CLINICAL AND VIROLOGICAL STUDIES ON α-INTERFERON TREATMENT OF CHRONIC HEPATITIS TYPE B

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KLINISCH EN VIROLOGISCH ONDERZOEK NAAR α-INTERFERON BEHANDELING BIJ CHRONISCHE HEPATITIS B

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Aan mijn ouders

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Contents

Abbrevations

ALT	alanine aminotransferase
anti-HBc	antibodies against hepatitis B core antigen
anti-HBe	antibodies against hepatitis B e antigen
anti-HBs	antibodies against hepatitis B surface antigen
ARA-AMP	adenine arabinoside monophosphate
AST	aspartate aminotransferase
AZT	3'-azido-3'-deoxythymidine
CAH	chronic active hepatitis
CPH	chronic persistent hepatitis
CI	confidence interval
cpm	counts per minute
DNA	deoxyribonucleic acid
DNA-p	deoxyribonucleic acid polymerase
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HDV	hepatitis delta virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IFN	interferon
IgG	immunoglobulin G
IgM	immunoglobulin M
MU	mega-units
PCR	polymerase chain reaction
pre-S1 Ag	hepatitis B pre-S1 antigen
pre-S2 Ag	hepatitis B pre-S2 antigen
RIA	radioimmunoassay
RNA	ribonucleic acid

CHAPTER 1

INTRODUCTION

The Hepatitis B Virus

The hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the group of hepadna viridae (1). The replication of HBV is believed to occur preferentially in the hepatocyte. Analysis of the nucleotide sequence of the virus revealed 4 open reading frames, regions of the genome which may code for viral antigens (1). Although the HBV genome contains only 3200 nucleotides its compactness and circular composition, employing overlapping genes for production of several viral proteins, make the virus highly efficient for replication. The replication cycle of HBV is illustrated in figure 1. After entry in the hepatocyte the virus is uncoated and the genomic DNA is converted to a supercoiled form of covalently closed fully double stranded DNA which is transcribed to pregenomic and messenger RNA. Viral messenger RNA is transported to the cytoplasm

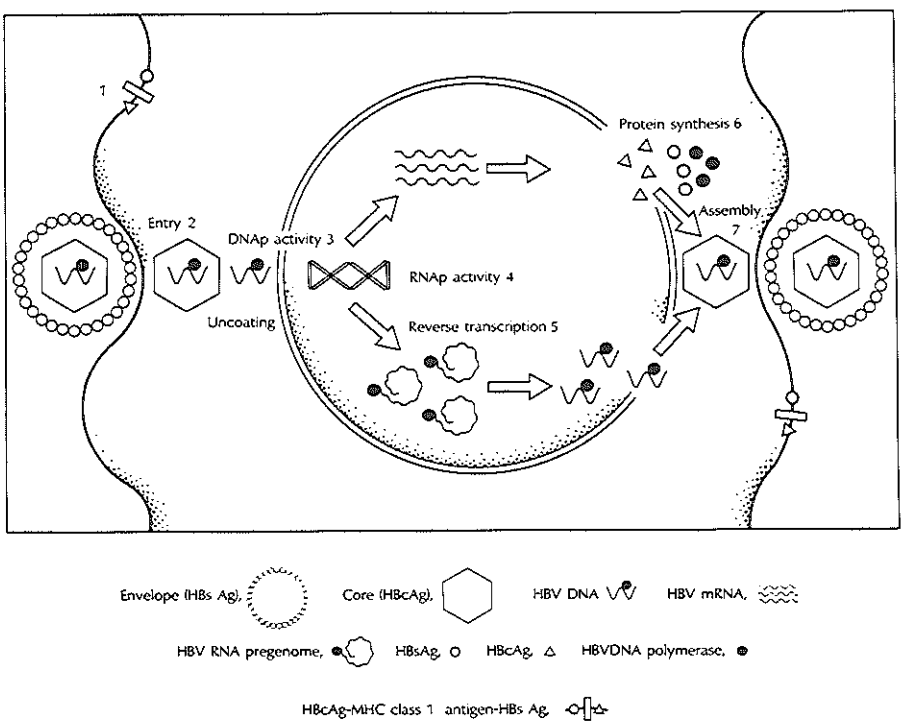

Figure 1. HBV replication cycle in hepatocytes. DNA-P: DNA polymerase; HBcAg: hepatitis B core antigen; HBsAg: hepatitis B surface antigen; HBV: hepatitis B virus; MHC: major histocompatibility complex; RNA-p: RNA polymerase. (from L. Berk et al. Current Opinion in Infectious Diseases 1989;2:419-23; with permission).

