 57% of patients after 3 years of treatment (13).

There is increasing evidence that only a complete and vigorous HBV-specific immune response is capable of achieving control and elimination of the virus, preventing disease progression (14). This suggests that induction of a host immune response is necessary for sustained response to HBV treatment, which can only be reached by immunomodulatory therapy, such as interferon.

The introduction of pegylated interferons (peginterferons) in the treatment of hepatitis C has led to greater treatment efficacy than that achieved with standard interferon (15), and a preliminary study has been conducted in the treatment of CHB (16). Increased efficacy with peginterferons may be due to more continuous levels of interferon in the serum than that achieved with standard interferon. The improvements in efficacy are combined with a similar safety profile and simpler and more convenient dosing (once weekly) compared with standard interferon (TIW). The convenience of dosing is significant because, across the therapeutic spectrum, simplified dosing regimens have been shown to enhance compliance, which in turn increases treatment efficacy (17).

To date, most studies of standard interferon combination regimens for the treatment of HBV disease have investigated the efficacy of 16-week regimens (3,18). However, studies indicate that prolonged regimens may result in higher rates of sustained response for the treatment of HBV disease (19). This study is based on the rationale that combining the immunomodulatory properties of interferon and the

strong antiviral potency of lamivudine in a prolonged treatment regimen may enhance the sustained response rate in CHB. This is the first study to assess whether prolonged treatment with peginterferon α-2b, alone or in combination with lamivudine, can improve sustained treatment response in patients with HBeAg-positive CHB.

Methods

Patients

Patients with CHB aged 16 years or over were enrolled after central evaluation of their eligibility. Patients were included if they were positive for hepatitis B surface antigen (HBsAg) for more than six months, were HBeAg positive on two occasions within 8 weeks before randomization and had two episodes of elevated serum ALT levels (2 X upper limit of normal) within the 8 weeks before randomization. Patients were excluded for the following reasons: presence of serum antibodies against hepatitis C, hepatitis D or human immunodeficiency virus; antiviral therapy or immune suppressive therapy within the preceding 6 months; pregnancy or inadequate contraception; substance abuse in the last 2 years; other acquired or inherited causes of liver disease; coexisting serious medical or psychiatric illness; uncontrolled thyroid disease; inadequate level of leukocytes ($\leq$3,000/mm^3), granulocytes ($\leq$1,800/mm^3), or platelets ($\leq$100,000/mm^3); radiological evidence of hepatocellular carcinoma; advanced liver disease with a prothrombin time prolonged by more than 3 seconds, serum albumin under 35 g/l, bilirubin more than 34 μg/L, or a history of either ascites, variceal bleeding and hepatic encephalopathy.

Study design

This was an investigator-initiated, multicenter, randomized, double-blind controlled trial conducted at 42 centers in 15 countries (in Europe, East Asia and North America). Recruitment began in February 2000 and the study ended in October 2003. The study compared the efficacy and safety of peginterferon α-2b monotherapy with peginterferon α-2b and lamivudine combination therapy for treatment of patients with HBeAg-positive chronic HBV infection. Patients were randomly assigned in a 1:1 ratio to receive combination therapy with weekly doses of 100 μg peginterferon α-2b (PegIntron, Schering-Plough) and 100 mg lamivudine (Zeffix, GlaxoSmithKline) daily or monotherapy with 100 μg/week peginterferon α-2b and placebo. Total duration of treatment was 52 weeks. To limit the probability of early treatment discontinuation peginterferon was reduced to a dose of 50 μg/week in both treatment arms after 32 weeks. Patients with $\leq$ 55 kg body weight received weight-adjusted dosing of peginterferon α-2b of 1.5 μg/kg/week for the first 32 weeks and 0.75 μg/kg/week for the remainder of the treatment period. All patients were followed-up for 26 weeks after treatment. During

treatment and follow-up patients attended the outpatient clinic every 4 weeks for routine examination and laboratory assessments. Patients were randomized centrally and stratified according to investigational center. Treatment was allocated in blocks of six per center. The study was conducted in accordance with the Declaration of Helsinki and formally approved by the ethical committee of each participating centre. Patients were required to give their written, informed consent before entering the study. Predefined data entry and data analysis were performed at the Clinical Research Unit of the coordinating center at the Erasmus MC, Rotterdam. The sponsor of this study was the Rotterdam Foundation for Liver Research (SLO).

Evaluation

Outcome measures were assessed at the end of treatment (Week 52) and at the end of follow-up (Week 78). The primary outcome measure was loss of HBeAg from serum (AxSYM, Abbott). Secondary outcome measures were HBV-DNA levels below 200,000 copies/ml, HBV-DNA below the level of detection, which was 400 copies/ml (in-house Taqman PCR assay based on the Eurohep standard) (20) , ALT normalization and presence of mutations in the YMDD motif of HBV polymerase (Inno-Lipa assay; Innogenetics). HBsAg (AxSYM, Abbott) and genotype (Inno-Lipa assay; Innogenetics) were also assessed. Routine biochemical and hematological tests were performed at the participating centers using automated techniques. To correct for heterogeneity of local assays, levels of serum ALT were expressed as values representing a ratio to the local upper limit of normal (ULN). All HBV markers were assessed centrally. Liver histology was assessed at baseline and an optional biopsy was taken at the end of treatment. The biopsies were scored centrally by one experienced pathologist who was blinded to the treatment regimen and chronological order of the biopsy. Histological scoring was performed according to the histological activity index, as described by Ishak (21). Improvement of histology was defined as a reduction of at least two points for the necroinflammatory score (range 0–18) and one point for the fibrosis score (range 0–6).

Statistical analysis

The study was powered to account for a mixed population with 50% of patients previously non-responsive to interferon therapy and 50% interferon-naïve, with a baseline ALT level of >2 X ULN. To obtain a power of greater than 80% (α=0.05), an estimated 270 patients needed to be included, assuming HBeAg loss at end of follow up of 20% for peginterferon α-2b monotherapy versus 36% for lamivudine combination therapy. The sample size calculation was based on an estimated drop out rate of 20%, which were to be considered as treatment failures. The effect of peginterferon α-2b monotherapy and combination therapy with lamivudine on response rates were compared by the χ^2 test. Response rates are those assessed by percentage HBeAg loss, HBV-DNA below 200,000 copies/ml, HBV-DNA negative by PCR or ALT normalization at the end of treatment and end of

follow-up. A response was considered to be significantly different between the two treatment groups if it achieved a 0.05 level of significance (χ^2 test). Patients with missing data at Week 52 or at Week 78 were regarded as nonresponders at end of treatment and end of follow up, respectively. The relationship between the patients' baseline characteristics and HBeAg loss at end of follow up (sustained response) was examined by logistic regression analyses. Univariate analysis was used to assess the importance of prognostic factors. To determine the independence of these factors, multiple logistic regression analyses were performed using all baseline characteristics given in table 1. All P values were two sided.

Results

Patient demographics

Of the 307 patients who met the entry criteria and were randomized to receive peginterferon α-2b and lamivudine combination therapy or peginterferon α-2b and placebo, 266 were included in the final modified intent-to-treat analysis (figure 1).

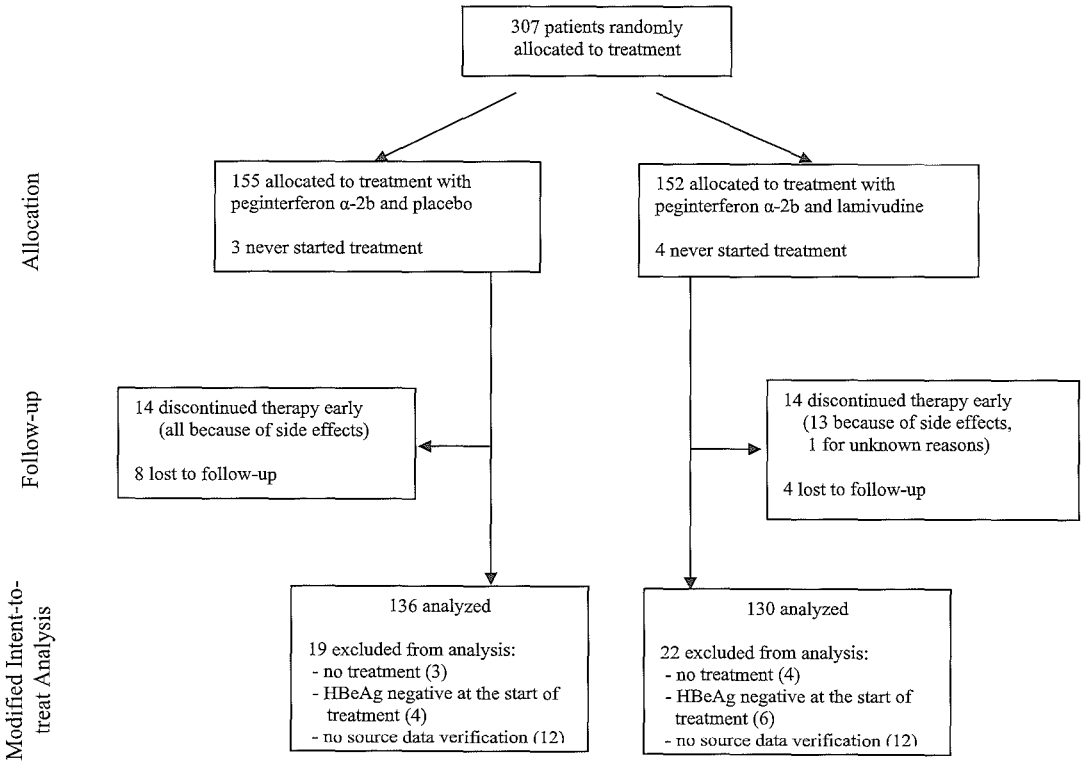


Figure 1. Flow diagram of patients through each stage of the study

Patients were excluded from the final analysis if they were, in retrospect, HBeAg negative at the start of treatment (n=10), if they did not take any study medication (n=7), and patients recruited by one center were excluded because the source data could not be verified (n=24). Patients excluded from the final analysis were equally distributed among assigned treatment groups. Fewer interferon-experienced patients were recruited than expected (21%); therefore, with the total of 266 patients in the modified intent-to-treat group, the study remained more than adequately powered. All patients in the final analysis were comparable with respect to baseline demographics and disease characteristics (table 1).

Table 1. Baseline disease characteristics of the modified intent-to-treat population

Characteristic	**Peginterferon-α 2b + lamivudine (n=130)**	**Peginterferon-α 2b + placebo (n=136)**
Age (year); mean ± SD	34 ± 12	36 ± 14
Sex: male, n (%)	98 (75)	107 (79)
Weight (kg); mean ± SD	74 ± 16	72 ± 13
Ethnic background, n (%)		
Caucasian	98 (73)	101 (74)
Asian	32 (18.5)	29 (21)
Other/Mixed	11 (8.5)	6 (4)
Transmission, n (%)		
Vertical	29 (22)	31 (23)
Sexual / parenteral	32 (25)	33 (24)
Unknown	69 (53)	72 (53)
ALT X ULN (U/l); mean ± SD	4.4 ± 3.9	4.3 ± 3.1
Log HBV DNA (copies/ml); mean ± SD	9.1 ± 1.0	9.1 ± 0.8
Genotype, n (%)		
A	43 (33)	47 (35)
B	11 (9)	12 (9)
C	18 (14)	21 (15)
D	52 (40)	51 (38)
Other	6(4)	5(4)
Cirrhosis, n (%)	13 (12)	11 (10)
Previous interferon therapy, n (%)	27 (21)	28 (21)
Previous lamivudine therapy, n (%)	17 (13)	16 (12)

Table 2. Response to treatment at the end of treatment and the end of follow-up

	End of therapy			End of follow-up		
	Peginterferon-α 2b +lamivudine n=130	**Peginterferon-α 2b +placebo n=136**	**P-value**	**Peginterferon-α 2b + lamivudine N=130**	**Peginterferon-α 2b +placebo N=136**	**P-value**
Virological response in serum						
HBeAg loss	57 (44%)	40 (29%)	0.01	46 (35%)	49 (36%)	0.91
HBeAg seroconversion	33 (25%)	30 (22%)	0.52	38 (29%)	39 (29%)	0.92
HBV DNA < 200.000 geq/ml	96 (74%)	40 (29%)	< 0.001	41 (32%)	37 (27%)	0.44
HBV DNA < 400 geq/ml	43 (33%)	13 (10%)	< 0.001	12 (9%)	9 (7%)	0.43
HBsAg loss	9 (7%)	7 (5%)	0.62	9 (7%)	9 (7%)	0.92
HBsAg seroconversion	8 (6%)	6 (4%)	0.53	9 (7%)	7 (5%)	0.54
Biochemical response in serum						
ALT normalization	66 (51%)	46 (34%)	0.005	46 (35%)	44 (32%)	0.60
Histological response	**n=52**	**n=58**				
Necroinflammatory score						
improved	25 (48%)	31 (53%)	0.57†	-	-	
no change	22 (42%)	21 (36%)		-	-	
worse	5 (10%)	6 (10%)		-	-	
Fibrosis score						
improved	17 (33%)	13 (22%)	0.22†	-	-	
no change	15 (29%)	23 (40%)		-	-	
worse	20 (38%)	22 (38%)		-	-	

† Improvement versus no change or worsening

HBeAg response

As summarized in table 2, the response at the end of treatment (Week 52), as assessed by serum HBeAg loss, was greater in the combination therapy group (44% of patients), compared with the monotherapy group (29%; P=0.01). However, this differential was not sustained at the end of follow up (Week 78). Peginterferon α-2b monotherapy resulted in a response rate of 36% after the end of follow up but only 35% of patients had a sustained response to peginterferon α-2b and lamivudine combination therapy (P=0.91).

HBV-DNA response

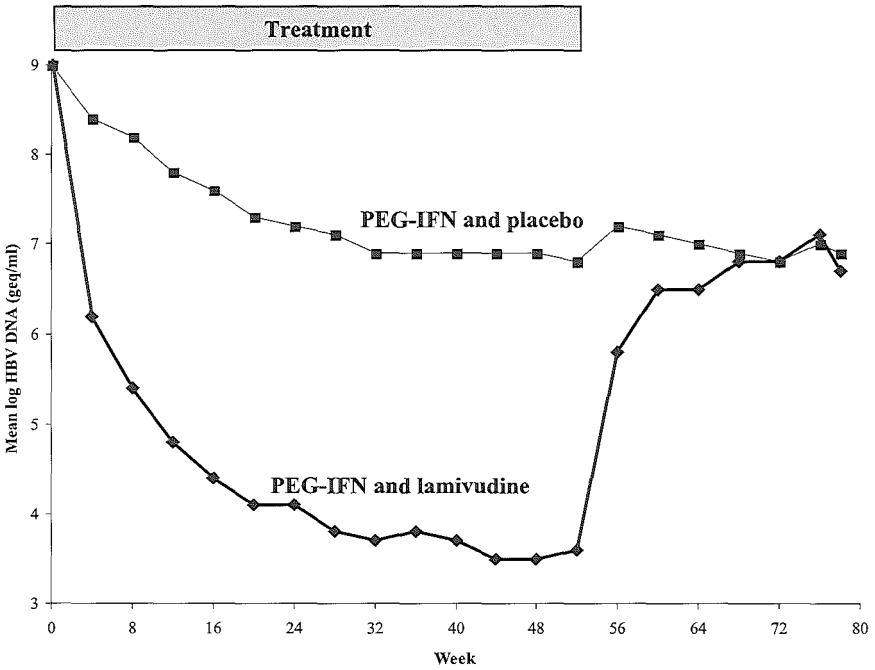
At the end of treatment, more patients (74%) had HBV DNA levels suppressed to below 200,000 copies/ml in the group treated with combination therapy than in those treated with peginterferon α-2b alone (29%; P<0.001) (table 2). The higher levels of HBV DNA suppression achieved in the patients treated with lamivudine combination therapy were not sustained over the follow-up period, with 32% of patients retaining HBV DNA suppression in the combination group, compared with 27% in the group treated with peginterferon α-2b monotherapy (P=0.44). Similarly, when patients with undetectable HBV DNA (HBV negativity by PCR) were assessed, although more patients on the combination therapy than on monotherapy initially responded at the end of treatment (33% compared with 10%; P<0.001), at the end of follow up there was no difference between the treatment groups (9% compared with 7%; P=0.43) (table 2). These response patterns were also reflected by longitudinal serum HBV DNA levels, which revealed greater reduction of viral load in the group treated with combination therapy than those on monotherapy, followed by a post-treatment rebound of HBV DNA only for those treated with combination therapy (figure 2).

ALT response

The response assessed by ALT levels followed a similar pattern to HBV DNA suppression and loss of HBeAg (table 2). At the end of follow-up, there was no difference in response between patients treated with monotherapy (32% of patients responded) and those treated with combination therapy (35%; P=0.60) despite a difference at the end of treatment (34% compared with 51%; P=0.005).

HBsAg response

There was no difference in the proportion of patients with HBsAg loss between the treatment groups at the end of treatment and at the end of follow up (table 2). At the end of follow up 7% of patients in both groups had HBsAg loss (P=0.92).



Patients evaluated											
PEG-IFN/ Placebo	130	125	127	121	122	117	119	108	104	108	108
PEG-IFN/ lamivudine	125	123	120	113	118	115	104	109	104	100	107

Figure 2. Serum levels of hepatitis B virus DNA (HBV DNA)

Histological response

Data on the change in histology of the liver from before treatment to the end of therapy were available for 110 patients (biopsies at the end of treatment were optional). Fibrosis scores improved in 33% of patients on combination therapy and 22% of patients on monotherapy (P=0.22; table 2). With respect to inflammatory changes in the liver, there was little difference between the two treatment groups. Improvements were seen in 48% of patients on combination therapy at the end of treatment and 53% of patients on monotherapy (table 2).

Lamivudine resistance

Of the 130 patients treated with combination therapy (patients who received lamivudine), 14 (11%) patients had the YMDD mutant at the end of treatment. Two patients responded to therapy with loss of HBeAg. Seven of them had been previously treated with lamivudine and harbored a mutant from the

start of therapy.

Response by genotype

There was a significant difference in sustained response rate according to HBV genotype (by univariate analysis, P=0.01). For patients with genotype A, 47% lost HBeAg at the end of follow up, 44% of patients with genotype B responded, compared with 28% with genotype C and 25% with genotype D (figure 3). There was no difference in HBeAg loss according to HBV genotype between the two treatment groups. To assess the independence of genotype as a prognostic factor, multivariate analysis was performed. With this analysis, the odds ratios show that patients infected with genotype A are more likely to respond to treatment than those with genotype D (odds ratio: 2.4, 95% CI 1.3–4.6, P<0.01) or C (odds ratio: 3.6, 95% CI 1.4–8.9, P<0.01). There was a trend for patients infected with genotype B to be more likely to respond than those with genotype C (odds ratio: 2.2, 95% CI 0.7–7.0, P=0.18). Other baseline factors that were predictive of response with multivariate analysis were low viral load (odds ratio: 1.6, 95% CI 1.3–1.8, P<0.01), high ALT levels (odds ratio: 1.1, 95% CI 1.0–1.2, P=0.02), and absence of prior interferon therapy (odds ratio: 2.2, 95% CI 1.1–4.5, P=0.04).

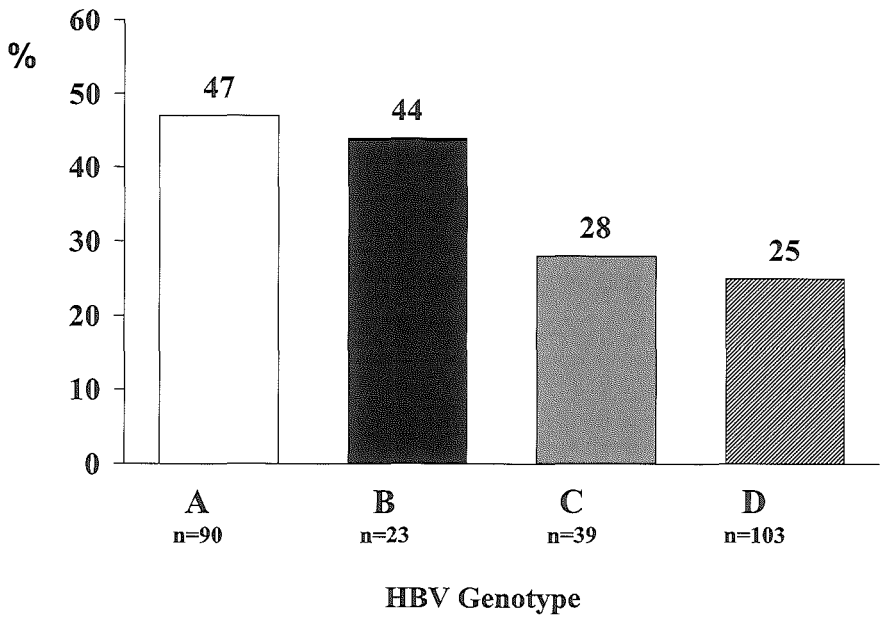


Figure 3. Proportion of patients responding to treatment (serum HBcAg loss) by HBV genotype.

A

Adverse event	Peginterferon + lamivudine n=130 (%)	Peginterferon + placebo n=136 (%)
Body as whole		
Flu-like syndrome	96(74)	84(62)
Headache	59(45)	55(40)
Fatigue	54(42)	59(43)
Myalgia	42(32)	41(30)
Abdominal pain	25(19)	26(19)
Arthralgia	20(15)	22(16)
Digestive tract		
Weight loss (>10%)	25(19)	28(21)
Anorexia	21(16)	22(16)
Diarrhea	14(11)	15(11)
Nausea	14(11)	25(18)
Dermatological		
Local reaction	38(29)	36(27)
Alopecia	35(27)	26(19)
Pruritus	18(14)	14(10)
Psychiatric		
Depression*	28(22)	29(21)
Insomnia	20(15)	11(8)
Hematological		
Neutropenia (<1.5x10^9)	34(26)	29(21)
Thrombocytopenia (<75x10^9)	14(11)	17(13)

- includes depression, mood changes and irritability

B

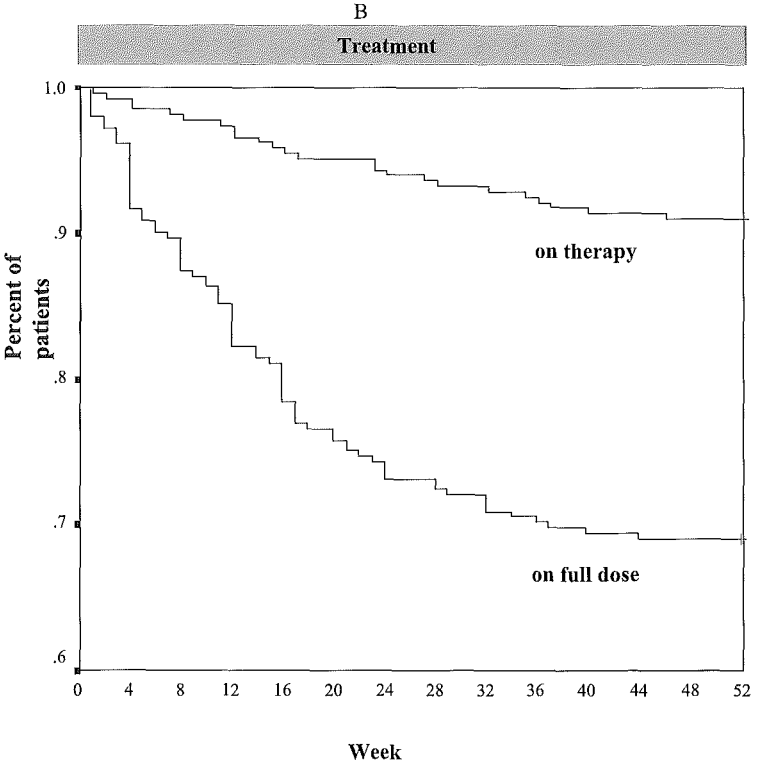


Figure 4. Safety and tolerability: A. Common adverse events indicating number of patients (percent). B. Rates of discontinuation of treatment and dose reduction during treatment

Safety

With respect to safety and tolerability, there was no difference between patients treated with peginterferon α-2b alone and those treated with peginterferon α-2b and lamivudine combination therapy. The side effect profile was similar to that seen with standard interferons and there were no new side effects that could be attributable to peginterferon α-2b. Overall, the incidence and severity of adverse events were comparable between the treatment groups. Common side effects included flu-like symptoms, headache, fatigue and local reaction at the injection site (figure 4A). There were 32 serious adverse events (12% of patients), of which 17 were probably related to therapy and all were reversible. The adverse events were: hepatitis flare (4), depression (3), severe neutropenia (3), psychosis (1), seizures (1), pancreatitis (1), anxiety (1), dizziness (1), diarrhea (1) and syncope (1). All serious adverse events were reversible after treatment was stopped. At the end of treatment 91% of patients remained on treatment and 69% of patients remained on full dose treatment (figure 4B). There were no significant differences in dose reductions between the two treatment groups.

Discussion

The current standard initial therapy for patients with chronic HBV infection is with interferon-α or lamivudine (22,23). The introduction of peginterferons, with their improved pharmacokinetic profiles, have led to higher response rates in the treatment of chronic hepatitis C (24,25) and a preliminary study suggested improvements in the response rates in HBeAg positive CHB patients (16). In this large, randomized study, sustained response (HBeAg loss) in HBeAg-positive CHB patients treated with peginterferon α-2b monotherapy was 36%. Treatment with peginterferon α-2b and lamivudine combination therapy was superior to peginterferon α-2b monotherapy at end of treatment, but not at end of follow up.

Two earlier, randomized controlled trials comparing combination and monotherapy in HBeAg-positive HBV patients include a shorter duration of lamivudine therapy in the group that received combination therapy than those who received monotherapy (3,18). This and the timing of primary efficacy endpoint (post-treatment for combination therapy or interferon alone versus on-treatment for lamivudine alone) prevented a definitive conclusion of the efficacy of combination therapy being reached. The authors concluded that the potential benefit of combining lamivudine with interferon therapy should be investigated further with different regimens of combination therapy.

The study reported here progressed these investigations and helps towards drawing a definitive conclusion on the efficacy of combination versus monotherapy for two major reasons. Firstly, it compares equivalent duration of treatment in the monotherapy and combination therapy arms. Secondly, the prolonged follow-up period extended further beyond the end of treatment with

lamivudine than previous studies, which enabled the extent of relapse to be monitored after 26 weeks follow up.

Our study highlights differences in response to interferon and to nucleoside analogues. Despite patients initially responding to lamivudine, the response (HBeAg seroconversion and HBV DNA reduction) is unsustained. This agrees with previous reports, which noted HBeAg relapse rates of 49% to 54% (11, 26). Lack of durability may be due to the mechanism of action of lamivudine, which suppresses viral replication without inducing the HBV-specific immune response necessary for sustained viral eradication.

Prolonged therapy with lamivudine is not an option because it usually leads to drug resistance (22, 27). This is a particular problem for hepatitis B patients because many patients develop CHB at a relatively young age and it is difficult to treat them for several decades with resistance-prone medication for which long-term toxicity is unknown. More recently, a study with the nucleotide analogue adefovir dipivoxil suggested that response was achieved with development of phenotypic resistance in less than 1.6% of the patients (9). Future studies are needed to determine whether this response is sustained beyond the end of therapy and whether, with continued therapy, clinically relevant drug resistance remains absent.

The study reported here reveals prospectively, for the first time, the importance of HBV genotype as independent predictor of treatment response for CHB using interferon. It corroborates with earlier retrospective studies, which indicated that HBV genotypes C and D are more difficult to treat than genotypes A and B (28-30). Our study indicates that future intervention studies for CHB may need stratification according to genotype.

The side effects and frequency of adverse events observed with peginterferon α-2b monotherapy treatment of CHB were similar to those encountered with standard interferon therapy. The rate of dose reductions (31%) and discontinuations (9%) was comparable to that reported with peginterferons in patients with chronic hepatitis C (15, 24, 25), and with peginterferon α-2a in CHB (16).

Conclusions

Peginterferon α-2b is effective and well tolerated for CHB. Sustained clearance of serum HBeAg and reduction of viral load are as high or higher than those that have previously been reported for any other therapy in this indication. Combination therapy with peginterferon α-2b and lamivudine is not superior to peginterferon α-2b monotherapy and HBV genotype is an important predictor of response to treatment.

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Chapter 3

PEG-interferon improves liver histology in patients with HBeAg positive chronic hepatitis B. No additional benefit of combination with lamivudine.

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Abstract

The effect of pegylated interferon or its combination with lamivudine on liver histology of patients with chronic hepatitis B (CHB) is unknown. In a double-blinded, randomized, multi-center study we assessed histological changes in 110 HBeAg-positive CHB patients treated for 52 weeks with Pegylated interferon alpha-2b (PEG-IFN)100μg/week in combination with either lamivudine 100 mg/day or placebo. The dose of PEG-IFN was decreased to 50 μg/week in all patients after 32 weeks of treatment. Liver biopsies were taken before and at the end of treatment. All biopsies were blinded and scored according to the Ishak system which includes a necroinflammatory score (0-18) and a fibrosis score (0-6). Necroinflammatory score improved (defined as a decrease of at least 2 points) in 25 patients (48%) of the PEG-IFN/ lamivudine combination therapy group and in 31 patients (53%) of the PEG-IFN monotherapy group. The fibrosis score improved (decrease of at least 1 point) in 17 patients (33%) of the combination therapy group versus 13 patients (22%) of the PEG-IFN monotherapy group (p=0.23). Responders (n=42), defined as serum HBeAg negative at the end of therapy, showed a larger decline in necroinflammatory score than non-responders (mean decline 2.3 and 1.2 points, respectively, p=0.02). Among patients receiving PEG-IFN monotherapy necroinflammation improved more frequently in responders (78% of responders vs 43% of non-responders, p=0.01) and in patients who showed normalization of ALT (76% of patients with normal ALT vs 40% of patients with abnormal ALT, p=0.01). Fibrosis score in the PEG-IFN monotherapy group improved more often in responders (39%) than in non-responders (15%, p=0.04). In the PEG-IFN/ lamivudine combination therapy group we found no significant association between virological and biochemical endpoints and histological improvement.

In conclusion, treatment with PEG-IFN therapy improves liver necroinflammation in HBeAg positive CHB patients, particularly in responders to therapy. PEG-IFN also improves fibrosis in responders. Addition of lamivudine to PEG-IFN did not further improve the histological outcome.

Introduction

The aim of treatment of chronic hepatitis B (CHB) is to prevent progression of liver disease to cirrhosis, liver failure and hepatocellular carcinoma. Lamivudine, which strongly suppresses HBV replication, has been shown to improve liver inflammation and slow the progression of fibrosis (1-3). Histological improvement (decrease of at least two points in total or necroinflammatory Knodell HAI score) was found in 49-67% of patients after one year of lamivudine treatment. Interferon therapy for 16 weeks has also been shown to improve liver histology (3-5). Long-term follow-up studies in Caucasian patients treated with interferon showed that the risk of HBV-related complications is reduced and survival is better in patients who lose hepatitis B e-antigen (HBeAg) (6, 7). In this study we investigated the effect of pegylated interferon alpha-2b (PEG-IFN) therapy alone and in combination with lamivudine on liver inflammation and fibrosis. Furthermore, we investigated whether virological and biochemical treatment endpoints were associated with improvement of liver histology.

Patients and methods

Patients

A total of 266 patients were included in an international randomized double-blinded study (HBV 99-01 study) reported previously (8). Eligible patients included men and women over 18 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over six months, and detectable serum HBV DNA levels. All patients were HBeAg positive and had ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization. Patients were excluded if they had decompensated liver disease (prothrombin time prolonged by $\geq$ 3 seconds, serum albumin below 35 g/l, ascites, encephalopathy or a history of variceal bleeding) or any other cause of liver disease. Also patients who were coinfected with hepatitis C, hepatitis D or Human Immunodeficiency Virus and patients with other significant comorbidity were excluded.

Study design

Treatment

Patients received PEG-IFN 100 μg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN was reduced to 50 μg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and then followed for an additional 24 weeks. During treatment and follow-up patients were evaluated every 4 weeks through routine physical examinations and laboratory assessments. Because liver histology was assessed at the end of therapy, all other treatment endpoints (virological, serological and biochemical) were also defined at the end of therapy.

Response was defined as HBeAg negativity at the end of therapy. Relapse was defined as reappearance of HBeAg during follow-up.

Histology

Liver biopsies were taken in all eligible patients before the start of therapy. If a recent biopsy (taken less than 1 year prior to the start of therapy) was available, no new biopsy was required. A second liver biopsy at the end of therapy was optional. All biopsies were blinded and scored by one experienced liver pathologist (P.Z.) according to the Ishak system which includes a necroinflammatory score (0-18) and a fibrosis score (0-6). The Inflammatory score was defined as "improved" if there was a decrease of ≥2 points, as "worse" if it increased by ≥2 points. For the fibrosis score improvement was defined as a decrease of ≥1 point, and worsening as an increase of ≥1 point. Overall sample length and the number of portal tracts were recorded. Biopsy samples were considered adequate for evaluation if they were at least 0.5 cm long, contained at least four evaluable portal tracts and were not so fragmented as to preclude recognition of acinal architecture.

Statistical analysis

Comparisons between groups were made using the chi-square test for categorical variables, and the Mann-Whitney test for continuous variables. We used logistic regression for multivariate analysis. The following baseline variables were evaluated as prognostic factors for improvement of necroinflammation and fibrosis, or with worsening of fibrosis: Sex, race, age, body mass index, transmission route, genotype of HBV, ALT, log HBV DNA, previous therapy with lamivudine or interferon. Also a separate multivariate analysis was performed to evaluate which treatment endpoints were associated with improvement of necroinflammation and fibrosis, or with worsening of fibrosis. The following treatment endpoints were considered in the model: ALT normalization, HBV DNA below 20000 copies/ml, HBV DNA PCR negativity, HBeAg loss and HBeAg seroconversion.

Results

Study population

The 266 patients analyzed in the original international study were eligible for the present histological study. Patients were encouraged to undergo a second biopsy at the end of therapy, but this biopsy was optional. We obtained paired biopsies from 151 patients (Figure 1). Sixteen biopsies were considered not evaluable, because they were too small (<0.5 cm). Of the remaining biopsies, 29 biopsies were not evaluable because they contained not enough portal tracts (n=12), were too fragmented (n=14) or for technical reasons (n=3). These 45 unevaluable biopsies, taken either pre-or post-treatment, came from 41 patients. Thus, 110 patients with evaluable biopsy pairs were included in the present study. There were no significant differences in baseline characteristics between patients for whom histological

assessment was feasible or not, therefore the population in the histological study was representative of the original study population. Baseline characteristics were comparable for both treatment groups (Table 1).

Table 1 Baseline characteristics

	PEG-IFN/ lamivudine (n= 52)	PEG-IFN/ placebo (n=58)
Age*	34±12	33±12
Sex M/F (%)	38/14 (73%/ 27%)	47/11 (81%/ 19%)
Weight (kg)*	74±16	72±13
Race (%)		
Caucasian	37 (71%)	43 (74%)
Asian	10 (19%)	12 (21%)
Other/ Mixed	5 (10%)	3 (5%)
Geographical distribution (%)		
Europe	42 (81%)	49 (85%)
Asia	5 (9.5%)	6 (10%)
North America	5 (9.5%)	3 (5%)
Transmission (%)		
Vertical	13 (25%)	16 (28%)
Sexual/ parenteral	14 (27%)	13 (22%)
Unknown	25 (48%)	29 (50%)
ALT x ULN (U/l)*	4.7±3.3	4.1±3.0
Log HBVDNA (copies/ ml)	9.1±0.9	9.1±0.8
Genotype (%)		
A	11 (21%)	16 (28%)
B	5 (10%)	3 (5%)
C	8 (15%)	10 (17%)
D	25 (48%)	27 (47%)
Previous IFN therapy (%)	10 (19%)	12 (21%)
Previous lamivudine therapy (%)	3 (6%)	6 (10%)
Liver Histology		
Necroinflammatory score*	5.4±2.0	5.6±2.2
Fibrosis score*	2.6±1.5	2.3±1.6
Cirrhosis (%)	6 (12%)	7 (12%)

* mean ± SD

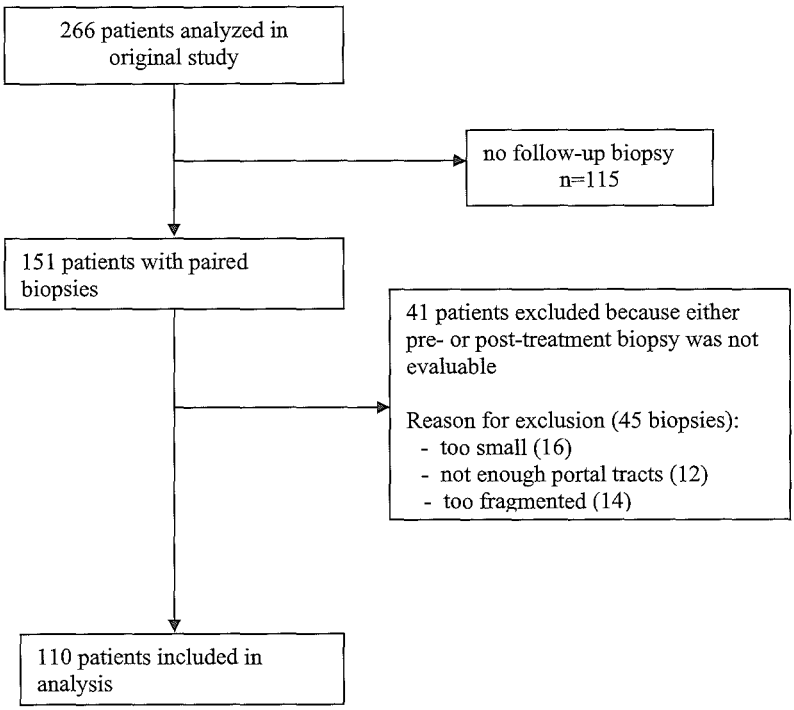

Figure 1 patient population

Effect of PEG-IFN and PEG-IFN/lamivudine combination therapy on liver histology

Necroinflammatory score

The mean necroinflammatory score at baseline was 5.2 (range 1-10). Scores were not different between treatment groups. Overall, the mean necroinflammatory score improved by 1.6 points ($p<0.001$). In both treatment groups we found a significant improvement in necroinflammatory score ($p<0.001$), which was not different between the two groups (Table 2). The necroinflammatory score improved in 51% of the patients (decrease ≥2 points), and only 10% showed worsening (increase of ≥2 points) of necroinflammation. The proportion of patients in each treatment group showing changes in liver histology is shown in figure 2. Inflammation improved in 48 % of patients in the PEG-IFN/lamivudine combination therapy group and in 53% of patients in the PEG-IFN monotherapy group ($p=0.57$).

Fibrosis score

At baseline, the mean fibrosis score was 2.4 (range 0-6). Overall the mean fibrosis score increased by 0.3 points ($p=0.03$). The fibrosis score improved in 27% of the patients (decrease ≥1 point). The mean fibrosis score increased by 0.2 points in the combination therapy group, and by 0.4 points in the PEG-

IFN monotherapy group (p=0.59) (Table 2). Improvement of fibrosis was found in 33% of patients in the combination therapy group and in 22% of patients in the PEG-IFN monotherapy group (p=0.23).

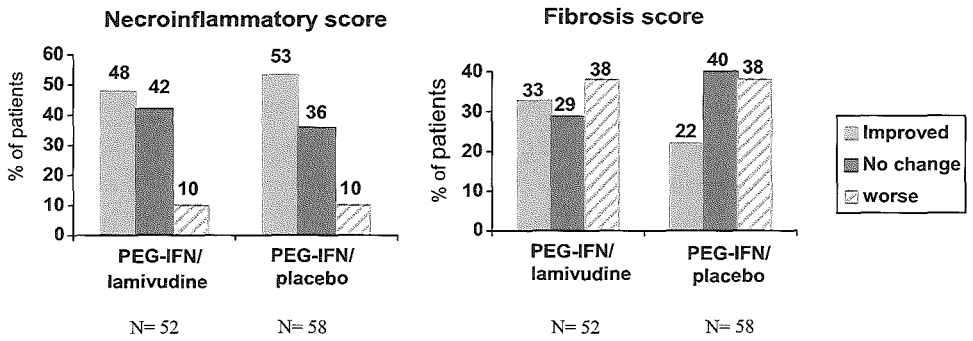

Figure 2 Histological response in patients receiving PEG-IFN-lamivudine combination therapy and PEG-IFN monotherapy

Table 2 Histological changes in patients receiving PEG-IFN/ lamivudine combination therapy or PEG-IFN alone

	PEG-IFN/ lamivudine (n=52)	PEG-IFN/ placebo (n=58)
Necroinflammatory score		
Pretreatment*	5.4± 2.0	5.6 ± 2.2
	(2-9)	(1-10)
Posttreatment*	3.7 ± 2.0	4.1 ± 1.8
	(1-8)	(1-9)
p (pre- vs posttreatment)	<0.001	<0.001
change*	-1.7 -± 2.6	-1.5 ± 2.3
	(-7/+3)	(-7/+4)
Fibrosis score		
Pretreatment*	2.6 ± 1.5	2.3 ± 1.6
	(0-6)	(0-6)
Posttreatment*	2.8 ± 1.8	2.7 ± 1.6
	(0-6)	(0-6)
p (pre- vs posttreatment)	0.23	0.07
change*	0.2 ± 1.4	0.4 ± 1.5
	(-3/+3)	(-2/+5)

* mean ± SD (range)

Baseline factors associated with histological improvement

Multivariate analysis showed that two baseline factors were associated with improvement of necroinflammatory scores: low HBV DNA levels (p=0.002), and low body mass index (0.02). Both factors were also associated with stable or improved fibrosis stage. The effect of HBV genotype on histological response is shown in figure 3. Patients with genotype A showed a significantly larger decrease in necroinflammatory scores, compared to genotype D (mean decrease 2.4 vs. 1.1, p=0.02). Improvement of necroinflammation was found in 67% of patients with genotype A, 63% of patients with genotype B, 50% of patients with genotype C and 42% of patients with genotype D (p=0.04 genotype A vs D). The difference in histologcal improvement between patients with genotype A and D can only partly be explained by a higher (serological) response rate of genotype A. When we analyzed responders separately, patients with genotype A showed a mean decrease in necroinflammatory score of 3.3, whereas this was only 1.8 for patients with genotype D (p=0.08). Changes in fibrosis score were not different between genotypes.