where it codes for production of viral proteins. Pregenomic RNA is reverse transcribed into a minus strand of DNA which is utilized as a template for completion of the plus strand of the newly synthesized viral DNA. The HBV-DNA-polymerase enzyme which exerts both a DNA polymerase and a reverse transcriptase activity plays a crucial role in the replication cycle of the virus.

Chronic Hepatitis B

Inoculation with the virus causes hepatocellular necrosis and inflammation which ranges in severity from an asymtomatic infection that resolves completely to severe, symptomatic infection with progressive or even fatal illness (2). It is still not clear why certain patients progress to a chronic hepatitis B infection while the majority clears HBV after an acute infection. One of the possibilities is that chronic HBV carriage results in part from a deficiency in IFN production. Several studies have showed a lower endogeneous IFN response in individuals with chronic hepatitis B (3). This low IFN production is likely to impair the display of HLA class I proteins and thereby impede the clearance of hepatocytes harbouring replicating virus (4).

In the patients who develop a chronic hepatitis there is a substantial risk of disease progression to cirrhosis, portal hypertension and hepatocellular carcinoma (5,6; figure 2). It is unlikely that HBV causes its damaging effect on liver cells by direct cytopathogenic changes because large quantities of HBsAg and HBcAg are found in the hepatocytes of many asymptomatic and appearantly healthy HBV carriers. Both for the pathogenesis of the liver damage and for succesful eradication of the disease the interaction between various structural components of the virus and the immune system appear to be essential.

In untreated chronic hepatitis B patients HBV probably replicates less vigorously as time passes, maybe because of an increasing immunological attack on virus-infected hepatocytes (7,8). This phenomenon is accompanied by a fall of serum HBV-DNA and increased liver damage as signalled by abnormal liver function tests. Cessation of HBV replication as indicated by an HBeAg serovonversion and a marked suppression of HBV-DNA, usually heralds a last and quiescent phase of the disease which may in time be followed by a total eradication of the virus (HBsAg seroconversion) (9-11). The process of HBeAg seroconversion is often preceded by intense hepatic inflammation and symptoms of fatigue. The aim of antiviral therapy with α-IFN is to assist the host in eliminating HBV replication early in the course of the chronic infection.

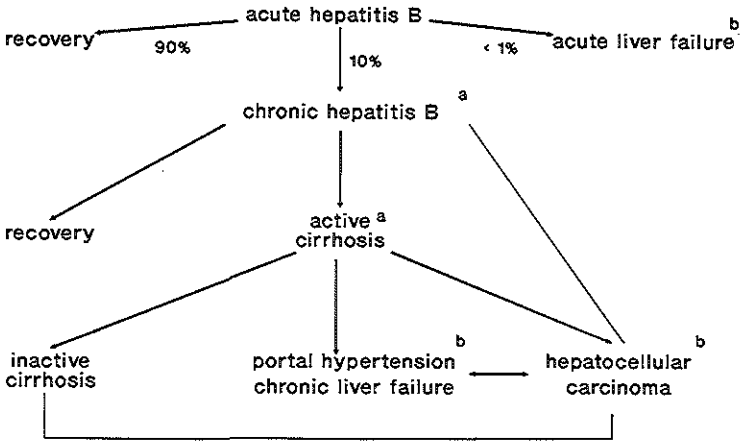

Figure 2. Course of hepatitis B infection.