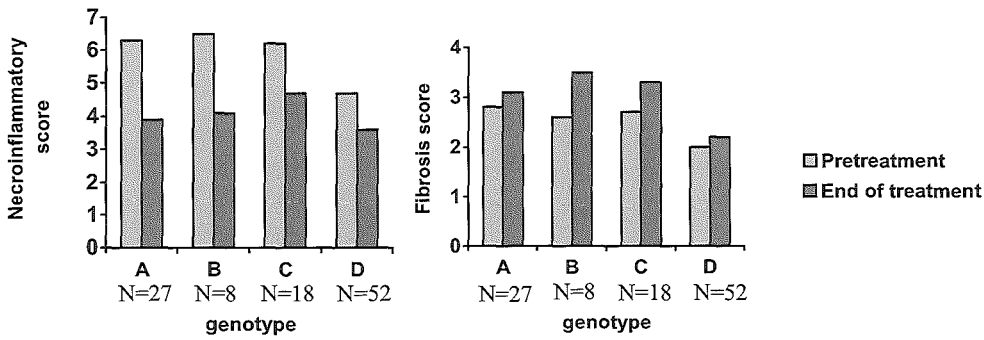

Figure 3 Changes in mean necroinflammatory and fibrosis scores within the different genotypes

Relation of liver histology with virological and biochemical response at the end of therapy

Loss of HBeAg

Loss of HBeAg (response) was achieved in 24 patients (47%) in the combination therapy group and in 18 patients (33%) in the PEG-IFN monotherapy group. The mean necroinflammatory and fibrosis scores at baseline were higher among patients who showed HBeAg loss (Table 3). Both responders and non-responders showed a significant decline in necroinflammatory scores. However, this decline was larger in patients who lost HBeAg (mean decline 2.3 vs 1.2 points, p=0.02). Necroinflammation improved in 62% (26/42) of responders, but only in 44% (30/68) of non-responders (p=0.07). Improvement of fibrosis score was found in 31% (13/42) of responders and 25% (17/68) of non-responders (p=n.s.). HBeAg loss was significantly associated with improvement of necroinflammation (78% of responders vs. 43% of non-responders, p=0.01) and reduction of fibrosis (39% of responders

vs. 15% of non-responders, p=0.04) in the PEG-IFN monotherapy group, but not in the combination therapy group (Table 4).

Table 3 Histological changes in patients showing HBeAg loss (responders) and patients who remained HBeAg positive at the end of therapy (non-responders)

	Responders (n=42)	Non-responders (n=68)
Necroinflammatory score		
pretreatment	6.0 ± 2.0†	5.2 ± 2.1†
	(2-9)	(1-10)
posttreatment	3.7 ± 1.6	4.0 ± 2.1
	(1-8)	(1-9)
p (pre- vs posttreatment)	<0.001	<0.001
change	-2.3 ± 2.4‡	-1.2 ± 2.4‡
	(-7/+3)	(-7/+4)
Fibrosis score		
pretreatment	2.8 ± 1.4*	2.2 ± 1.6*
	(0-6)	(0-6)
posttreatment	3.2 ± 1.9**	2.5 ± 1.6 **
	(0-6)	(0-6)
p (pre- vs posttreatment)	0.18	0.07
change	0.4 ± 1.4	0.3 ± 1.5
	(-2/+3)	(-3/+5)

† p=0.07 responders vs. non-responders

‡ p=0.02 responders vs. non-responders

* p=0.02 responders vs. non-responders

** p=0.07 responders vs. non-responders

Loss of HBV DNA

Among patients that had lost HBV DNA at the end of treatment (PCR negative, detection limit 373 copies/ml), necroinflammation improved in 64% (16/25), as compared to 47% (40/85) for patients who remained PCR positive (p=0.14). In the combination therapy group the proportion of patients that showed improvement in necroinflammatory scores was higher in patients that lost HBV DNA than in patients that remained PCR positive (62% vs 39 % respectively). In the PEG-IFN monotherapy group only 4 of the 58 patients lost HBV DNA. (Table 4). We found no significant association between loss of HBV DNA and fibrosis scores.

ALT normalization

At the end of therapy ALT levels had normalized in 30 patients (58%) in the combination therapy group and in 21 patients (36%) in the PEG-IFN monotherapy group. Among patients with normal ALT levels at the end of therapy 63% showed improved necroinflammatory scores, compared to 41% of patients with persistently abnormal ALT levels (p=0.02). Among patients receiving PEG-IFN monotherapy, the necroinflammatory score improved in 76% of patients who normalized ALT, compared to 41% of patients who did not (p=0.009) (Table 4). In the PEG-IFN monotherapy group progression of fibrosis occurred more often in patients with persistently elevated ALT levels than in patients whose ALT levels normalized (49% vs. 19% respectively, p=0.08). We found no significant association between ALT normalization and histological changes (necroinflammation or fibrosis) in the combination therapy group.

Table 4 Relation of virological and biochemical endpoints at the end of therapy and histological improvement

Endpoint	Improvement of inflammation		Improvement of fibrosis	
	PEG-IFN/ lamivudine	PEG-IFN/ placebo	PEG-IFN/ lamivudine	PEG-IFN/ placebo
HBeAg loss				
Yes	12/24 (50%)	14/18 (78%)	6/24 (25%)	7/18 (39%)
No	13/28 (46%)	17/40 (43%)	11/28 (39%)	6/40 (15%)
p*	0.80	0.01	0.27	0.04
HBV DNA loss (PCR negative)				
Yes	13/21 (62%)	3/4 (75%)	6/21 (21%)	2/4 (50%)
No	12/31 (39%)	28/54 (52%)	11/ 31 (36%)	11/54 (20%)
p*	0.10	0.37	0.60	0.17
ALT normalization				
Yes	16/30 (53%)	16/21 (76%)	11/30 (37%)	6/21 (29%)
No	9/22 (41%)	15/37 (40%)	6/22 (27%)	7/37 (19%)
p*	0.38	0.01	0.48	0.40

* p-value represents difference between patients who do and do not reach the endpoint

Multivariate analysis of treament endpoints

Overall, ALT normalization was the only variable independently associated with improved necroinflammation (relative risk (RR) 2.5, p=0.02). In the PEG-IFN monotherapy group two factors were independently associated with improved necroinflammatory scores: HBeAg loss (RR 3.7, p=0.04) and ALT normalization (RR 3.8, p=0.03). In this group, HBeAg loss was also associated with improved fibrosis (RR 3.6, p=0.05) and ALT normalization with a decreased risk of worsening of

fibrosis (RR 0.25, p=0.03). In the combination therapy group we found no significant association between treatment endpoints and histological response.

Effect of lamivudine resistance on liver histology

Four patients in the combination therapy group developed lamivudine resistant HBV variants (YMDD mutants) during therapy. An additional patient had previously been treated with lamivudine and harbored a YMDD mutant from the start of therapy. Two of these 5 patients responded to therapy (HBeAg loss). Among the 5 patients with YMDD mutants, 3 showed improvement in necroinflammatory score, and 2 showed improvement in fibrosis score. None of the patients showed worsening of necroinflammation or fibrosis.

Relation of liver histology with relapse

Relapse (defined as recurrence of HBeAg during post-treatment follow-up) occurred in 9 of the 24 (38%) responders in the combination therapy group, but only in 2 of the 18 responders in the PEG-IFN monotherapy group (11%) (p=0.05). Among sustained responders 71% had improved necroinflammatory score at the end of therapy, compared to 36% of patients who relapsed (p=0.04) (figure 4). There were no significant differences in improvement or worsening of fibrosis between sustained responders and patients who exhibited a relapse after treatment discontinuation.

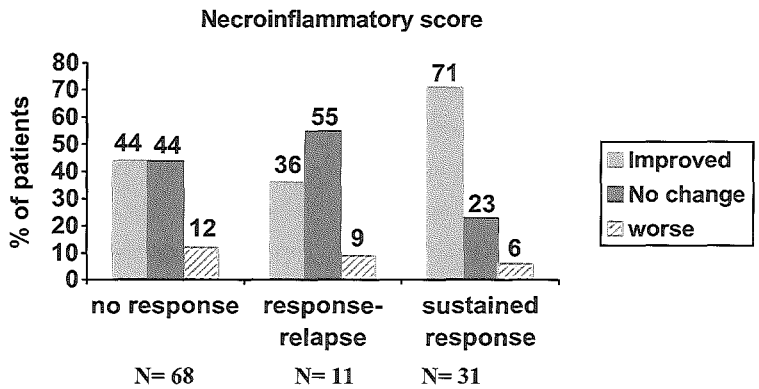

Figure 4 Histological response in patients with a sustained virological response, relapsers and non-responders

Discussion

This report is the first to describe the histological response to a pegylated interferon in CHB patients. Recently, PEG-IFN has shown to be effective in CHB patients, and here we report the histological results in the patient group described earlier (8). We found a significant reduction in

necroinflammatory activity, and an improved necroinflammatory score (reduction of at least 2 points) in 51% of patients. In addition, 27% of the patients showed reduction of fibrosis. We did not find a significant difference in histological outcome between patients treated with PEG-IFN alone or PEG-IFN in combination with lamivudine.

Several studies have shown that liver histology improves in CHB patients who respond to standard IFN therapy (4, 5, 9-11). Perrillo et al. found improved HAI scores in responders and reported improvement in liver histology (ranked assessment) in 18 of 30 (60%) patients who responded to standard IFN compared to 8 of 24 (33%) untreated controls (4). Previously, we also found improved HAI scores in responders to standard IFN (5). In the current study with PEG-IFN monotherapy 78% of responders showed improved necroinflammatory score and 39% of responders showed reduction of fibrosis.

Histological improvement was significantly associated with HBeAg loss and ALT normalization at the end of PEG-IFN therapy. We could not find an additional benefit of combination therapy on liver necroinflammation, despite the greater proportion of patients showing improvement in the virological and biochemical markers of the disease at the end of treatment. This might be explained by a larger proportion of patients showing a post-treatment relapse in the lamivudine group. Most of these patients showed no improvement in necroinflammation, which suggests that an adequate immune response, rather than virus suppression alone may be required to achieve histological improvement and sustained virological remission.

Prolonged treatment with lamivudine leads to a sustained improvement of necroinflammation and has been shown to substantially improve fibrosis and even reverse cirrhosis in the majority of patients (12, 13). However the efficacy of lamivudine therapy is limited by the increasing incidence of YMDD mutants, which rises to 70-71% after 4 years of lamivudine treatment (14, 15). In this study we also demonstrated that improvement in fibrosis occurred more often in the PEG-IFN/lamivudine combination therapy group. In the PEG-IFN monotherapy group improvement of fibrosis strongly depended on response, while in the combination therapy group improved fibrosis scores were found in 37% of non-responders. This might indeed be due to the effect of lamivudine (1-3). Whether this beneficial effect of combination therapy is durable after stopping of treatment is still uncertain and should be investigated further in future studies.

We conclude that PEG-IFN therapy improves liver necroinflammation in CHB patients, particularly in responders to therapy. Fibrosis improved significantly more often in responders to PEG-IFN. Adding lamivudine did not further improve the histological outcome .

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Chapter 4

Viral decline during PEG-interferon alone and in combination with lamivudine in HBeAg- positive chronic hepatitis B. Early prediction of response.

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Submitted

Abstract

Treatment of chronic hepatitis B is still only effective in a minority of patients. Attempts are being made to improve the efficacy of interferon alpha (IFN) therapy by using pegylated interferons and combining its immunomodulatory effect with the potent virussuppressive effect of nucleoside analogues. To evaluate viral decline during therapy with pegylated interferon alpha-2b (PEG-IFN) and establish if combination therapy with lamivudine has an additive effect, we analyzed viral decline in 266 HBeAg positive chronic hepatitis B patients treated with PEG-IFN monotherapy or PEG-IFN/ lamivudine combination therapy. Serum levels of HBV-DNA were measured monthly. Response (HBeAg negativity at the end of follow-up) was achieved in 35% of patients (46/130) in the combination therapy group and in 36% of patients (49/136) in the PEG-IFN monotherapy group. Throughout the study period, we found a significantly faster HBV DNA decline in the combination therapy group, compared to the monotherapy group. In the combination therapy group a HBV DNA level below 10^7 geq/ml after 4 weeks of therapy predicted response (sensitivity 95%, specificity 65%, positive predictive value 45%, negative predictive value 93%). In the PEG-IFN monotherapy group the decline of HBV DNA was less predictable. In this group more complex HBV DNA decay patterns were found. A HBV DNA level below 10^9 geq/ml after 20 weeks of therapy predicted response in this group (sensitivity 94%, specificity 71%, positive predictive value 45%, negative predictive value 88%).

We conclude that combination therapy with PEG-IFN and lamivudine is more effective in suppressing HBV replication than PEG-IFN alone. This did not result in enhanced sustained response rates. Only for patients treated with combination therapy initial decline of HBV DNA was indicative of response. For patients treated with PEG-IFN alone response was associated with a delayed HBV DNA decline, probably due to the necessary enhanced immune reactivity. In both groups HBV DNA levels during therapy could be used to predict response.

Introduction

Treatment of HBeAg-positive chronic hepatitis B is still only effective in a minority of patients. Interferon alpha (IFN) has been shown to induce HBeAg seroconversion in one third of patients (1), but has considerable side effects. Lamivudine strongly suppresses viral replication and induces HBeAg-seroconversion in 16-18% of patients (2-5). However the response to lamivudine is not durable after discontinuation of therapy (6) and prolonged therapy leads to the emergence of YMDD mutant strains in an increasing proportion of patients (7, 8). The response rate to both lamivudine and IFN monotherapy is dependent of baseline ALT levels (9, 10). The limited efficacy of standard therapy makes new approaches necessary. The efficacy of IFN therapy may be improved by using pegylated interferons, which have been shown to produce higher response rates in patients with chronic hepatitis C (11-15), and possibly in chronic hepatitis B (16). Combining the virussuppressive and immunomodulatory effects of lamivudine and IFN may be more effective than monotherapy with either drug (2, 17), particularly in patients with moderately elevated ALT levels (2). To evaluate whether viral decline during therapy predicts sustained response (HBeAg loss 6 months posttreatment) we analyzed viral decline in HBeAg-positive chronic hepatitis B patients treated with pegylated interferon alpha-2b (PEG-IFN) alone or in combination with lamivudine.

Patients and methods

Patients

A total of 266 patients were included in an international multicenter randomized double-blinded study reported previously(18). Eligible patients were men and women over 18 years of age with chronic hepatitis B, documented by liver biopsy and HBsAg positivity for over six months, and positive serum HBV DNA levels. All patients were HBeAg-positive and had ALT levels of at least 2 times the upper limit of normal on two occasions within eight weeks before randomization. Patients were excluded if they had decompensated liver disease (prothrombin time prolonged by $\geq$ 3 seconds, serum albumin below 35 g/l, ascites, encephalopathy or a history of variceal bleeding) or any other cause of liver disease. Also patients who were coinfected with hepatitis C, hepatitis D or Human Immunodeficiency Virus and patients with other significant comorbidity were excluded.

Study design

Patients received PEG-IFN 100 μg once weekly and were randomized to receive either lamivudine 100 mg once daily or placebo. The dose of PEG-IFN was reduced to 50 μg once weekly after 32 weeks of therapy. Patients were treated for 52 weeks and followed for 6 months posttreatment. Serum samples for HBV DNA measurement were taken at the start of therapy (day 0), and thereafter monthly until the

end of follow-up. HBV DNA levels were measured using an in-house developed TaqMan real-time PCR test. (dynamic range $4x10^2$-10^{10} copies/ml) (19). The Eurohep HBV DNA standard was used for validation of HBV DNA levels (20). HBeAg was measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, Il). Response was defined as HBeAg negativity at the end of follow-up.

Statistical analysis

Comparisons between groups were done using the chi-square test for categorical variables, and the Mann-Whitney test for continuous variables. To investigate whether response could be predicted at an early timepoint during therapy, HBV DNA levels and HBV DNA decline from baseline to each timepoint from week 4 to 32 were assessed as possible predictors of response. For each test areas under the receiver operating characteristic (ROC) curves were calculated, and compared using the method of DeLong et al.(21). Also for each timepoint different levels (in half-log steps) of HBV DNA and decline from baseline were assessed to determine which cut-off level best excluded patients who failed to respond.

Results

Patient characteristics

Patient characteristics are shown in table 1. Of the 266 patients, 205 were male (77%) and 199 were of Caucasian origin (75%). The PEG-IFN monotherapy and PEG-IFN/ lamivudine combination therapy groups were comparable with respect to all baseline parameters. Previously 55 patients (21%) had been treated with standard IFN, and 33 patients (12%) with lamivudine. None of the patients had received any antiviral therapy within 6 months of entering the study.

Viral decline with PEG-IFN alone and PEG-IFN/lamivudine combination therapy

The decline of HBV DNA during therapy is shown in figure 1. The combination therapy group showed a biphasic decline, whereas the decline in the PEG-IFN monotherapy group showed a less consistent pattern. In the PEG-IFN monotherapy group 57 patients (43%) showed a decline of at least 0.5 log during the first month of therapy. Another 36 patients (27%) showed no initial decline, but a delayed decline of at least 2 log from baseline during week 4-32 of therapy (n=17), week 32-52 of therapy (n=8) or during follow-up (n=11). The monthly HBV DNA decline was significantly less in the monotherapy group than in the combination therapy group during the first 24 weeks ($p<0.01$) and again during weeks 24-28 of therapy ($p=0.01$). Patients in the combination therapy group had significantly lower mean HBV DNA levels at all timepoints during therapy. After de end of therapy, however, HBV DNA levels for both groups became comparable

Table 1 Baseline characteristics

	PEG-IFN/ lamivudine (n= 130)	PEG-IFN/ placebo (n=136)
Age*	34±12	36±14
Sex M/F (%)	98/32 (75/ 25)	107/29 (79/ 21)
Weight (kg)*	74±16	72±13
Race (%)		
Caucasian	98 (73)	101 (74)
Asian	32 (18.5)	29 (21)
Other/ Mixed	11 (8.5)	6 (4)
Transmission (%)		
Vertical	29 (22)	31 (23)
Sexual/ parenteral	32 (25)	33 (24)
Unknown	69 (53)	72 (53)
ALT x ULN (U/l)*	4.4±3.9	4.3±3.1
Log HBVDNA (copies/ ml)*	9.1±1.0	9.0±1.0
Genotype (%)		
A	43 (33)	47 (35)
B	11 (9)	12 (9)
C	18 (14)	21 (15)
D	52 (40)	51 (38)
Cirrhosis (%)	13/107 (12)	11/106 (10)
Previous IFN therapy (%)	27 (21)	28 (21)
Previous lamivudine therapy (%)	17 (13)	16 (12)

*mean ±SD

Relation of viral decline and response

Response (HBeAg negativity at the end of follow-up) was achieved in 35% of the PEG-IFN/lamivudine combination therapy group and 36% of the PEG-IFN monotherapy group (p=n.s.). The viral decline for responders and non-responders treated with PEG-IFN alone or the combination is illustrated in figure 2. For the combination treatment group both responders and non-responders showed a significant drop in HBV DNA whereas in the PEG-IFN monotherapy group HBV DNA was minimal in non-responders. In the combination therapy group most patients showed a biphasic decline. During the first month there was a rapid decline in HBV DNA, which was significantly faster in responders (3.2 log) thanin non-responders (2.8 log, p=0.01). Thereafter we found a slower decline in viral load, which was not different between responders and non-responders. In the PEG-IFN

monotherapy group 57 patients (43%) showed a decline of HBV during the first month of therapy. We defined HBV DNA decline as a decrease of at least 0.5 log during the first month, regarding the variability of the test. This immediate decline resulted in response in 51% (Table 2). Among patients who showed no such decline during the first month of therapy we found a different decline pattern with a sudden fast HBV DNA decline after a variable delay. This delayed HBV DNA decline, defined as a drop of HBV DNA to at least 2 logs below the baseline level after the first month of therapy, was found in 25 patients (19%). Among the 17 patients who showed this delayed HBV decline within weeks 4-32 of therapy, 76% responded.

Figure 1 HBV DNA levels during therapy: PEG-IFN monotherapy (dashed line) versus combination therapy of PEG-IFN and lamivudine (solid line).

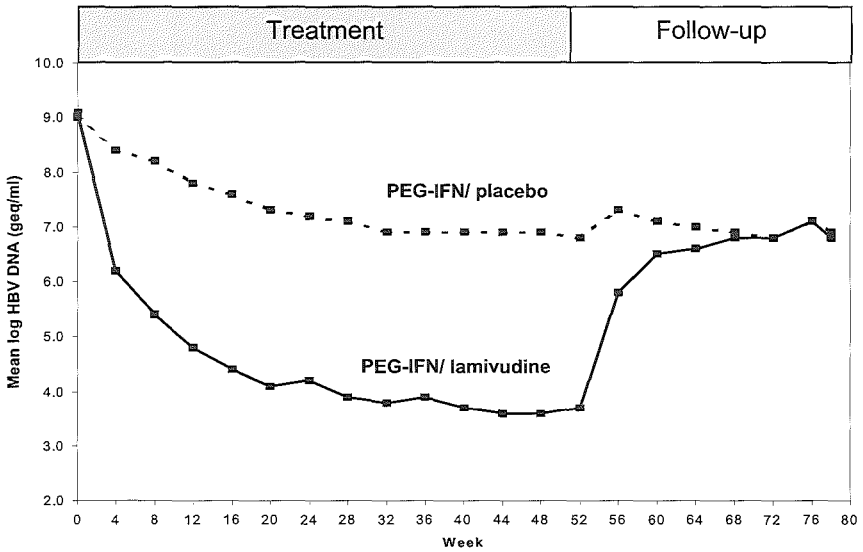

Table 2 Association of pattern of viral decline during therapy with response in the PEG-IFN monotherapy group

Pattern	*% of patients responding*	*% of responders showing pattern*	*% of non-responders showing pattern*
Immediate decline (n=57)*	51%	59%	33%
Delayed decline (n=25)**			
Week 4-32 (n=17)	76%	27%	5%
Week 32-52 (n=8)	25%	4%	7%
No decline (n=52)	10%	10%	55%

* ≥ 0.5 log during first month of therapy

** ≥ 2 log from baseline after first month of therapy

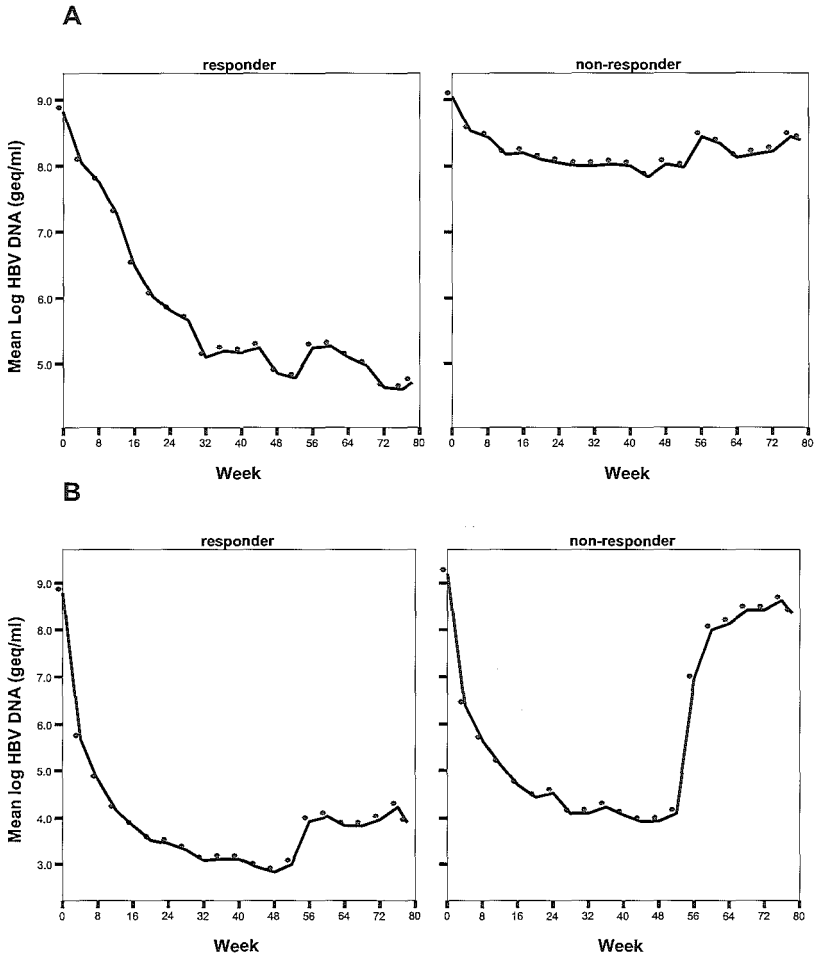

Figure 2 HBV DNA levels for responders (defined as HBeAg negative at week 78) and non-responders to PEG-IFN monotherapy (A) and PEG-IFN/lamivudine combination therapy (B)

Prediction of response

PEG-IFN and lamivudine. When we analyzed HBV DNA levels and decline at the different timepoints for discrimination between responders and non-responders, the largest area under the ROC curve was found for viral load at week 12. To find the earliest possible moment for prediction we compared this with earlier timepoints. We found that the area under the ROC curve was comparable for testing at week week 4 (0.69) and week 12 (0.75, p=0.17). The ROC curves showing the relation between viral load at weeks 4 and 12 and response are shown in figure 3. The association between response and

HBV DNA levels at week 4 is shown in table 3. Response could best be predicted by a log HBV DNA level below 7.0 at week 4, which included 95% of responders, and excluded 35% of non-responders.

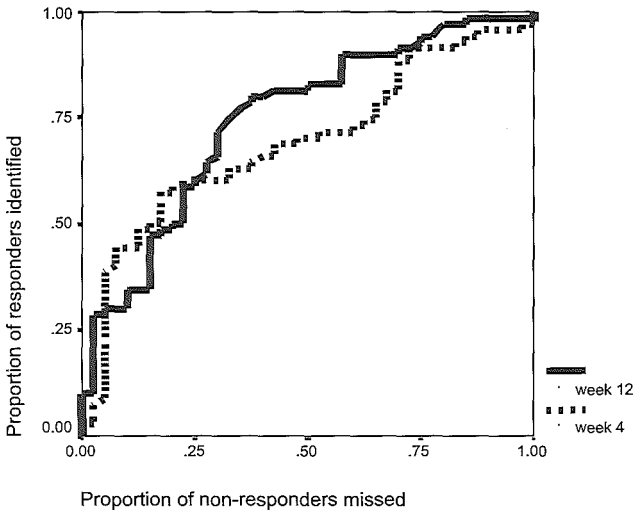

Figure 3 ROC curve for prediction of response in the PEG-IFN/lamivudine therapy group by testing HBV DNA level at weeks 4 and 12 of therapy. The area under the curve was not significantly different between the two tests.

Table 3 Prediction of response in the combination therapy group at week 4 of therapy

Log HBV DNA level at week 4 of therapy	*% of patients reaching level*	*Positive predictive value*	*Negative predictive value*	*% of responders included*	*% of non-responders excluded*
< 7.5	85%	40%	89%	95%	21%
< 7	**76%**	**45%**	**93%**	**95%**	**35%**
< 6.5	66%	48%	88%	88%	47%

PEG-IFN and placebo. In the group of patients receiving PEG-IFN monotherapy, analysis of response showed the largest area under the ROC curve for HBV DNA levels at week 32 of therapy. Here we also compared with earlier timepoints. To be able to make a prediction as early as possible, we selected week 20, which was the earliest timepoint with area under the ROC curve (0.80) was not significantly different from the area under the ROC curve at week 32 of therapy (0.83, p=0.20). The ROC curves showing the relation between response and viral load at weeks 20 and 32 are shown in figure 4. Response could best be predicted by a log HBV DNA level below 9.0 at week 20 of therapy, which included 94% of responders and excluded 29% of the non-responders (Table 4).

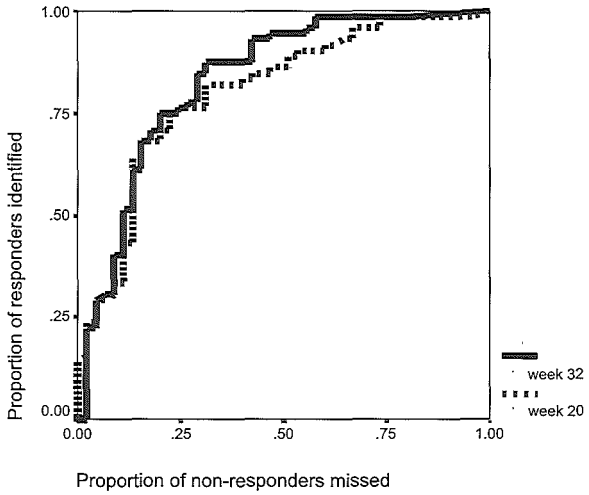

Figure 4 ROC curve for prediction of response in the PEG/IFN placebo group by testing HBV DNA level at weeks 20 and 32 of therapy. The area under the curve was not significantly different between the two tests.

Table 4 Prediction of response in the PEG-IFN monotherapy group at week 20 of therapy

Log HBV DNA level at week 20 of therapy	*% of patients reaching level*	*Positive predictive value*	*Negative predictive value*	*% of responders included*	*% of non-responders excluded*
< 9.5	86%	43%	94%	98%	21%
< 9	**80%**	**45%**	**88%**	**94%**	**29%**
< 8.5	62%	51%	83%	83%	51%

Discussion

Throughout the treatment period, we found a significantly faster decline of HBV DNA in the PEG-IFN/ lamivudine combination therapy group, compared to the PEG-IFN monotherapy group. HBV DNA in the combination therapy group showed a biphasic decline, as has previously been described with conventional IFN or PEG-IFN in chronic hepatitis C patients (22-26), and in chronic hepatitis B patients treated with nucleoside analogues (27-31). In the PEG-IFN monotherapy group there was a less consistent pattern of HBV DNA decline. Despite the difference in initial HBV DNA decline, in our group of 266 HBeAg positive patients there was no difference in sustained off-treatment HBeAg response between PEG-IFN monotherapy and PEG-IFN/ lamivudine combination therapy (18). In the

combination therapy group both responders and non-responders showed a marked decline of HBV DNA during therapy, with a fast relapse in non-responders but not in responders after the end of therapy. With PEG-IFN monotherapy only responders showed a HBV DNA decline, which was sustained during follow-up. Non-responders in this group showed no or a minimal decline of HBV DNA.

Looking at the individual patients, HBV DNA decline in the PEG-IFN monotherapy group followed a variable pattern. In this group 27% of patients initially showed a very slow decline or no change in viral load, followed after a variable delay by phases of sudden fast HBV DNA decline during treatment. This delayed viral decay probably reflects immune activation induced by PEG-IFN. We found that in patients treated with PEG-IFN alone a delayed viral decline during weeks 4-32 was associated with response in 76% of the patients. After 20 weeks of therapy a prediction of response could be made on the HBV DNA level at that time. A HBV DNA level below 10^7 geq/ml after 20 weeks predicted response in 45%; If HBV DNA remained above this level the chance of response was 5%. In the combination therapy group responders had a faster decline of HBV DNA during the first month of therapy than non-responders. The HBV DNA level after 4 weeks of therapy could be used to predict response in this group. A HBV DNA level below 10^9 geq/ml predicted response in 45% of patients. If HBV DNA was above this level the chance of response was 6%. These results might give a useful tool to decide whether or not to continue PEG-IFN therapy. It provides the opportunity to discontinue therapy in patients who are unlikely to respond, which limits the side effects and burden of treatment for these patients and also enhances cost-effectiveness. Also it motivates patients who reach the early goal of treatment to continue therapy, knowing they have a good chance of responding. Figure 5 shows the proposed stopping rules for both treatment groups.

We conclude that combination therapy with PEG-IFN and lamivudine is more effective in suppressing HBV replication than PEG-IFN alone. This did not result in enhanced rates of sustained HBeAg loss. Only for patients treated with combination therapy initial decline of HBV-DNA was indicative of response. For patients treated with PEG-IFN alone response was associated with HBV-DNA decline after 12 weeks, probably due to enhanced immune reactivity.

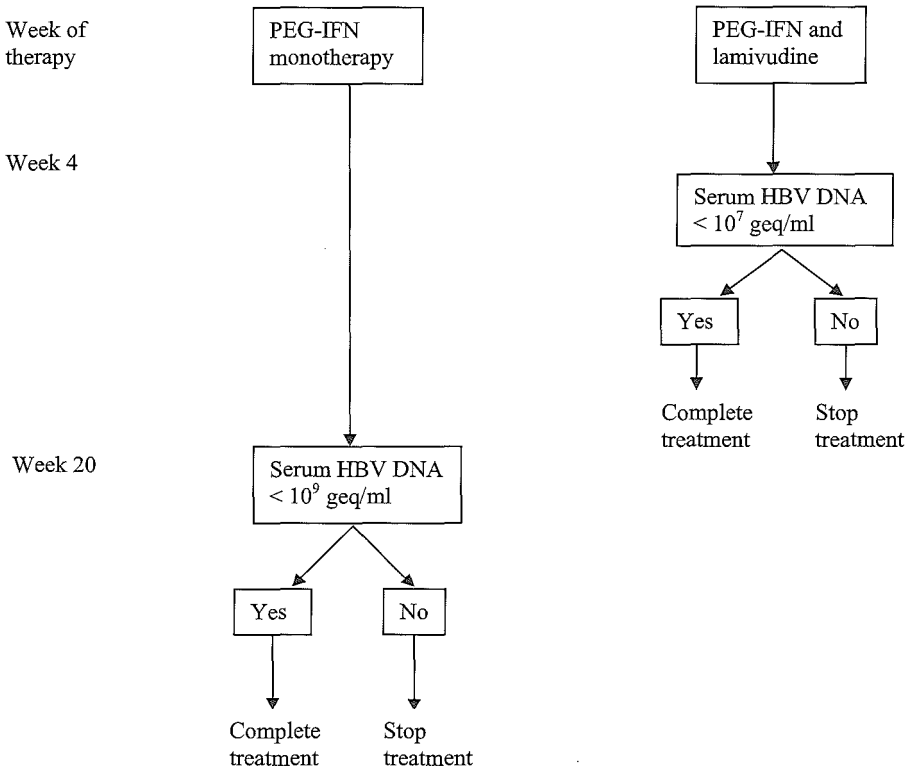

Figure 5 Algorithm for HBV DNA testing to identify patients with a high chance of response

Acknowledgments

The study was organised and sponsored by the Foundation for Liver Research (SLO), Rotterdam, The Netherlands. Financial support and study medication was provided by: Schering-Plough International, Kenilworth, NJ, USA; GlaxoSmithKline, Research and Development, Greenford, UK. Monitoring was coordinated by Denys Research Consultants bvba, De Haan, Belgium. Data collection and data management was done by Elke Verheij and Eva Leeuwenhoek, Clinical Research Bureau, Department of Gastroenterology & Hepatology, Erasmus MC, University Medical Center Rotterdam, The Netherlands. Dr. Janssen is a Clinical Research Fellow from The Netherlands Organisation of Scientific Research (NWO). We are endebted to Suzan Pas for performing the virological tests and to Avidan Neumann for his helpful advice.

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Chapter 5

The safety of pegylated interferon alpha-2b in the treatment of chronic hepatitis B. Predictive factors for dose reduction and treatment discontinuation

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Submitted

Abstract

Treatment with interferon-alpha (IFN) has been shown to be effective in one third of HBeAg-positive chronic hepatitis B patients, but is clinically associated with relevant adverse events. We studied the safety of pegylated interferon alpha-2b (PEG-IFN) in three hundred HBeAg-positive patients with compensated liver disease. Patients were treated with PEG-IFN for 52 weeks combined with either lamivudine 100mg/day or placebo. PEG-IFN was administered for 100μg 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. 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 PEG-IFN 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 PEG-IFN combined with lamivudine or placebo. In 69 (22%) patients the dose of PEG-IFN was reduced prematurely. Of these dose reductions 36 (52%) were due to 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. We conclude that in patients with chronic hepatitis B and compensated liver disease prolonged PEG-IFN therapy is safe and that preexistent 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 is worldwide the single most common cause of liver cirrhosis and hepatocellular carcinoma (HCC). (1). Interferon alpha (IFN) is effective in about one third of patients(2). Reported HBeAg seroconversion rates range from 15 to 37 percent (3-5), depending on baseline characteristics such as ALT levels and viral load. Response to IFN therapy has been shown to result in sustained clearance of HBeAg, HBV DNA and normalisation 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 clinical 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 reduce the number of side effects, newer forms of IFN have been developed. "Pegylated" forms of IFN (with a polyethylene glycol moiety attached to it) have an improved pharmacokinetical 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 chronic hepatitis B are limited to the study of Cooksley et al.(14), who investigated the safety of peginterferon alpha-2a in chronic hepatitis B patients. In this study we assessed the safety of pegylated interferon alpha-2b (PEG-IFN) alone or in combination with lamivudine in HBeAg-positive chronic hepatitis B.

Materials and methods

Patients

In a randomized multicenter trial reported previously (15), three hundred and seven HBeAg-positive CHB patients with compensated liver disease were treated with PEG-IFN in combination with either lamivudine or placebo. Inclusion criteria were hepatitis B surface antigen (HBsAg)-positivity for at least 6 months, age ≥ 16 years, alanine aminotranferase (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 within 6 months or any investigational drug within 30 days of entry to this protocol, were coinfected with hepatitis C, hepatitis D or HIV, had alcoholic hepatitis or other causes of liver disease, had pre-existent leukopenia or thrombocytopenia (white blood cell count (WBC) $\leq 3{,}000/mm^3$, neutrophils $\leq 1{,}800/mm^3$, platelets $\leq 100{,}000/mm^3$), had decompensated liver disease (prothombin time prolonged by ≥ 3 sec, serum albumin < 35 g/l, ascites, encephalopathy, history of variceal bleeding) or had hypo- 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 centers 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 for 52 weeks. Patients were treated with a dose of 100 µg PEG-IFN once a week until week 32, whereafter the dose was reduced to 50 µg once a week. In addition, patients received either placebo or100 mg lamivudine orally.

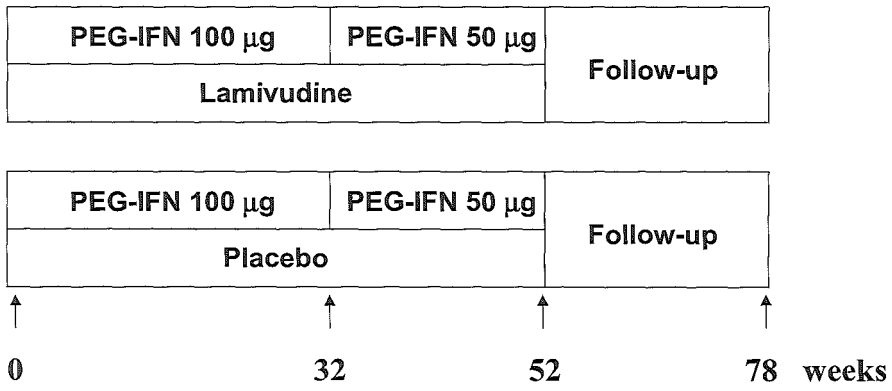


Figure 1 Treatment schedule

Patients were followed every four weeks during treatment and during the post-treatment follow-up period of six months..At visits routine physical examination was performed and blood samples were obtained for hematological and biochemical screening (hemoglobin, white blood cells, neutrophils, platelets, ALT and aspartate aminotranferase(AST)). All adverse events were reported by the treating physician on standard case record forms and verified by the trial coordinating center. All participating investigators were instructed, received a protocol and were monitored every 3 months in order to 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 judged 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 which are life-threatening, require or prolong inpatient hospitalisation, 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 alanine aminotransferase (ALT) to at least 3 times the baseline level. Guidelines on discontinuation of therapy in case of flares were provided to each investigator before start of therapy. The decision to stop therapy in patients with flares and signs of diminished liver function was left at the responsibility of the participating doctor 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-value <0.2) were included in a multivariate analysis. Cox regression was used for multivariate analysis. Chi-square was used to compare frequencies of adverse events. All statistical analyses were performed in SPSS version 10 (SPSS Inc, Chicago, Il).

Results

Baseline characteristics

A total of 307 patients were randomized to treatment with PEG-IFN and either lamivudine or placebo. The analysis of efficacy, that was reported previosly, was done for 266 patients, and excluded patients that never started treatment (n=7), were HBeAg negative at the start of treatment (n=10), and patients from one single center 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 that received at least one dose of study medication were included in the present safety analysis. The baseline characteristics of these patients are shown in table 1. A total of 228 men (76%) and 72 women were treated with PEG-IFN in combination with either lamivudine or placebo. The mean age of the patients was 35 years (range 16-72). The majority of patients (76%) were Caucasians. For 230 patients pretreatment biopsies were available and of sufficient quality to be evaluated. Of these patients 26 patients (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

mean ± SD

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/ placebo group and the PEG-IFN/ lamivudine group. Also there was no difference in occurrence of SAE and in 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). 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 (Fig 2). During therapy leucopenia (< 3.0 x 10^9 u/L, grade II), neutropenia (< 1.5 x 10^9 u/L, grade II) and thrombocytopenia (< 75 x 10^9 u/L, grade II) occurred in 42, 22 and 12 percent of the patients, respectively (Table 2). All adverse events were reported less frequently after dose reduction and were completely reversible after the end of therapy (Figure 2).