Alpha-Interferon Treatment of Chronic Hepatitis B

Intervention treatment of chronic hepatitis B is targeted at patients with active viral replication, preferably at a stage before signs and symptoms of cirrhosis or significant disease activity have occurred. Although the genetic heterogeneity of HBV, recently disclosed by molecular biology (12), has necessitated a reconsideration of the classical serologic distinctions between active virus replication and viral latency, the presence of HBeAg and HBV-DNA is still considered to be the main indication for antiviral therapy.

α-IFN probably interferes directly with HBV production by activation of intracellular enzymes like 2'-5' oligoadenylate synthetase or protein kinase, leading to an activation of ribonucleases that destroy viral messenger RNA. As immune modifying substance α-IFN augments the natural killer cell activity and causes enhanced expression of HLA class I proteins on the hepatocyte surface thereby facilitating recognition and lysis of virus-infected cells by the cellular immune system (13). In addition, α-IFN may boost the production of the HBV transcripts that code for HBcAg thereby promoting an increased hepatocyte membrane expression of this antigen which is one of the key viral proteins recognised by the cytotoxic T cells (14).

Although α-IFN is the first agent which has repeatedly shown efficacy for chronic hepatitis B in large randomized controlled trials, more than half of the patients do not respond and are left with continuing disease activity (15,16). Because several individual variables (e.g. high level of inflammatory activity, low level of virus replication and a short duration of infection) are associated with a higher response rate (17,18) and because the adverse effects of α-IFN are often a cause dose reduction, timing and dosage of α-IFN are important in determining the potency of this therapy. The scope of this thesis is comprised to the question how we could safely improve the indication for and antiviral effect of α-IFN therapy in chronic hepatitis type B.

The objectives of the study are:

1. To assess the prognostic role of HBeAg seroconversion in patients with HBV-related cirrhosis of the liver (chapter 2).

2. To evaluate the efficacy of modifications (prolonged intermittent therapy, combination therapy with zidovudine and α-IFN retreatment) of α-IFN treatment in chronic hepatitis B (chapter 3, 4 and 5).

3. To evaluate the usefulness of quantitated HBV-DNA and HBsAg assessments for monitoring of chronic hepatitis B patients undergoing α-IFN therapy (chapter 6 and 7).

4. To review the frequency and clinical aspects of serious side effects (fatal hepatic decompensation, seizures and suicidal behaviour) associated with α-IFN therapy for chronic viral hepatitis (chapter 8, 9 and 10).

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CHAPTER 2

SURVIVAL AND PROGNOSTIC INDICATORS IN HBsAg-POSITIVE CIRRHOSIS OF THE LIVER. THE ROLE OF HBeAg SEROCONVERSION.

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Abstract

To evaluate indications for new therapies, such as liver transplantation and antiviral therapy, we assessed survival of histologically proven HBsAg-positive cirrhosis of the liver in a cohort of 98 patients, followed for a mean period of 4.3 years. The overall survival probability was 92% at 1 year, 79% at 3 years and 71% at 5 years.

Variables significantly associated with the duration of survival were age, serum aspartate aminotransferase levels, the presence of esophageal varices and all 5 components of the Child-Pugh index (bilirubin, albumin, coagulation factors, ascites and/or encephalopathy). Multivariate analysis showed that only age, bilirubin and ascites were independently related to survival.

Survival for patients with decompensated cirrhosis (determined by the presence of ascites and/or jaundice and/or encephalopathy and/or a history of variceal bleeding) and compensated cirrhosis at 5 years was 14 and 84%, respectively.

For patients with compensated liver cirrhosis, HBeAg positivity was also a prognostic factor with a 5-year survival of 72% for HBeAg-positive cirrhosis and 97% for HBeAg-negative cirrhosis; the risk of death was decreased by a factor 2.2 when HBeAg seroconversion occurred during follow-up.

We conclude that liver transplantation should be considered for patients with decompensated HBsAg-positive liver cirrhosis and antiviral therapy for patients with HBeAg-positive compensated cirrhosis.