Table 2 Frequencies of adverse events

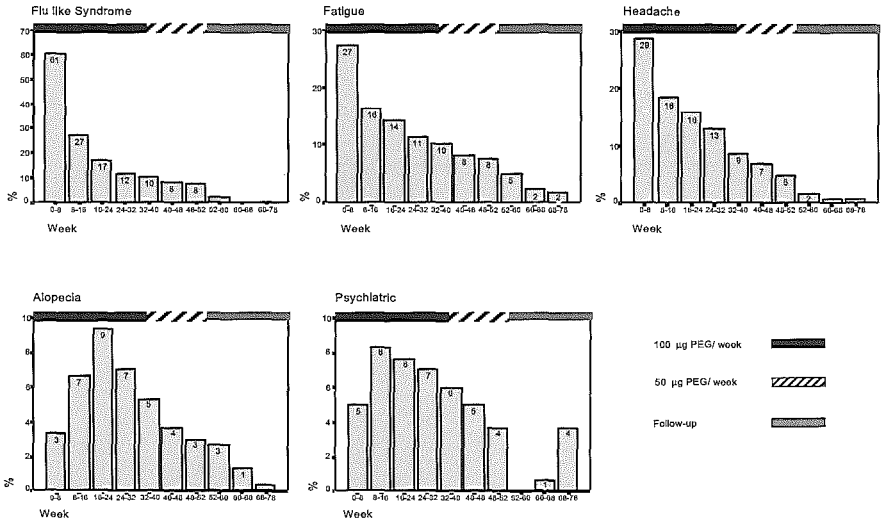
Adverse event	**Freq %**
Flu like Syndrome	68
Headache	40
Fatigue	39
Myalgia	29
Local reaction	29
Alopecia	22
Weight loss (> 10 %)	19
Psychiatric*	20
Abdominal pain	15
Haematologic events	
Leucopenia (< 3.0 x 10^9 u/L)	42
Neutropenia (< 1.5 x 10^9 u/L)	22
Thrombocytopenia (< 75 x 10^9 u/L)	12

*Includes depression, mood changes, irritability

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/ lamivudine group and in 22% (34/152) of patients in the PEG-IFN/ placebo group (p=0.5). The frequency of on-treatment flares and post-treatment flares was not different between groups. Two flares led to discontinuation of therapy. None of the hepatitis flares resulted in sustained decompensated liver disease or death.

A



B

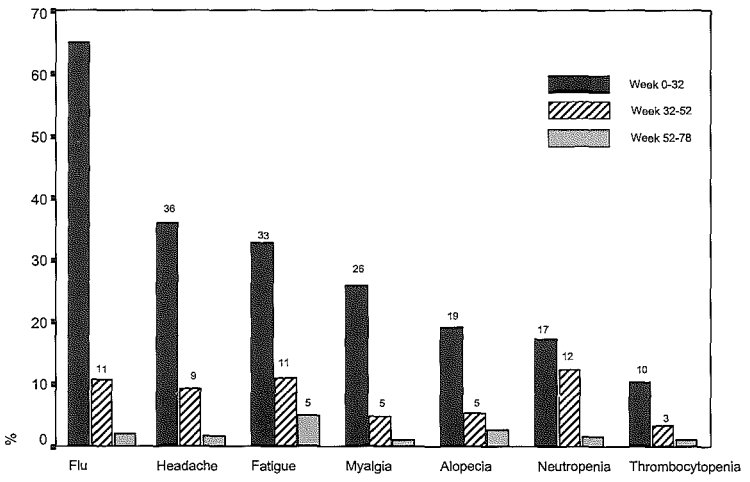


Figure 2 Course of frequent adverse events during therapy and follow-up (A) and adverse events: Frequency of events in relation to PEG-IFN dose (B).

Serious adverse events

During the study 33 SAE were reported. Of these SAE 17 were judged as probably related to therapy. Four patients had a serious hepatitis flare, and three patients developed severe depression. Neutropenia (grade III or IV) was reported in three cases. Other SAE that were probably related to therapy were syncope, seizures, psychosis, pancreatitis, diarrhea, 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 x 10^9/L) (Table 3). The risk of infection increased with an increasing severity of neutropenia. Patients with neutrophil counts below 1.5 x 10^9/L had a significantly increased risk of infection compared to patients with higher neutrophil counts (13.6% versus 1.3% respectively, p<0.001). Infections included urinary tract infection (n = 4), gastro-enteritis (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 x 10^9 /L. The third patient had a salmonella gastro-enteritis while his neutrophil count was 0.7 x 10^9/L. In four cases (pneumonia, appendicitis and gastroenteritis (n=2)) the infection necessitated hospital admission. All infections resolved without lasting complications.

Table 3 Bacterial infections in patients with low neutrophils.

Neutrophils (U/L)	*n*	**Bact. Infections (%)**
> 1.5 x 10^9	234	3 (1.3)
< 1.5 x 10^9	66	9 (13.6)
< 1.0 x 10^9	24	2 (8.3)
< 0.5 x 10^9	9	1(11.1)

Reported bacterial infections: urinary tract infection (n = 4), gastro-enteritis (n = 2), tonsillitis (n = 2), gingivitis (n = 2), pneumonia (n =1), appendicitis (n=1)

The risk of bleeding complications was increased in patients with thrombocytopenia, especially grade III (< 50 x 10^9/L) or IV. Reported bleeding complications in these patients were epistaxis (n = 2), GI 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 (developing 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 4 Bleeding complications in patients with low platelets

A Bleeding complications according to level of thrombopenia

Platelets (U/L)	n	Bleeding complications (%)
$> 100 \times 10^9$	222	6(2.7)
$< 100 \times 10^9$	78	5(6.4)*
$< 75 \times 10^9$	37	3(8.1)
$< 50 \times 10^9$	9	2(22.2)

B Bleeding complications in cirrhotic patients

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

*Reported bleeding complications in patients with platelets $< 100 \times 10^9$ U/L: epistaxis (n = 2), GI 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 was reduced prematurely (Table 5). Of the dose reductions 37 (54%) occurred in the PEG-IFN/ lamivudine group and 32 (47%) in the PEG-IFN/ 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 due to psychiatric symptoms and in seven patients due to flu-like-syndrome. Fifty percent of the dose reductions occurred within the first ten weeks. Hereafter the number of dose reductions decreased. Only two dose reductions were reported after week 32.

PEG-IFN therapy was discontinued prematurely in 28 patients (9%). In 24 patients the blinded drug (lamivudine/ placebo) was also discontinued. In ten 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 at week 32. In univariate analysis, a low neutrophil count ($<3 \times 10^9$/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 below 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, leukocyte 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 in patients with chronic hepatitis B. 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 unexpected events were reported. The frequency of the most common adverse events- flu-like-syndrome (66%), headache (39%), fatigue (37%) and myalgia (28%) -was comparable to those previously reported in chronic hepatitis B patients treated with standard IFN in a dose of 4.5 MU (14) or 10 MU t.i.w.(17) for 16-32 weeks. Obviously, these comparisons with

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	

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. Hematological abnormalities (especially neutropenia) occur more frequently in chronic hepatitis C patients treated with pegylated interferons (PEG-IFN or peginterferon alpha-2a) than with standard IFN. The rate of dose reductions (22%) and therapy discontinuations (9%) in our study is comparable to the frequencies reported with pegylated interferons in patients with chronic hepatitis C (8, 12, 13, 20) and with 24 weeks of peginterferon alpha-2a in patients with chronic hepatitis B (14). The proportion of patients that is withdrawn from therapy with pegylated interferons is similar to that of conventional IFN, but, as compared to standard IFN, the proportion of patients requiring dose reductions is higher with PEG-IFN in most hepatitis C studies (8, 11, 12, 18). In chronic hepatitis B patients the frequency of dose reduction with peginterferon alpha-2a was higher than with conventional IFN (22-30% versus 10%, respectively)(14). The increased rate of dose reductions with pegylated interferons seems mainly due to the increased occurrence of neutropenia.

Untill now it was unknown whether PEG-IFN-induced neutropenia and thrombocytopenia indeed lead to an increase of bacterial infections and bleeding complications, respectively. In our study a neutropenia of less than $<1.5x10^9$ /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 preexistent cirrhosis and platelets dropping below 75 x 10^9 u/L. However bleeding complications were generally mild (epistaxis). Only one potentially life-threatening bleeding complication (bleeding duodenal ulcus) 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 or depression in some cases necessitated dose reduction. Informing patients about the course of these adverse events and adequate treatment with paracetamol and specific serotonine reuptake inhibitors (SSRI's) may lead to an increased proportion of patients able to complete the treatment. Neutropenia and thrombocytopenia are another frequent cause for dose reduction. Considering the mildness and rareness of complications of neutropenia in our patients, it might be worth wile to investigate in a randomized study if it is safe to accept grade 3 neutropenia without dose reduction during PEG-IFN treatment for chronic hepatitis B. Since, in our study, particularly cirrhotic patients were at an increased risk of thrombocytopenia and (minor) bleeding complications we would recommend to monitor them closely and avoid a decrease in platelet count

below 75x 10^9/L. In a previous study in 217 cirrhotic patients with chronic hepatitis C treated with peginterferon alpha-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, treatment with PEG-IFN for chronic hepatitis B is safe and has a comparable side effect profile to conventional IFN. All adverse events were reversible after treatment discontinuation. Adding lamivudine to PEG-IFN did not affect PEG-IFN related side-effects. Hematological abnormalities during PEG-IFN 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 be reluctant to initiate PEG-IFN treatment in cirrhotic patients.

Acknowledgments

The study was organised and sponsored by the Foundation for Liver Research (SLO), Rotterdam, The Netherlands. Financial support and study medication was provided by: Schering-Plough International, Kenilworth, NJ, USA; GlaxoSmithKline, Research and Development, Greenford, UK. Monitoring was coordinated by Denys Research Consultants bvba, De Haan, Belgium. Data collection and data management was done by Elke Verheij and Eva Leeuwenhoek, Clinical Research Bureau, Department of Gastroenterology & Hepatology, Erasmus MC, University Medical Center Rotterdam, The Netherlands. Dr. Janssen is a Clinical Research Fellow from The Netherlands Organisation of Scientific Research (NWO).

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Chapter 6

Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B

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Hepatology 2004; 39: 804-810

Abstract

Data on the long-term effects of alpha-interferon (IFN) treatment on disease progression and mortality in patients with chronic hepatitis B (CHB) are limited. To evaluate factors that influence clinical outcome and survival, we performed a follow-up study on 165 hepatitis B e antigen (HBeAg)-positive CHB patients, treated with IFN in Rotterdam between 1978 and 2002. The median IFN dose was 30 megaunits (MU)/week (range 2-70 MU/week), the median duration of therapy 16 weeks (range 1-92 weeks). Response to treatment was defined as HBeAg loss within 12 months after the end of IFN therapy. Median follow-up was 8.8 years (range 0.3-24 years). Fifty-four patients (33%) responded to IFN treatment. Relapse (HBeAg reactivation) occurred in 7 of the 54 (13%) responders. Fifty-two percent of the responders lost Hepatitis B surface antigen (HBsAg) as compared to 9% of the non-responders ($p<0.001$). Liver histology showed a decreased necroinflammatory activity and less progression of fibrosis in responders. Twenty-six patients died during follow-up. Hepatocellular carcinoma (HCC) was found in 8 patients, of which 6 were non-responders. Of the two responders that developed HCC, one patient had relapsed after discontinuation of therapy. Multivariate analysis showed a significantly improved survival (Relative risk (RR) of death 0.28, 95% CI 0.10-0.78) and reduced risk of developing hepatocellular carcinoma (HCC) (RR 0.084, 95% CI 0.09-0.75) in responders. In conclusion, response to IFN therapy results in a prolonged clinical remission with an increased rate of HBsAg seroconversion and improved liver histology. Our results indicate that, after correction for baseline factors, response to IFN therapy inreases survival and reduces the risk of developing HCC.

Introduction

Chronic hepatitis B (CHB) affects 400 million people worldwide and is a major cause of morbidity and mortality. Patients with CHB are at an increased risk of developing liver cirrhosis and its complications such as hepatic decompensation and hepatocellular carcinoma (HCC), compared to matched controls from the normal population (1-5). During their lifetime, 25%-40% of CHB patients will develop serious complications (5). Treatment of CHB aims at inactivation of liver disease as indicated by hepatitis B e antigen (HBeAg) seroconversion and disappearance of HBV DNA, measured by hybridization techniques. Spontaneous HBeAg seroconversion occurs in about 8-12 % per year (6-8). Interferon alpha (IFN) has been reported to increase the HBeAg seroconversion rate to 33% (8). Particularly in Caucasians data on the long-term effects on disease progression and mortality are limited. To further evaluate factors that influence clinical outcome and survival, we performed a retrospective cohort study on all HBeAg-positive CHB patients treated with IFN in our liver unit.

Patients and methods

Patients

Patients were identified through a search of our trial databases and our computerized hospital-wide patient information system. All HbeAg-positive chronic hepatitis B (CHB) patients that were treated with interferon between 1978 and 2002 were included in the study. CHB was documented by hepatitis B surface antigen (HBsAg) positivity for more than 6 months and by a liver biopsy. We identified a total of 165 HBeAg positive patients that were treated with IFN in our center. One hundred thirty-two patients participated in one or more trials (9-16), the remaining 33 patients were treated electively. Sixty-two patients (38%) received treatment with nucleoside analogues as combination therapy with IFN (zidovudine (n=12), aciclovir (n=10) and lamivudine (n=40), one patient received two courses of combination therapy). Forty-eight non-responders were retreated and received two or more courses of IFN. Patients were seen every 2-4 weeks during treatment. Thereafter patients were scheduled for visits every 3-12 months. Response was defined as loss of HBeAg at two consecutive measurements at least one month apart, during therapy or within 12 months after the end of the first IFN therapy. All other patients were considered non-responders.

Follow-up

Data were collected by review of patient records. We contacted all patients and asked them to return for evaluation and laboratory examination. If a patient could not be traced, relevant information was obtained from the patient's primary care physician. Data were collected on survival, occurrence of decompensated liver disease (defined as ascites, encephalopathy, variceal bleeding or bilirubin> 34

μmol/l) and HCC. Of all patients that died the exact date and cause of death were documented. Follow-up time was calculated from the start of IFN treatment until death or until the last visit. Secondary end points were occurrence of liver disease related complications (HCC, decompensated liver disease). For calculation of survival times liver transplantation was combined with death. Of 16 patients no recent information was available. Only one of these patients had cirrhosis at the last biopsy, but minimal inflammatory activity. None of the patients that were lost to follow-up showed any signs of decompensated liver disease or HCC at the last visit.

Measurements

We reviewed biochemical data (ALT, AST, bilirubin and albumin) and virological data from the patient records. These tests were routinely performed at each visit to our outpatient clinic. For measurement of HBV DNA samples obtained pretreatment, one year posttreatment and at follow-up were retested. HBV DNA was assessed by TaqMan real-time PCR assay (17) (dynamic range 373 – 10^{10} geq/ml, Eurohep standard). HBeAg, HBsAg, antibodies to HBeAg and antibodies to HBsAg were measured using enzyme-linked immunnnosorbent assays or solid-phase radioimmunoassays (Abbott Laboratories, Abbott Park, Il., USA). Liver biopsies were obtained at the start of therapy. For follow-up the most recent liver biopsy was used. All liver biopsies were scored for inflammatory activity (combined score for portal inflammation and interface hepatitis) and fibrosis by two experienced pathologists using predefined criteria. Inflammatory activity was scored as none(0), minimal(1), mild(2), moderate(3) and severe(4). Fibrosis was scored as none(0), mild(1), moderate(2), severe(3), probable cirrhosis(4) and cirrhosis(5).

Statistics

The Chi- square test was used to compare frequencies, the Mann-Whitney test to compare means between groups for various variables. The Kaplan-Meier (KM) method was used to estimate survival, and time to HBeAg and HBsAg seroconversion. The following variables were considered for univariate analysis: age; sex; baseline AST, ALT, albumin, bilirubin and HBV DNA levels; necroinflammatory and fibrosis score on liver biopsy; liver cirrhosis; mean weekly dose, total dose of IFN therapy and combination therapy with other antivirals. Response was analyzed as a time dependent factor. Patients who responded might have longer survival times, because they lived at least until response. To avoid this bias response was entered as a time-dependent covariate, i.e. all patients were entered in the non-responder group at the start of therapy, and, in case of response, were censored from the non-responder group at the time of response and included in the responder group (18). Cox regression analysis was applied to determine which factors were independently associated with survival. First, all baseline factors that were related to survival in the univariate analysis with $p<0.10$ were assessed by both the forward stepwise and backward stepwise method. Then baseline factors that predicted death were entered in a multivariate model together with the time-dependent factor response

to therapy. The same analysis was performed for the secondary endpoints decompensated liver disease and HCC. P-values are based on likelihood-ratio statistics. To exclude a confounding effect of coinfection with HCV, HDV or HIV survival analyses were repeated after exclusion of patients with these coinfections. All analyses were performed using SPSS version 10.1 (SPSS inc, Chicago, Il, USA).

Results

Baseline characteristics

Baseline characteristics of the patients are shown in table 1. The patient group consisted of 165 patients, of which 118 (72%) were male and 123 (75%) were Caucasian. The median ALT level was 89 U/L (range 12-730). The median dose of IFN was 30 MU/week (range 2-70 MU /week), the median duration of therapy was 16 weeks (range 1-92 weeks).

Table 1 Baseline characteristics

	Total (n = 165)	Responders (n= 54)	Non-responders (n= 111	p-value
Age (years)*	34 (10-67)	37 (10-64)	32 (16-67)	0.081
Male (%)	118 (71.5)	41 (75.9)	77 (69.4)	0.38
Race (%)				0.056
Caucasian	123 (74.5)	45 (83.3)	78 (70.3)	
Asian	32 (19.4)	6 (11.1)	26 (23.4)	
Other	10 (6.0)	3 (5.6)	7 (6.3)	
Mode of transmission (%)				0.91
Vertical	13 (7.9)	3 (5.6)	10 (9.0)	
Parenteral	13 (7.9)	4 (7.4)	9 (8.1)	
Sexual	60 (36.4)	21 (38.9)	39 (35.1)	
Unknown	79 (47.9)	26 (48.2)	53 (47.7)	
ALT (U/l)*	89 (12-730)	102 (21-730)	81 (12-654)	0.020
AST (U/l) *	46 (12-367)	59 (15-264)	41 (12-367)	0.002
Bilirubin (μmol/l) *	11 (2-45)	12 (5-32)	11 (2-45)	0.91
Albumin (g/l) *	43 (14-54)	42 (14-50)	44 (28-54)	0.057
Log HBV DNA	9.0 (3.5- 10.1)	8.5 (3.5-9.9)	9.1 (4.2-10.1)	0.080
Cirrhosis (%)	30 (19)	15 (29)	15 (14)	0.034
IFN dose (MU) *	510 (33-3130)	504 (55-3130)	510 (33-1650)	0.864
Mean weekly IFN dose *	30 (2-70)	30 (9-36)	30 (2-70)	0.254
Follow-up (years) *	8.8 (0.3- 23.9)	10.6 (0.8-19.5)	7.9 (0.3-23.9)	0.2756

* median (range)

Forty-eight patients, 45 non-responders and 3 responders who relapsed, were retreated with IFN: 33 patients received 2 courses of IFN, 8 patients 3 courses, 5 patients 4 courses, and 2 patients 5 courses. Median follow-up time was 8.8 years (range 0.3-23.9 years). Coinfection with HCV, HDV and HIV was present in 3, 2, and 7 patients, respectively.

Follow-up

Virological response: Of all 165 patients 54 patients (33%) exhibited a response. Recurrence of HBeAg was seen in 7 of the responders (13%). Responders had a significantly higher pretreatment ALT level than non-responders (median 102 U/l (range 21-730 U/l) vs 81 U/l (range 12-654 U/l), respectively, p=0.02). Patients with preexisting cirrhosis (n=30) had a higher response rate than patients without cirrhosis (50% vs 29%, p=0.026). At the end of follow-up HBV DNA was negative by PCR in 61 patients (43%). Responders significantly more often exhibited loss of HBsAg (52%) and HBV DNA (70 %) than non-responders (9% and 30% respectively, p<0.001). The time to loss of HBeAg and HBsAg in responders and non-responders is shown in figure 1. Among the 111 non-responders, 57 patients lost HBeAg during follow-up. Of these patients 17 lost HBeAg within a year after retreatment with IFN and 40 patients exhibited a spontaneous loss of HBeAg.

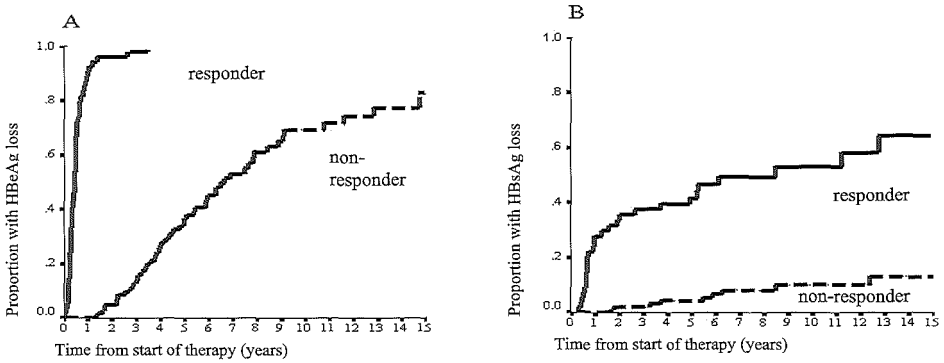

Figure 1 Time to HBeAg-loss (A) and HBsAg-loss (B) of responders compared to non-responders. Responders exhibited HbeAg-loss during or within a year after the first course of IFN.