Introduction

Chronic hepatitis B is associated with substantial morbidity and mortality depending on the subgroups as healthy carrier, chronic hepatitis or cirrhosis (1,2). Primary hepatocellular carcinoma and liver failure probably account for more than 50% of all deaths among HBsAg carriers (3,4). Follow-up studies show that mortality is linked predominantly to cirrhosis (1,5-12). After the development of liver cirrhosis the five-year survival rate varies between 52 and 80% (9,13-16). Major causes of death for cirrhotic patients are, in order of frequency: hepatocellular carcinoma, liver failure and upper gastrointestinal bleeding (17-20).
Little data on the prognosis of HBsAg-positive cirrhosis for West-European patients are available. The present paper reports on mortality of HBsAg-positive liver cirrhosis in a predominantly white West-European population. A total of 12 possible determinants of survival were analyzed by univariate and multivariate methods. In addition, the prognostic role of the hepatitis B e antigen (HBeAg) and the influence of HBeAg-seroconversion on the risk of death were determined. Such analyses may help to define indications for and timing of new treatment modalities such as antiviral therapy and liver transplantation for HBsAg-positive cirrhosis.

Patients and Methods

Patients.
From January 1970 to June 1990 almost 450 patients with chronic hepatitis B virus (HBV) infection visited the Department Hepatogastroenterology of our institution, which serves a tertiairy referral function. Eighty-three percent of these patients underwent a liver biopsy. Cirrhosis was consecutively diagnosed in 98 patients. The criteria for the diagnosis of HBV-related cirrhosis were the presence of serum HBsAg and a liver biopsy that showed cirrhosis or "probably" cirrhosis (21). Of the non-biopsied patients 4 exhibited signs that could be related to advanced liver disease (albumin $<$ 34 g/l n=3; ascites n=2; jaundice n=2). Because these signs cannot unequivocally be linked to cirrhosis we did not include these patients in the study.

Design of the study.
We calculated the actuarial survival of the 98 patients with HBsAg-positive cirrhosis who visited our department. The following characteristics, present at the beginning of follow-up, were evaluated to determine their prognostic significance for survival: sex, age, subjective symptoms, alcohol intake, ascites, encephalopathy, esophageal varices, HBeAg status,

serumaspartate aminotransferase (AST), bilirubin, albumin and coagulation factors. In addition, the effect of HBeAg-seroconversion during follow-up on mortality was analyzed.

Follow-up.

Follow-up started after histopathological confirmation of HBsAg-positive liver cirrhosis or - when histopathological confirmation had already been obtained elsewhere - at the time of the first visit to our department. Clinical and laboratory parameters were followed at regular intervals (at least every 6 months). Survival from entry into the study was evaluated up to 1 September 1990. Three patients who had moved abroad were lost to follow-up. The mean follow-up time was 4.3 years (range 0.1 to 18 years).

Clinical and laboratory assessment.

HBsAg, HBeAg, antibodies to HBsAg, antibodies to HBeAg and antibodies to the hepatitis C and D virus were measured with commercially available enzyme-linked immunosorbent assays or solid-phase radioimmunoassays (Abbott Laboratories, North Chicago, Ill., USA). The presence of antibodies to hepatitis C was confirmed by a recombinant immunoblot assay (Ortho). To achieve complete and uniform HBeAg testing, sera obtained before 1980 were collected and retested with the presently used radioimmunoassay test system (Abbott, North Chicago, Ill., USA). HBeAg-seroconversion was defined as the absence of serum HBeAg for at least six months. AST, bilirubin and albumin were determined with the sequential multiple autoanalyzer (12-panel SMA; Technicon Instruments Corp., Tarrytown, N.Y.). Coagulation factors were assessed by Normotest® and Trombotest®.

All biopsy specimens - including those from referring hospitals - were judged by a single experienced pathologist according to international standards (21). Ascites was diagnosed by physical examination and ultrasonography of the abdomen which was carried out routinely. Esophageal varices were present when clearly demonstrated by standardized radiologic examination or endoscopy (grade II, III or IV) (22). Twelve patients did not undergo esophageal examination; esophageal varices were assumed not to be present in these cases. Presence of hepatic encephalopathy was confirmed by spectral analysis of the electroencephalogram (23).

Statistical analysis.