ALT normalization: ALT levels at the end of follow-up were significantly lower in the group of responders (median ALT 21 U/L vs 35 U/L, p=0.001). In the responder group 39 of the 54 patients (72%) had a normal ALT at the end of follow-up, compared to 63 of the 111 (57%) non-responders. This difference tended towards significance (p= 0.055)

Liver histology: At the start of therapy, 155 patients underwent a liver biopsy. Of 123 patients paired liver biopsies were available (mean follow-up time 3.1 years, range 0.4-14.6 years). At baseline, responders had higher inflammatory activity (mean score 2.4 vs. 2.1, p=0.006) and more advanced fibrosis (mean score 2.4 vs. 1.6, p=0.001) than non-responders. Inflammatory score improved in 30 of 43 responders (70%) and in 27 of 81 non-responders (33%) (p<0.001), mean score at follow-up was 1.6 in responders vs. 2.0 in non-responders (p=0.011). In the responder group, less patients had progression of fibrosis (8 of 42 responders (19%) vs. 35 of 81 non-responders (43%), p=0.039). Also more responders showed an improvement in fibrosis score (12 of 42 responders (29%) vs 13 of 81 non-responders (16%)), but this difference did not reach statistical significance (p=0.1).

Survival

In 18 patients complications of CHB occurred (HCC and/or decompensation of liver disease). Two patients underwent liver transplantation, one of whom died of transplantation related complications. One patient underwent a partial liver resection for HCC, but had a recurrence thereafter. Two patients died on the waiting list for liver transplantation. The other patients had inoperable HCC (n=6) or were not eligible for transplantation because of comorbidity, high age or clinical condition (n=7). Twenty-six patients died during follow-up, 16 of complications of liver disease, and 10 of unrelated causes. Overall, five-year survival was 90% (95% CI: 86%-94%), ten-year survival was 84% (95% CI: 78%-91%). The survival of responders and non-responders is shown in figure 2. Overall, not corrected for baseline factors, there was no difference in survival between responders and non-responders (Fig 2A).

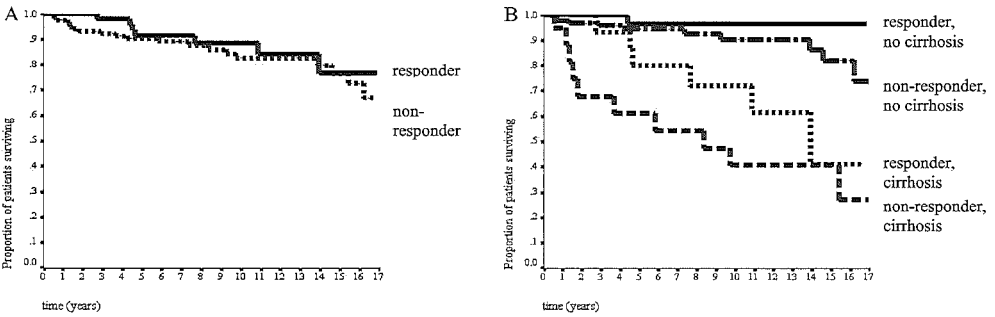

Figure 2 Survival among responders and non-responders to IFN, overall (A) and stratified for preexisting cirrhosis (B). All patients were entered as non-responders at start of therapy, with response as a reason for data censoring. Survival of responders was calculated from time of response.

However, the percentage of patients with cirrhosis in the responder group was twice as high as in the non-responders (29% and 14%, respectively), giving the responders a priori a substantially higher mortality risk. When analyzed separately for patients with and without preexisting cirrhosis, survival

was improved in both groups for patients responding to IFN (Fig 2B). In the univariate analysis of all 165 patients, the baseline factors sex, age, AST, bilirubin, albumin, cirrhosis, liver inflammation and mean weekly IFN dose were related to survival ($p<0.1$) (Table 2). To correct for possible changing treatment schedules and inclusion criteria over time we stratified for 5-year cohorts, but survival was not different between these cohorts. To evaluate the effect of combination therapy with lamivudine (n=40), zidovudine (n=12) or aciclovir (n=10), we stratified for these therapies. Only zidovudine combination therapy was associated with survival (worse survival with zidovudine), but this can be explained by the fact that this group included 3 patients with HIV coinfection. The factors related to survival in univariate analysis were then included in the multivariate analysis. In this analysis the baseline factors sex, age, albumin and cirrhosis were independently associated with death. Then response was entered in a multivariate model as a time-dependent factor together with these baseline factors. Corrected for the above baseline factors, response to therapy significantly improved survival (Table 3). Response was associated with a significantly decreased risk of HCC, but not of decompensated liver disease. When we repeated the analysis for patients without cirrhosis, survival was still significantly improved in responders (p=0.007). Exclusion of the small number of patients with HCV (n=3), HDV (n=2) and HIV (n=7), did not influence the analysis. Also excluding patients treated with low (<15 MU) and high (>45 MU) weekly doses of IFN did not influence the outcome. We then analyzed whether spontaneous HBeAg loss influenced survival in the non-responder group. Among the non-responders survival was significantly improved in patients that exhibited spontaneous HBeAg loss compared to patients that remained HbeAg-positive (RR 0.11, p=0.04). The time to HBeAg loss was not a predictor of survival.

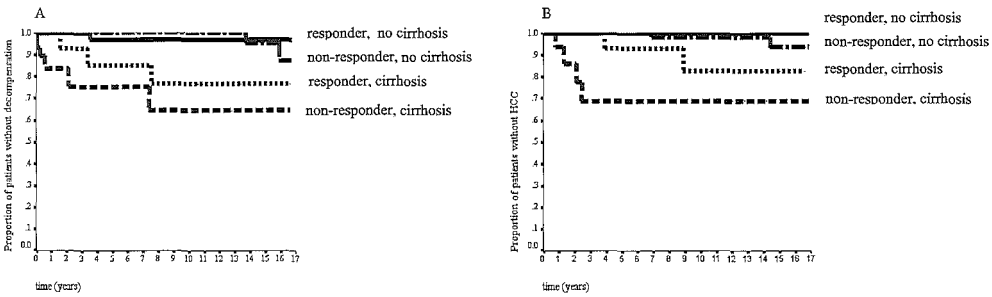

Figure 3 Development of decompensated liver disease (A) and HCC (B) among responders and non-responders to IFN, stratified for preexisting cirrhosis. All patients were entered as non-responders at start of therapy, with response as a reason for data censoring. Survival of responders was calculated from time of response.

The development of decompensated liver disease and HCC is shown in figure 3. HCC occurred in 8 male patients. Among the 111 non-responders six patients (5.4 %) developed HCC after a median interval of 2.2 years (range 0.8-14.4 years). In the responder group two of the 54 patients (3.7%)

developed HCC after 9.5 and 4.1 years of follow-up. Of the two responders that developed HCC, only one patient had a lasting response to therapy, the other relapsed after discontinuation of therapy. As mentioned above, response also significantly reduced the risk of developing HCC in a time-dependent multivariate analysis (Table 3).

Table 2 Univariate analysis of baseline factors. Kaplan-Meier estimates of survival compared by log-rank testing.

		n	5-year survival (%)	p-value
Sex	Male	118	87	0.009
	Female	47	100	
Age	<35	90	95	0.003
	≥35	75	84	
Race	Caucasian	123	88	0.20
	Asian	32	97	
Transmission	vertical	13	100	0.16
	sexual / parenteral	73	86	
AST	< 1.5x ULN	93	93	0.087
	≥ 1.5x ULN	70	86	
ALT	< 2x ULN	66	94	0.17
	≥ 2x ULN	95	88	
Bilirubin	<10	62	97	0.02
	≥10	97	87	
Albumin	<40	38	70	<0.001
	≥40	121	97	
Log HBV DNA	<9	66	89	0.35
	≥9	67	92	
Inflammation (biopsy)	None-Mild	100	96	0.003
	Moderate- Severe	56	80	
Cirrhosis	Absent	125	95	<0.0001
	Present	30	67	
Combination therapy	No	102	91	0.66
	Yes	62	89	
Mean weekly IFN dose	< 30 MU	59	88	0.08
	≥ 30 MU	105	92	
Total dose of IFN	< 510 MU	81	88	0.47
	≥ 510 MU	83	94	

Table 3 Predictive factors for death, decompensated liver disease and HCC: multivariate Cox analysis

	Death (n=27)		Decompensated liver disease (n=16)		HCC (n=8)	
Variables	RR (95%CI)	p-value	RR (95%CI)	p-value	RR (95%CI)	p-value
Age	1.04 (1.01-1.07)	0.022	1.10 (1.03-1.18)	0.003	1.07 (1.01-1.13)	0.032
Sex	0.17 (0.02-1.28)	0.027	-		-	
Albumin	0.88 (0.82-0.94)	0.002	-		0.79 (0.67-0.92)	0.003
Cirrhosis	2.68 (1.12-6.37)	0.025	8.06 (1.97-33.0)	0.003	9.62 (1.76-52.5)	0.009
Response	0.28 (0.10-0.78)	0.008	-		0.08 (0.01-0.75)	0.027

Discussion

Treatment with IFN has been reported to increase the HBeAg seroconversion rate and induce disease remission in patients with CHB. In our study 33% of patients lost HBeAg within one year after the end of therapy, which is comparable to the response rates found in other studies (7,8,19). We also found a high rate of HBsAg loss (52%) in patients who exhibited HBeAg loss after IFN therapy , as has been described before particularly in Caucasians (20,21). However, the long-term effects of IFN therapy on morbidity and mortality are controversial. In our study, we found a significantly prolonged survival in patients who responded to IFN. A number of studies have reported the long-term clinical course of CHB patients treated with interferon. Most studies compared the survival of IFN treated patients to untreated controls (2,18, 21-23). Among these studies, two were randomized controlled trials (21-22), the others were cohort studies with a control group. Di Marco et al. reported a better overall survival and complication-free survival after IFN treatment in Italian patients (2). However this study included both HBeAg-positive and -negative patients, and patients with coinfections. Also, the different groups were not fully comparable, as treated patients had significantly higher baseline ALT levels than untreated controls. In Asian patients Lin et al. found a prolonged survival, and a decreased incidence of HCC in IFN treated patients (22). In our study the majority of deaths were due to complications of liver disease. We that the risk of developing HCC was decreased in responders to IFN, when all independent predictors of complications of liver disease were included in the multivariate analysis. As we found no significant difference in occurrence of decompensated liver disease, the survival benefit of response in our patients may be explained by the decreased incidence of HCC. Because of the small number of patients developing HCC in our study (n=8) the analysis of this endpoint should be confirmed in future studies. Considering the disease remission following response to IFN treatment, one might expect a prolonged survival and less HBV-related complications in IFN treated patients.

Particularly, in Caucasian patients even after long-term follow-up the proportion of patients with disease remission (HBeAg-negative, HBV DNA-negative, normal ALT levels) remains higher in IFN treated patients (18-20). In patients with preexisting cirrhosis HBeAg clearance and biochemical remission have been reported to be predictors of survival (24,25). Niederau et al also reported prolonged survival and a decreased risk of complications of liver disease after HBeAg-clearance (18). Nevertheless, two large studies, one in a predominantly Caucasian patient group (21), one in Asians (23), found no difference in survival or liver related complications between IFN treated patients and controls. The difference between the results of these studies and our current study may be explained by several factors. Firstly, the limited follow-up of studies and the slow natural course of the disease. In patients with active disease and minimal fibrosis who remain untreated or do not respond to IFN therapy the development of liver cirrhosis and its complications may take considerably longer than the mean follow-up in most of these previous studies. Only the study of Yuen et al. (23) had a comparable follow-up time to our study (median follow-up 9 years), but due to the very young patient group (median age 27 years, much lower than in all other studies) the incidence of complications was low. Secondly, the different study design of most previous studies. Several studies compared IFN treated patients to untreated controls. However, a long-term benefit of IFN therapy might only occur in patients who exhibit IFN-induced HBeAg loss. As a consequence of the limited response rate to IFN (30-40%) this would make the benefit of therapy difficult to prove when the whole treated group is compared to untreated controls. Our study aims to overcome these difficulties by a longer follow-up time than in previous studies, and by comparing IFN responders to non-responders. In this approach one has to take into account that responders to IFN tend to have more disease activity than non-responders (higher ALT, more inflammation on liver biopsy). In addition, the percentage of cirrhotic patients among responders was two times higher than in non-responders. These patients with preexisting cirrhosis clearly exhibited a substantially increased risk of complications and mortality. Using a multivariate time-dependent analysis, correcting for cirrhosis and other baseline factors, we found a significantly better survival in responders to IFN. Also if we analyzed patients without cirrhosis separately we found an improved survival after response to IFN. To exclude a confounding effect of retreatment with IFN in non-responders survival analyses were repeated after data censoring at the start of a second IFN course. After censoring patients at the start of retreatment with IFN, response remained significantly associated with survival. Also the survival of patients who received one course of therapy was not significantly different from patients who received two or more courses of IFN. Our results are supported by the findings of Lau et al (20), who reported a better complication-free survival in patients who responded to IFN treatment compared to non-responders, when cirrhosis was considered in the analysis. In our opinion the durability of response to IFN, high HBsAg-seroconversion rate after response, combined with the improved long-term clinical outcome justifies the use of IFN as first-line therapy for HBeAg-positive CHB patients with compensated liver disease.

We conclude that in CHB patients response to IFN therapy results in a prolonged clinical remission with an increased rate of sustained HBsAg seroconversion and improved liver histology. Our results indicate that, after correction for baseline factors, response to IFN therapy increases survival and reduces the risk of developing HCC.

Acknowledgements

Dr. Janssen is a clinical research fellow of the Netherlands Organisation for Scientific Research (NWO).

The authors wish to thank Dr RA Heijtink from the department of Virology, Erasmus MC Rotterdam, for providing serum samples of study patients.

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Chapter 7

Polyarteritis Nodosa associated with hepatitis B virus infection. The role of antiviral treatment and mutations in the hepatitis B genome.

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Eur J Gastroenterol Hepatol 2004;16:801-807

Abstract

Polyarteritis nodosa (PAN) is a systemic inflammatory disease causing vasculitis of medium sized and small arteries. Circulating immune complexes containing viral proteins have been implicated in the pathogenesis of HBV-related PAN and several immunosuppressive and antiviral regimens have been used with varying success. In our hospital seven HBV positive patients with a confirmed diagnosis of PAN could be identified between 1984 and 2001. Most patients had an acute HBV infection and all patients were treated with prednisone. Only in the last four patients prednisone was combined with antiviral therapy with alpha-interferon (IFN). HBV DNA was isolated from serum samples obtained pre-treatment from the four IFN-treated patients and amplified by polymerase chain reaction (PCR). None of the patients without, but 2 of 4 with antiviral therapy exhibited HBsAg seroconversion. In 3 out of 4 patients HBV DNA decreased rapidly after starting IFN-therapy. Clinical remission of PAN was observed in 3 of the 4 treated patients, but in none of the 3 patients who lacked treatment with antiviral medication. Analysis of the HBV genome revealed no mutations, which could be associated with PAN. In one patient a stop codon in the precore region and a double mutation A17662T-G1764A were found during antiviral therapy. We did not find HBV heterogeneity predisposing to the development of PAN.

In our group of patients it appeared that clinical remission of PAN was primarily related to - spontaneous or therapy-induced - loss of HBV DNA replication. The combined administration of a priming steroid course and IFN appears an improvement over prednisone monotherapy and should be considered for every patient with HBV-related PAN. The efficacy of new generation nucleoside analogues should be further elucidated in future studies.

Introduction

Polyarteritis nodosa (PAN) is an uncommon systemic inflammatory disease caused by a necrotizing vasculitis of the medium sized and small arteries. The predominant clinical manifestations of PAN are abdominal pain, fever, weight loss, hypertension, arthritis, neuropathy and renal failure (1,2). The diagnosis of PAN is based on criteria described by the American College of Rheumatology (ACR), including histologic or angiographic evidence of vasculitis and a spectrum of clinical findings (3). Nevertheless, the varying clinical presentation and the rarity of the disease often lead to a considerable interval between appearance of initial symptoms and diagnosis of PAN. If left untreated PAN has a poor prognosis with a 5-year survival rate of 20% (4). Approximately 7% of the patients with PAN are hepatitis B surface antigen (HBsAg) positive. Most of them are diagnosed with PAN in the acute phase of the hepatitis B virus (HBV) infection. Although viral circulating immune complexes have been implicated in the pathogenesis of HBV-related PAN the exact cause of the vasculitic lesions remains obscure (5). A recent case of PAN and HBeAg-negative HBV infection due to a mutation in the pre-core promotor region, suggests that responsible immune complexes do not necessarily include HBeAg (6). PAN is a therapeutic challenge and several immunosuppressive and antiviral regimens have been used with different success. In this paper we report on our experience of combined therapy of prednisone and IFN in PAN associated with hepatitis B infection, and we address the question whether heterogeneity in the HBV genome could predispose to PAN.

Case Reports

Case identification

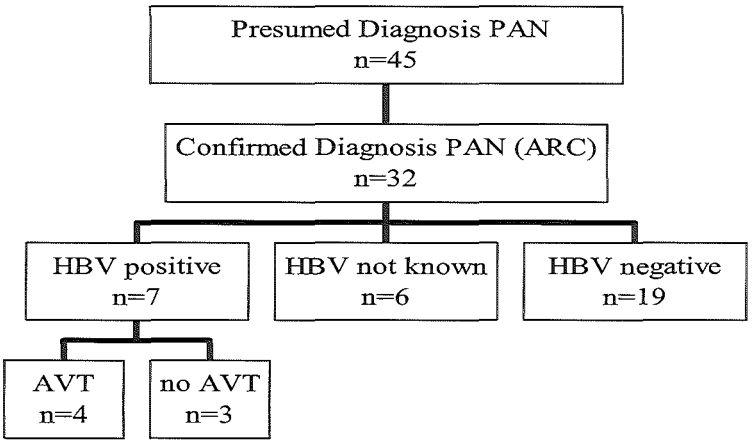


Figure 1 Identification of patients with HBV related PAN

We performed a search for patients registered with the diagnosis of PAN between 1984 and 2001 in our computerized hospital registration system. A total of 45 patients were identified. In 32 of them the diagnosis of PAN could be confirmed following the ACR criteria (3). Of these 32 patients 7 (22%) were HBV positive (figure 1). All HBV patients were treated with prednisone. In addition, four patients underwent antiviral therapy for HBV infection:

Table 1 Laboratory values at referral to our hospital.

	Case 1	**Case 2**	**Case 3**	**Case 4**	**normal values**
ESR (mm/hour)	82	80	18	51	0 -10
Hemoglobin (mmol/l)	7.5	6.6	7.8	6.8	8.2-10.2
Leukocytes (x 109/l)	17.9	23.0	6.8	12.5	4.0-10.0
Platelets (x 109/l)	369	114	280	505	140-360
ALT (U/l)	46	61	157	37	5 - 30
AST (U/l)	23	54	136	45	5 - 30
Alkaline fosfatase (U/l)	347	904	404	60	25 - 75
Gamma-GT (U/l)	128	213	115	33	5 - 35
Bilirubin (micromol/l)	11	26	19	10	4 - 14
Albumin (g/l)	29	20	36	35	36 - 48
Creatinin (micromol/l)	56	365	101	104	60 - 110
Urea nitrogen (U/l)	8.3	11.3	5.1	7.1	3.0 - 8.0

Case 1 was a 39-year old male admitted elsewhere with fatigue, fever and a weight loss of 20 kg. One year previously, he attempted suicide by taking an overdose of diazepam. Otherwise his previous history was unremarkable. Three weeks after onset of disease, he developed a tachycardia, hypertension (160/110 mmHg) and tetraparalysis with hyporeflexia and distal sensory impairment. He was referred to our hospital. Laboratory results showed a mild hepatitis (table 1). He was serum HBsAg, HBeAg and IgM anti-HBc positive indicating an acute hepatitis B infection. Serum HBV DNA level was 2.5×10^9 genomes per ml (Digene assay). Electromyography was suggestive of mononeuritis multiplex and muscle biopsy showed necrotising vasculitis of medium- and small-sized arteries. Liver

biopsy revealed a mild hepatitis and steatosis without evidence of vascultits. Abdominal angiography was normal. After psychiatric consultation he was started on prednisone 60 mg. Prednisone was tapered and discontinued after 3 months. One month after start of prednisone IFN 9 MU t.i.w. was introduced. There was a rapid decrease in HBV DNA level, which was accompanied by a transient rise in aminotransferase values and subsequently by an HBe- and HBsAg seroconversion (figure 2). Within the following months the patient exhibited a gradual but spectacular clinical improvement with disappearance of neurological symptoms. At present, 1.5 year later, he is in complete remission and able to walk again.

Case 2 was a 30-year old male who developed jaundice, arthralgias and anorexia after visiting a prostitute. Two months later the icterus had subsided but he complained of pain and hyposensibility in hands and feet. In addition he had developed fever, hypertension (160/100 mmHg) and paralysis of the peroneal nerve caused by mononeuritis multiplex. Laboratory findings showed severe renal impairment and slightly elevated liver enzymes (table 1). HBsAg, HBeAg, IgM anti-HBc and HBV-DNA ($9.9x10^9$ genomes/ml) in serum were positive. Liver biopsy revealed moderate hepatitis with minimal fibrosis. Renal angiography showed microaneurysms and small infarcts consistent with PAN. A few days after administration of 100 mg prednisone he experienced a grand-mal seizure for which depakine was given. One month after start of prednisone, IFN 9 MU t.i.w. and lamivudine 150 mg p.o. daily were added. Although HBV-DNA dropped considerable, HBeAg remained positive (figure 2). His complaints did not disappear. In the period thereafter prednisone was gradually reduced and although his neurological status slightly improved, renal function deteriorated. Two months after start of antiviral therapy he was readmitted with rapidly developing dyspnea and respiratory failure due to pneumocystis carinii infection. He died 3 days later.

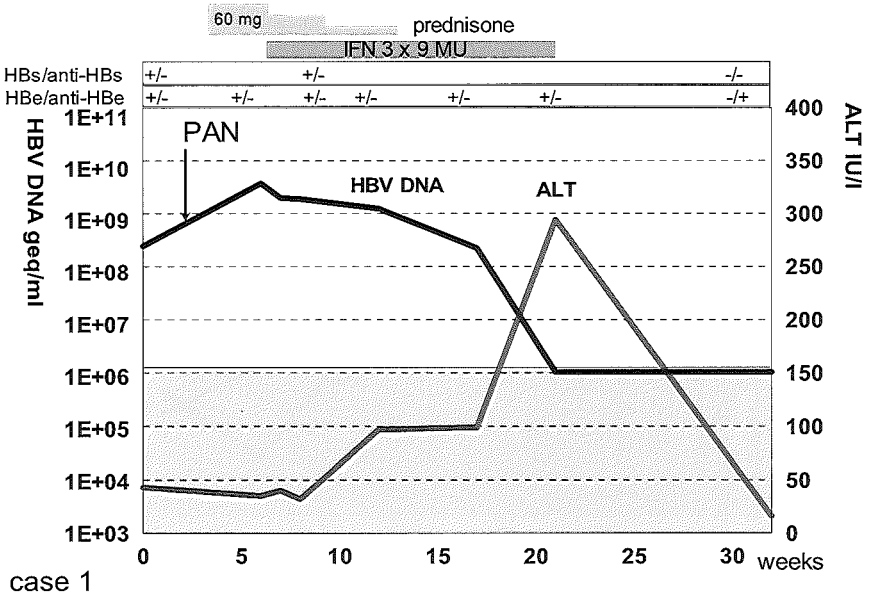
Case 3 was a 56-year old male who was hospitalized elsewhere with a hepatitis B infection, complicated by mononeuritis multiplex and a 5 kg weight loss. The duration of HBV infection was unknown. The patient was married to a heart transplantation recipient who was accidently infected with HBV. At referral to our hospital, 3 months after the initial symptoms, he was still invalidated by fatigue, hyposensibility and motoric disturbances of the extremities. Lab results showed a mild hepatitis and active HBV replication (HBeAg positive, HBV DNA $8.2x10^8$ genomes/ml) (table 1). Vasculitis was confirmed by sural nerve biopsy. Abdominal angiography was normal. He was treated with a course of prednisone (tapered from 60 mg per day) in combination with IFN 10 MU t.i.w. There was no response to IFN therapy, which had to be discontinued prematurely because of severe anorexia and weight loss (figure 2). Spontaneous HBeAg seroconversion occurred 2.5 years after IFN had been stopped. HBsAg remained positive. During the last year the patient exhibited a full clinical recovery.

Case 4 was a 29-year old homosexual male who developed abdominal pain and fatigue. At

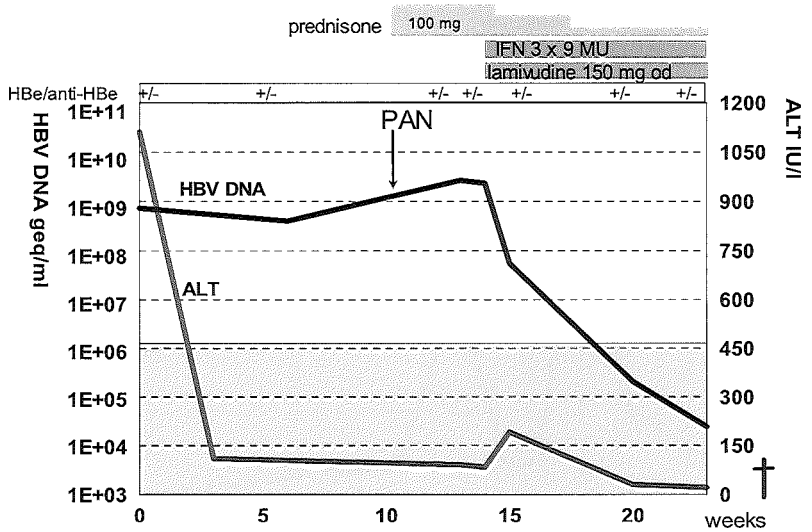
his local hospital acute hepatitis B infection was diagnosed (IgM anti-HBc positive). During the following two months he complained of dizziness, visual disturbances and periorbital numbness. In addition, he developed hypertension (160/110 mm Hg) with impaired renal function and proteinuria (2.4 g/l) (table 1). Minimal liver function abnormalities were found. HBeAg and HBV DNA ($1.2x10^9$ genomes/ml) in serum were positive. Magnetic resonance imaging of the brain demonstrated ischemic lesions in the medulla oblongata and the periventricular area. Retinal exsudates and sclerotic vessels were seen on ophtalmic examination. Electromyography showed mononeuritis multiplex and neuromuscular biopsy revealed necrotizing vasculitis. Abdominal angiography was unremarkable. Prednisone was started at 60 mg and slowly withdrawn in weeks. Simultaneous with the start of prednisone he received a 9-month IFN course with doses varying from 3 to 9 MU t.i.w. After prednisone was stopped there was a double transaminase flare leading to loss of serum HBV-DNA in combination with HBeAg and HBsAg seroconversion (figure 2). With virus eradication he underwent a clinical improvement. At the moment, 4 years later, he is still treated for hypertension. All other manifestations of PAN have disappeared.

Table 2 Characteristics and clinical outcome of patients with and without antiviral therapy

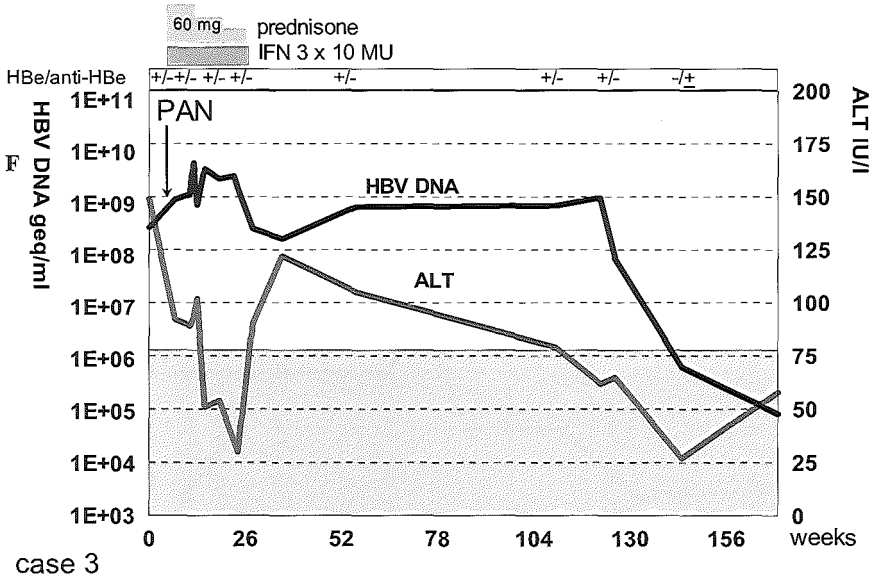
	Symptoms	Immunosuppression	antiviral therapy	Outcome PAN	outcome HBV
1	Weight loss, muscular weakness, mononeuritis multiplex, hypertension	Prednisone	IFN	Partially resolved	HBeAg/HBsAg seroconversion
2	Weight loss, myalgia, mononeuritis multiplex, hypertension, renal failure, micro-aneurysms	Prednisone	IFN/ Lamivudine	Died	HBeAg positive
3	Weight loss, muscular weakness, mononeuritis multiplex	Prednisone	IFN	Remission	HBeAg seroconversion
4	Weight loss, mononeuritis multiplex, hypertension, renal failure,	Prednisone	IFN	Remission, still treated for hypertension	HBeAg/HBsAg seroconversion
5	Hypertension, renal failure, vasculitis	Prednisone/ Endoxan	-	Renal transplantation	HBeAg seroconversion
6	Hypertension, renal failure, vasculitis	Prednisone	-	Renal transplantation	HBeAg seroconversion
7	Weight loss, polyneuropathy, testicular pain	Prednisone/ Endoxan	-	Died	unknown

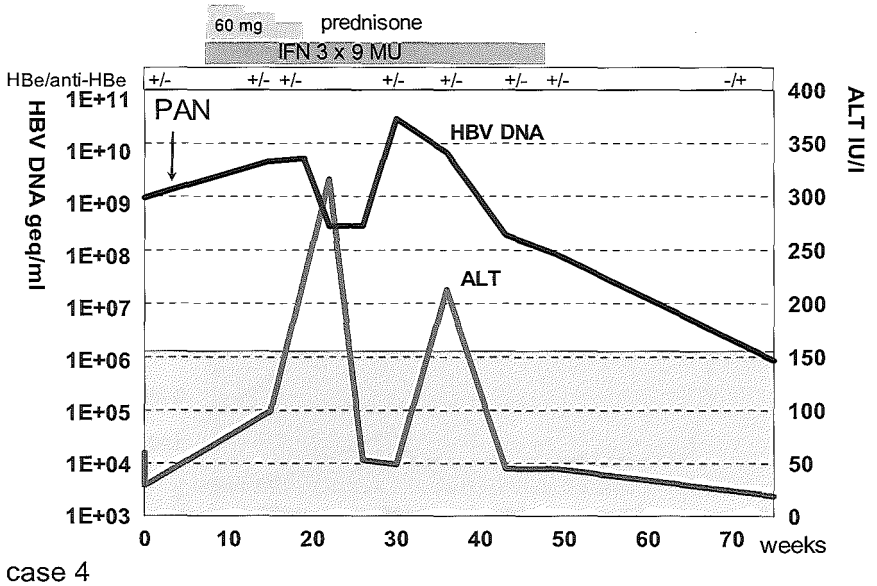

60 mg
prednisone
IFN 3 x 9 MU
HBs/anti-HBs
HBe/anti-HBe
+/-
-/-
-/+
PAN
HBV DNA
ALT
HBV DNA geq/ml
ALT IU/l
1E+11
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
weeks

case 1


prednisone
100 mg
IFN 3 x 9 MU
lamivudine 150 mg od
HBe/anti-HBe
+/-
PAN
HBV DNA
ALT
HBV DNA geq/ml
ALT IU/l
1E+11
1E+10
1E+09
1E+08
1E+07
1E+06
1E+05
1E+04
1E+03
1200
1050
900
750
600
450
300
150
0
0
5
10
15
20
weeks

case 2





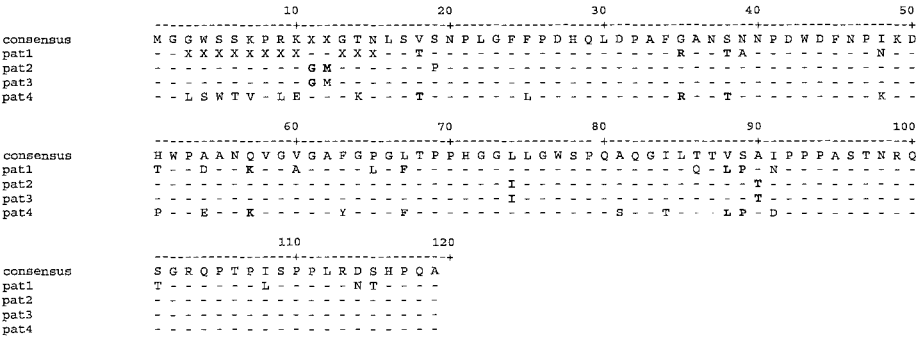
igure 2: Virological and biochemical response to antiviral therapy

Characteristics and clinical outcome of these 4 patients undergoing antiviral therapy and of the remaining 3 who were only treated with immunosuppressive agents are given in table 2. In summary, none of the patients without, but 2 of 4 with antiviral therapy exhibited HBsAg seroconversion. No clinical remission of the PAN was observed in the 3 patients with acute HBV infection who lacked treatment with antiviral medication. Two of them developed HBeAg seroconversion but none HBsAg seroconversion. This suggests that HBsAg seroconversion indicating viral eradication is best associated with clinical remission of PAN.

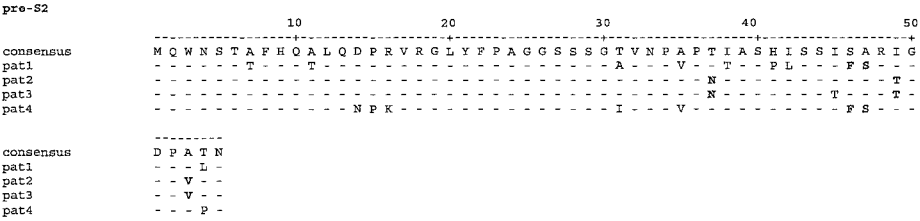
HBV DNA genome analysis

To investigate whether (a) HBV-related PAN is associated with specific HBV gene mutations and (b) treatment outcome in PAN is associated with HBV variants we sequenced the entire HBV genome from all 4 IFN-treated patients. Pre-treatment serum HBV DNA was amplified by polymerase chain reaction (PCR) using the primers HBPr108 and HBPr109. For case 4 only on-treatment serum was available for sequence analysis. Sequencing was performed on an automated DNA sequencer as previously described (7). The sequencing primers were used according to Stuyver et al. (8). The HBV genome of the four treated patients was compared to a consensus sequence. Genotype A was found in case 1 and case 2, genotype D (serotype ayw3) in case 3, and genotype G in case 4. The amino acid sequence of the pre-S1, pre-S2, HBsAg, precore, core and X open reading frame are shown in figure 3. No identical mutations were found in more than two patients. The following X gene mutations occurred in 2 patients: G1390A, G1437A, A1574T, A1676T, A1703C, T1741C, C1449T/A, a 1511G/T, G1634A/T and C1637G/T. In one patient (case 4) a stop codon mutation at nucleotide(nt) 1896 was found (G1896A) during antiviral therapy. The same patient also had a double mutation A1762T-G1764A in the basal core promoter gene. No identical repetitive mutations were found in the precore, core and polymerase gene.

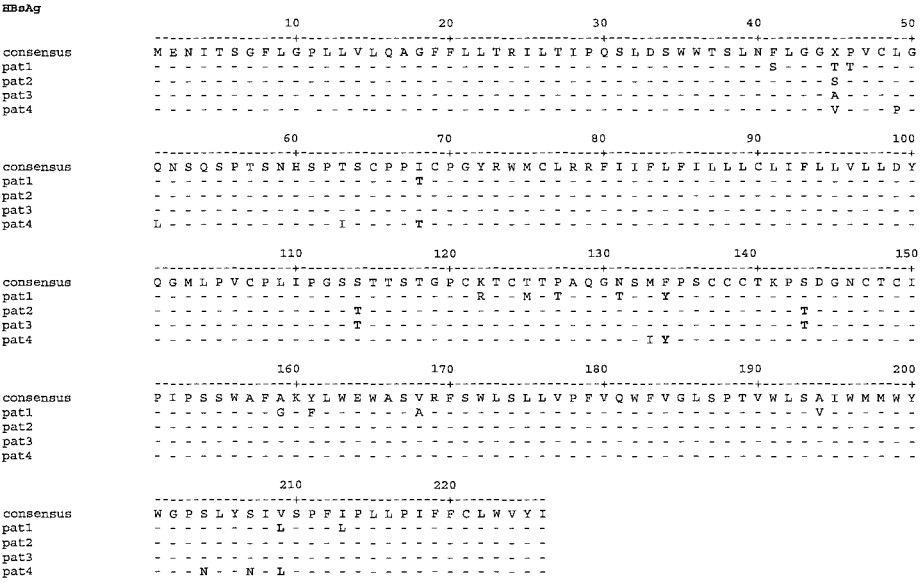
pre-S1



pre-S2



HBsAg



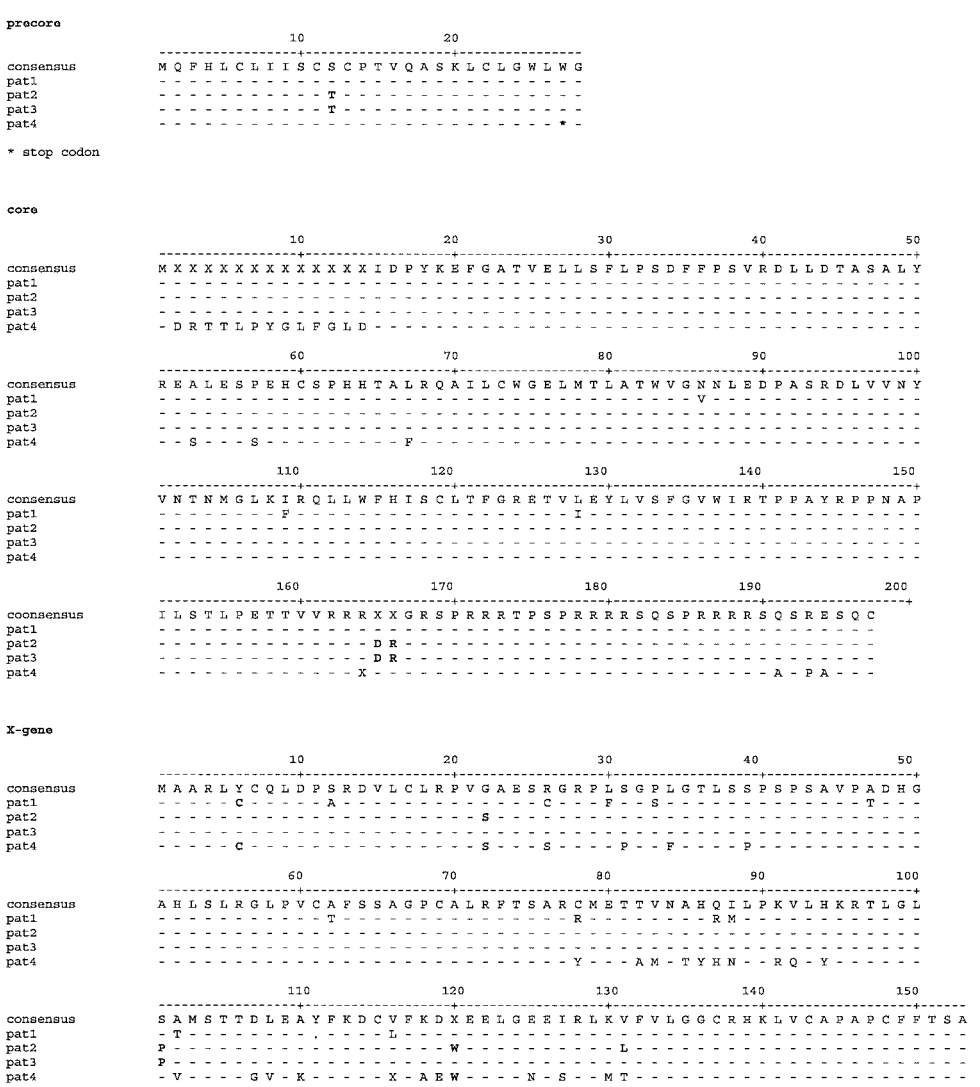


Figure 3 Amino acid sequence alignment of the pre-S1, pre-S2, HBsAg, precore, core and X open reading frame

Discussion

As circulating viral immune complexes are probably of pathogenetic importance for the vasculitic lesions, it is thought that eradicating HBV replication is essential in controlling the evolution of PAN. Conventional therapy of PAN consists of immunosuppression with steroids, cyclophosphamide and methotrexate, whether or not in combination with plasma exchanges (4,9). Immunosuppressive regimens have proven efficacy against the symptoms of vasculitis but perpetuate chronic HBV infection. About one decade ago antiviral treatment was introduced for HBV-related PAN. Prednisone combined with vidarabine led to a reduction of viral replication and to significant improvement of symptoms (10). Vidarabine, however, is not used anymore for this indication because of its neurotoxicity.

In our group of 7 patients it appeared that clinical remission of PAN was primarily related to - spontaneous or therapy-induced – control of HBV DNA replication. Recently the position of IFN as first-line standard therapy for HBV infection has been re-established. Previously, IFN-based regimens were found to be efficacious in several studies on HBV-associated PAN (11-13). Guillevin et al. reported that prednisone-IFN therapy in combination with plasma exchanges resulted in a 50% HBsAg seroconversion response and clinical remission (11). In our small series of patients with HBV-related PAN the simultaneous administration of steroids and IFN with early discontinuation of steroids, was efficacious in 2 of 4 patients. Priming treatment with prednisone should initially control the manifestations of vasculitis while subsequent withdrawal will activate the cellular immune system and thereby facilitate the immune modifying effect of IFN necessary to clear the HBV-infection. Previously, in two HBeAg-negative patients a significant improvement of clinical symptoms after IFN therapy was described, with HBsAg seroconvesion in one patient who had a precore promotor mutant HBV infection (6,14).

The clinical benefit of lamivudine on other novel nucleoside analogues in HBV-related PAN is as yet not well described. A recent case report describes successful lamivudine therapy for a kidney transplant recipient who developed cutaneous PAN associated with the reappearance of HBV 16 years after transplantation (16). Trepo et al. reported a good clinical response to lamivudine as single antiviral therapy (17). In contrast, Maclachlan reported a patient whose clinical signs worsened despite reduction of the viral load with lamivudine (18). If one considers sustained HBeAg and HBsAg seroconversion as a prerequisite for a full clinical response of PAN, lamivudine would appear less helpful than IFN (15). In contrast to IFN, therapy with lamivudine hardly augments a sustained immune response but merely suppresses viral replication. Therefore, lamivudine does not induce HBsAg seroconversion and continued therapy leads to selection of drug resistant mutants as manifested by reappearance of HBV DNA.

Successful combination therapy of IFN and lamivudine has been reported in patients with chronic hepatitis B and in two cases with HBV-related PAN (19-21). A patient who developed life-threatening

complications of PAN despite IFN (mono)therapy was successfully treated with a combination of IFN and lamivudine (22). The only patient from our group who was treated with both IFN and lamivudine did not respond and died.

The role of heterogeneity of HBV in the development of PAN remains unclear. In our patients no association between a specific mutation and the development of PAN was found. One patient developed both a precore stop codon mutation and a mutation in the basal core promoter during antiviral therapy. These mutations have been associated with increased virulence. Despite the fact that precore mutations have been shown to negatively influence response to IFN, our patient showed a response with HBsAg seroconversion.

In conclusion, our cases support the concept that clinical remission of PAN is primarily related to control of HBV replication. Based on our results and the available literature prednisone-IFN therapy maybe effective for HBV-associated PAN. The efficacy of IFN and new generation nucleoside analogues must be further investigated in long-term follow-up studies, preferably in a multi-center controlled setting. Our data do not lend support to the concept that specific mutations in the HBV genome predispose to the development of PAN.

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Chapter 8

Summary and conclusions

Samenvatting en conclusies

Dankwoord

Curriculum vitae

Bibliografie

Summary and conclusions

The treatment of chronic hepatitis B aims at the prevention of liver cirrhosis and its complications. Ideally, it would eliminate the virus completely (HBsAg seroconversion). The most frequently used measure of efficacy, however, is control of viral replication, either by (prolonged) suppression with antiviral drugs or without drugs after successful immune modulatory treatment. In the past years marked progress has been made in the treatment of chronic hepatitis B. At the start of this study in 1999, the efficacy of treatment was still limited. The two registered drugs at that time were interferon alpha (IFN) and lamivudine. IFN is only effective in 20-35 % of patients. For lamivudine the efficacy of treatment is limited by the emergence of resistant HBV mutants. Therefore there was a need for more effective treatment options.

The high response rates achieved with pegylated forms of IFN in chronic hepatitis C have led to a renewed interest in IFN treatment for chronic hepatitis B. We studied the effect of peginterferon alpha-2b (PEG-IFN) in a large randomzied trial, with participating centers from Europe, Asia and Canada. A total of 266 HBeAg-positive patients were evaluated after one year of treatment with PEG-IFN in combination with either lamivudine or placebo, and followed for six months thereafter. Our primary endpoint for the study was loss of serum HBeAg, because this is associated with durable virus suppression and normalization of ALT, as reported in earlier studies. We found that 36% of patients had lost HBeAg at the end of follow up (chapter 2). Although the rate of HBeAg loss at the end of therapy was higher in patients that received the combination of PEG-IFN and lamivudine (44%) than in patients receiving PEG-IFN alone (29%), this was not sustained during follow-up. At the end of follow-up there was no difference in response between patients treated with PEG-IFN alone or in combination with lamivudine. Response rates were dependent on HBV genotype, with genotype A and B responding better than genotype C and D. Because HBV genotype is an important predictor of response to treatment, future studies should take genotype into account. Like in chronic hepatitis C, different treatment regimens may be needed for different genotypes.

The effect PEG-IFN on liver histology was assessed at the end of treatment in 110 patients. We found that response (HBeAg negativity at the end of follow-up) to PEG-IFN therapy was also accompanied by histological improvement (chapter 3). At the end of therapy, necroinflammation had improved in half of the patients, but more often in responders (78%) than non-responders (43%). Fibrosis only improved in a minority of responders. However, the interval of one year between the biopsies may have been too short to achieve the full effect of therapy on liver fibrosis.

With combination therapy of PEG-IFN and lamivudine a fast and profound virus suppression was achieved (chapter 4). During therapy with PEG-IFN alone viral decline was significantly less. Particularly responders showed a viral decline, and in a proportion of responders this only occurred after a variable delay of up to 32 weeks. We assessed if HBV DNA decline or single HBV DNA levels at different timepoints during therapy could be used to predict response. We found that HBV DNA

level was best associated with response. Because of the different patterns of HBV DNA decline, a prediction of response could be made at week 4 of therapy for PEG-IFN combined with lamivudine and at week 20 of therapy for PEG-IFN alone. The chance of response was minimal if with combination therapy of PEG-IFN and lamivudine the HBV DNA level was above 10^7 geq/ml (negative predictive value 93%) after 4 weeks of therapy. With PEG-IFN therapy alone this was the case if the HBV DNA level was above 10^9 geq/ml after 20 weeks of therapy (negative predictive value 88%). These cut-off levels might be used as "stopping rule".

As PEG-IFN therapy has not been used previously in chronic hepatitis B patients we also assessed its safety in this patient population (chapter 5). The most common side effects were the same as those occurring with standard IFN (flu-like symptoms, headache, fatigue, myalgia, alopecia, gastrointestinal symptoms and psychiatric symptoms). These adverse events were most prominent during the first months of therapy. Hematological abnormalities were frequent but did not lead to serious complications. Dose reduction and discontinuation of treatment were necessary in 23% and 9% of patients, respectively. Half of the dose reductions were due to neutropenia, whereas the most frequent cause for treatment discontinuation was the occurrence of psychiatric symptoms (depression, psychosis). Neutropenia and liver cirrhosis at baseline were independent predictors of dose reduction and treatment discontinuation. Patients with preexistent cirrhosis were at an increased risk of thrombocytopenia and (minor) bleeding complications. All side effects were reversible after discontinuation of therapy.

Although the beneficial effects of IFN therapy on disease activity have been well described, the effects on the development of complications related to liver cirrhosis and mortality are still less well defined. Therefore we followed all hepatitis B e antigen (HBeAg)-positive CHB patients that were treated with standard IFN in Rotterdam between 1978 and 2002 (chapter 6). The median follow-up time in our study was 8.8 years. Of the 165 patients, 33% responded to IFN treatment. Responders to IFN showed an increased rate of HBsAg loss and less progression of liver fibrosis. Survival analysis showed that the risk of developing hepatocellular carcinoma was lower and survival was longer in responders to IFN.

Chapter 7 describes our experience with IFN treatment in hepatitis B associated polyarteritis nodosa (PAN). Seven patients that developed PAN after (acute) hepatitis B infection were treated with prednisone and in four of these patients prednisone was combined with IFN. Among the four patients treated with a priming steroid course and IFN, three showed a clinical remission of PAN, and two cleared the infection altogether. None of the patients that received only prednisone exhibited a clinical remission or HBsAg seroconversion. Why some patients develop PAN in the course of acute hepatitis B infection remains unclear. We could not find any mutations in the HBV genome predisposing to PAN. The combination of short-term steroid priming followed by IFN appears to be an improvement over prednisone monotherapy.

At the start of the studies discussed in this thesis, effective control of the hepatitis B virus was only possible in a proportion of patients. With the introduction of PEG-IFN and new powerful nucleoside analogues such as adefovir (approved recently), entecavir (phase III trials) and telbivudine (phase III trials), with a very small risk of resistance development, an adequate treatment for many chronic hepatitis B patients seems achievable.

Conclusions:

1. PEG-IFN is a safe and effective treatment for HBeAg positive chronic hepatitis B resulting in a sustained response in 36% of patients, which is associated with biochemical and histological improvement. The efficacy of PEG-IFN was not increased by combining PEG-IFN with lamivudine.

2. The combination of PEG-IFN and lamivudine leads to a faster and more profound virus suppression during treatment compared to PEG-IFN alone. However this is not sustained after cessation of treatment.

3. Patients who respond to standard IFN treatment have a decreased risk of developing hepatocellular carcinoma and a prolonged survival compared to non-responders.

4. A priming course of prednisone followed by IFN appears to be beneficial in hepatitis B-associated PAN.

Samenvatting en conclusies

Het doel van de behandeling van chronische hepatitis B is het voorkomen van levercirrhose en de complicaties daarvan. Ideaal zou zijn het geheel elimineren van het virus (HBsAg seroconversie). De meest gebruikte maat voor effectiviteit is echter controle van de virale replicatie, ofwel door (langdurige) onderdrukking met antivirale medicatie ofwel zonder medicatie na geslaagde immuunmodificerende behandeling. De afgelopen jaren is er sterke vooruitgang geboekt in de behandeling van chronische hepatitis B. Bij de start van dit onderzoek in 1999 was de effectiviteit van de behandeling van chronische hepatitis B nog beperkt. De twee toen geregistreerde middelen waren interferon alpha (IFN) en lamivudine. IFN is slechts effectief in 20-35% van de patienten. De effectiviteit van behandeling met lamivudine wordt vooral beperkt door de opkomst van lamivudine resistente HBV mutanten. Daarom was er dringend behoefte aan effectievere behandelingsmogelijkheden.

De hoge responspercentages die worden bereikt met gepegyleerde vormen van IFN bij patienten met chronische hepatitis C hebben tot een hernieuwde belangstelling geleid voor IFN behandeling van chronische hepatitis B. Wij bestudeerden het effect van peginterferon alpha-2b (PEG-IFN) in een grote gerandomiseerde studie, met deelnemende centra uit Europa, Azië en Canada. In totaal 266 HBeAg-positieve patienten werden geëvalueerd na een jaar behandeling met PEG-IFN in combinatie met lamivudine of placebo, en daarna nog zes maanden gevolgd. Ons primaire eindpunt voor de studie was het verlies van serum HBeAg, omdat dit meestal gepaard gaat met duurzame virussuppressie en normalisatie van ALT. Wij vonden dat 36% van de patiënten het serum HBeAg had verloren aan het eind van de follow-up periode (hoofdstuk 2). Hoewel het percentage patiënten dat HBeAg had verloren aan het einde van de behandeling hoger was bij patiënten die werden behandeld met de combinatie van PEG-IFN en lamivudine (44%) dan bij patiënten die werden behandeld met PEG-IFN alleen (29%), bleef dit verschil niet behouden tijdens de vervolgperiode. Aan het eind van de vervolgperiode was er geen verschil in respons tussen patiënten die werden behandeld met PEG-IFN alleen of in combinatie met lamivudine. De responspercentages werden mede bepaald door het HBV genotype, waarbij genotype A en B beter respondeerden dan genotype C en D. Omdat het HBV genotype een onafhankelijke voorspellende factor voor respons op behandeling is, zal er in toekomstige studies rekening gehouden moeten worden met het genotype. Zoals bij chronische hepatitis C, zullen mogelijk ook verschillende behandelingsschema's nodig zijn voor verschillende genotypes.

Het effect van PEG-IFN op de histologie van de lever werd bepaald aan het einde van de behandeling bij 110 patiënten. Wij vonden dat respons (HBeAg negativiteit aan het eind van de follow-up) op PEG-IFN therapy ook vergezeld ging met histologische verbetering (hoofdstuk 3). Aan het eind van de behandeling was de necroinflammatie verbeterd bij de helft van de patiënten, maar vaker bij responders (78%) dan bij non-responders (43%). Fibrose verbeterde alleen bij een minderheid van de

responders. Echter het interval van een jaar tussen de biopsieën is mogelijk te kort geweest om het volledige effect van de behandeling op de leverfibrose te bereiken.

Met de combinatiebehandeling van PEG-IFN en lamivudine werd een snelle en sterke virussuppressie bereikt (hoofdstuk 4). Tijdens behandeling met PEG-IFN alleen was de daling van de hoeveelheid virus significant minder. Met name responders lieten een daling van de hoeveelheid virus zien, en bij een deel van de responders gebeurde dit pas na een variabele periode, die wel tot 32 weken kon duren. Wij bepaalden of de daling van het HBV DNA of het actuele HBV DNA niveau op individuele tijdspunten tijdens de behandeling gebruikt kon worden om respons te voorspellen. Wij vonden dat het HBV DNA niveau het beste geassocieerd was met respons. Vanwege de verschillende patronen van HBV DNA daling kon de beste predictie van respons gedaan worden op week 4 van de behandeling voor combinatiebehandeling met PEG-IFN en lamivudine, en op week 20 voor PEG-IFN alleen. De kans op respons was minimaal als bij de combinatietherapie van PEG-IFN en lamivudine het HBV DNA niveau na 4 weken behandeling nog boven 10^7 geq/ml lag (negatief predictieve waarde 93%). Bij behandeling met PEG-IFN alleen was dit het geval als het HBV DNA niveau na 20 weken behandeling nog boven 10^9 geq/ml lag (negatief predictieve waarde 88%). Deze grens zou dan ook aangehouden kunnen worden als "stopping rule".

Omdat PEG-IFN behandeling niet eerder is onderzocht voor patiënten met chronische hepatitis B, onderzochten we ook de veiligheid ervan in deze populatie patiënten (hoofdstuk 5). De meest voorkomende bijwerkingen waren dezelfde als de bijwerkingen die optreden bij standaard IFN (griepachtige verschijnselen, hoofdpijn, vermoeidheid, spierpijn, haaruitval, gastrointestinale verschijnselen en psychiatrische symptomen). Deze bijwerkingen waren vooral aanwezig gedurende de eerste maanden van de behandeling. Hematologische afwijkingen traden vaak op maar leidden niet tot ernstige complicaties. Dosisreductie en stoppen met de behandeling was noodzakelijk bij respectievelijk 23% en 9% van de patiënten. In de helft van de gevallen waar de dosis werd gereduceerd, gebeurde dit vanwege neutropenie, terwijl de meest voorkomende reden voor het stoppen van de behandeling het optreden van psychiatrische symptomen (depressie, psychose) was. Neutropenie en levercirrhose bij het starten van de behandeling waren onafhankelijke predictoren voor dosisreductie en staken van de behandeling. Patiënten met preëxistente levercirrhose hadden een verhoogd risico op thrombocytopenie en (milde) bloedingscomplicaties. Alle bijwerkingen waren reversibel na staken van de behandeling.

Hoewel de gunstige effecten van IFN behandeling op de ziekteactiviteit goed beschreven zijn, zijn de effecten op het ontwikelen van lange-termijn complicaties van levercirrhose en mortaliteit minder goed gedefinieerd. Daarom volgden we alle HBeAg-positieve chronische hepatitis B patiënten die tussen 1978 en 2002 in Rotterdam werden behandeld met standaard IFN (hoofdstuk 6). De mediane follow-up duur in onze studie was 8,8 jaar. Van de 165 patiënten patients respondeerden 33% op IFN behandeling, gedefinieerd als verlies van HBeAg tijdens behandeling of binnen een jaar na behandeling. Responders op IFN lieten een hoger percentage HBsAg verlies en minder progressie van

leverfibrose zien dan non-responders. Survival analyse liet zien dat het risico op het ontwikkelen van hepatoellulair carcinoom lager en de overleving beter was bij responders op IFN.
Hoofdstuk 7 beschrijft onze ervaring met IFN behandeling bij hepatitis B geassocieerde polyarteritis nodosa (PAN). Zeven patiënten die PAN ontwikkeleden na een (acute) hepatitis B infectie werden behandeld met prednison, en bij vier van deze patiënten werd dit gecombineerd met IFN. Bij de vier patiënten die kortdurend werden behandeld met prednison gevolgd door IFN lieten er drie een clinische remissie van de PAN zien en twee klaarden de infectie volledig. Geen van de patiënten die alleen prednison kregen hadden een klinische remissie of HBsAg seroconversie. Waarom sommige patiënten PAN ontwikkelen in het beloop van een acute hepatitis B infectie blijft onduidelijk. Wij konden geen mutaties vinden in HBV genome die predisponeren voor het ontwikkelen van PAN. De combinatie van kortdurende behandeling met steroïden gevolgd door IFN lijkt een verbetering ten opzichte van prednison monotherapie.

Bij aanvang van dit onderzoek was slecht bij een deel van de patiënten effectieve controle van het hepatitis B virus mogelijk. Met de komst van PEG-IFN en nieuwe krachtige nucleoside analogen als adefovir (recent geregistreerd), entecavir (fase III trials) en telbivudine (fase II trials), waarbij het risico op resistentieontwikkeling zeer gering is, lijkt een adequate behandeling bereikbaar voor veel patiënten met chronische hepatitis B.

Conclusies:

1. PEG-IFN is een veilige en effectieve behandeling voor HBeAg positieve chronische hepatitis B resulteerend in een blijvende respons bij 36% van de patiënten, die geassocieerd is met biochemische en histologische verbetering. De effectiviteit van PEG-IFN wordt niet verhoogd door PEG-IFN met lamivudine te combineren.

2. De combinatie van PEG-IFN en lamivudine leidt tot een snellere en sterkere virussuppressie tijdens de behandeling dan PEG-IFN alleen. Dit is echter niet duurzaam na het stoppen van de behandeling.

3. Patiënten die responderen op behandeling met standaard IFN hebben een verlaagd risico op het ontwikkelen van hepatocellulair carcinoom en een langere overleving vergeleken met non-responders.

5. Kortdurende behandeling met prednison gevolgd door IFN lijkt een gunstig effect te hebben bij hepatitis B-gassocieerde PAN.

Dankwoord

Een promotieonderzoek is nooit het werk van één persoon. Ik heb het geluk gehad mee te mogen werken in een grote internationale studie, die alleen mogelijk was dankzij de samenwerking van zeer veel mensen.

Allereerst wil ik mijn promotor, professor Schalm, danken voor zijn grote inzet en gedrevenheid. Beste Prof. Schalm, ik bewonder uw energie en schijnbaar onuitputtelijke stroom van nieuwe ideeën.

Mijn co-promotor Harry Janssen wil ik bedanken voor de begeleiding gedurende de studie. Dankzij jouw kritische blik waarmee je keer op keer de manuscripen hebt doorgenomen en het duwtje in de rug dat ik soms nodig had om verder te komen zijn de studies tot een goed einde gekomen.

Rob de Man, bedankt voor de supervisie van de poliklinische patiënten en het kritisch lezen van mijn manuscripten.

De mensen van het trialbureau, Elke, Eva, Wanda en Maarten hebben zeer veel werk verzet bij het opzetten en begeleiden van de studie, en bij het ordenen en verwerken van de grote massa aan data.

Bettina, bedankt voor de statistische analyses en de vele uren achter de computer!

Dr. Zondervan, u heeft het histologie-artikel mogelijk gemaakt en mij tegelijkertijd een cursus leverhistologie gegeven!

Zonder de monitors Mieke Denys, Sarah, Gerd, Anneleen en Kathleen, was het nooit gelukt een studie die in zoveel centra loopt in goede banen te leiden en alle data te verzamelen. Bedankt voor de fijne samenwerking en de gezelligheid op de congressen.

De mensen van de CRU wil ik bedanken voor hun hulp bij de "visits" van de Rotterdamse patiënten. Wilma en Karin, bedankt voor het bijhouden van alle CRF's.

Op het MDL lab werd een enorme hoeveelheid serummonsters geordend en opgeslagen. Op het virologisch lab o.l.v. Bert Niesters werden alle serologische bepalingen en PCR's verricht.

Margriet en Marion, bedankt voor de hulp bij het submitten van de manuscripten en het "regelwerk" voor de promotie.

Nermin en Esther, het was een leuke tijd op de poli. Dank zij jullie liep het gesmeerd.

De dames van het "kippenhok"- Heleen, Cockie, Anneke en Gwen: Bedankt voor de gezelligheid!

Thjon, Jan-Maarten, Pieter, Rachel en Marjolein, heel veel succes met de opleiding. Wim, Hajo, Bart, Dave en Sarwa, jullie zijn al een eind op weg op het promotiepad. Succes, en ik kijk uit naar jullie boekjes! Annemiek, mijn kamergenootje, bedankt voor de gezelligheid en de vele kopjes thee.

Tot slot wil ik de leden van de promotiecommissie bedanken voor het beoordelen van mijn manuscript.

Curriculum vitae

Monika van Zonneveld werd op 10 maart 1972 geboren in Giessen (Duitsland). In 1990 behaalde zij het eindexamen VWO aan de Rijksscholengemeenschap JH Tromp Meesters in Steenwijk, waarna zij geneeskunde studeerde aan de Rijksuniversiteit Groningen. In 1997 behaalde zij het artsexamen. Gedurende een jaar werkte zij als AGNIO cardiologie in he Rijnland Ziekenhuis in Leiderdorp. Hierna werkte zij als AGNIO interne geneeskunde in het Groene Hart Ziekenhuis in Gouda en het Erasmus MC in Rotterdam. Van december 2000 t/m april 2004 was zij werkzaam als arts-onderzoeker op de afdeling maag-darm-leverziekten. Hier werd onder leiding van Dr. HLA Janssen onderzoek verricht naar de behandeling van chronische hepatitis B, wat resulteerde in dit proefschrift (promotor Prof. SW Schalm). In mei 2004 is zij begonnen met de opleiding tot internist.

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APPENDIX The HBV 99-01 Study Group

This thesis was based on the HBV 99-01 study.

In addition to the authors of chapter 2-5, the HBV 99-01 Study Group includes the following investigators:

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