Survival analysis - irrespective of the cause of death - was carried out by the Kaplan-Meier method. For univariate analysis the log rank test to compare survival curves was used. Variables that were statistically significant (two-sided, $P<0.05$) according to the univariate analyses were subsequently introduced into the multivariate analysis, as described by Cox

(24). The relation between HBeAg seroconversion and subsequent death rates was evaluated according to a statistical method that corrected for response-time bias for patients who underwent HBeAg seroconversion (25).

Results

The initial patient characteristics are presented in table 1. At presentation the median age was 46 years (range 24-81), the median bilirubin level was 12 μmol/l (range 8-602; normal < 14 μmol/l) and the median AST level 54 U/l (range 14-383; normal < 30 U/l). Seventy-six percent of the patients were West-European caucasians. The risk factors for transmission of the HBV were: HBV-positive heterosexual partner in 4%, intravenous drug abuse in 5%, blood transfusion in 6%, (para)medical work in 6%, homosexual contacts in 18%, stay in an endemic area for HBV in 21% and unknown in 40% of the patients.

Twenty-six of the 98 patients (27%) died during the follow-up period. Ten patients died of hepatocellular carcinoma, 10 due to liver failure or fatal bleeding of the upper gastrointestinal tract and 6 due to non-liver-related causes. Estimated survival for HBsAg-positive liver cirrhosis is illustrated in figure 1. The overall survival probability was 92% at 1 year, 79% at 3 years and 71% at 5 years (95% confidence interval (CI) 86-97%, 71-87% and 62-80% respectively).

Univariate analysis revealed eight variables that were significantly ($P < 0.05$) associated with the duration of survival (table 1). The survival was significantly reduced for patients with higher age, impaired coagulation status, esophageal varices, ascites, encephalopathy, high levels of serum AST and bilirubin, and low levels of serum albumin. AST-levels that exceeded twice the upper limit of normal (30 U/l) were associated with a more than twofold increase in death risk.

Thirteen patients were found to be positive for hepatitis delta antibodies (1 died), 2 for hepatitis C virus antibodies (0 died) and 2 for HIV antibodies (0 died); 6 patients underwent a liver transplantation (3 died). Since the transplantations and part of the testing for these co-infections were performed during follow-up we separately analyzed these factors; no significant effect on survival was found.

At entry into the study, 54 patients were seropositive for HBeAg. The estimated five-year survival rate was 59% for HBeAg-positive patients and 83% for HBeAg-negative patients. HBeAg status just failed to be a significant factor that influenced survival for the whole patient population ($P = 0.08$). After a mean interval of 16 months, 27 of the HBeAg-positive patients underwent HBeAg seroconversion; in 25 of them antibodies to HBeAg subsequently developed. The calculated annual HBeAg seroconversion rate was 27%. Almost half of all

Table 1. Initial patient characteristics and results of univariate analysis (n=98).

		n	5-year survival	p-value
Sex	Male	89	69 %	0.4
	Female	9	89 %	
Age*	<36 years	20	100 %	<0.001
	36-50 years	42	82 %	
	51-60 years	24	46 %	
	>60 years	12	41 %	
Symptoms	Absent	31	84 %	0.07
	Present	67	65 %	
Alcohol	≤40 g/day	86	69 %	0.4
	>40 g/day	12	92 %	
HBeAg	Negative	44	84 %	0.08
	Positive	54	60 %	
AST*	≤30 IU/l	22	81 %	0.01
	31-60 IU/l	38	76 %	
	>60 IU/l	38	48 %	
Bilirubin*	≤12 μmol/l	53	91 %	<0.001
	13-34 μmol/l	35	56 %	
	>34 μmol/l	10	0 %	
Albumin	≤34 g/l	22	32 %	0.003
	>34 g/l	76	83 %	
Coagulation factors	≤34 %	16	48 %	0.02
	>34 %	82	75 %	
Ascites	Absent	85	83 %	0.004
	Present	13	23 %	
Varices	Absent	59	86 %	0.002
	Present	39	48 %	
Encephalopathy	Absent	94	73 %	0.005
	Present	4	0 %	

*: tested for trend

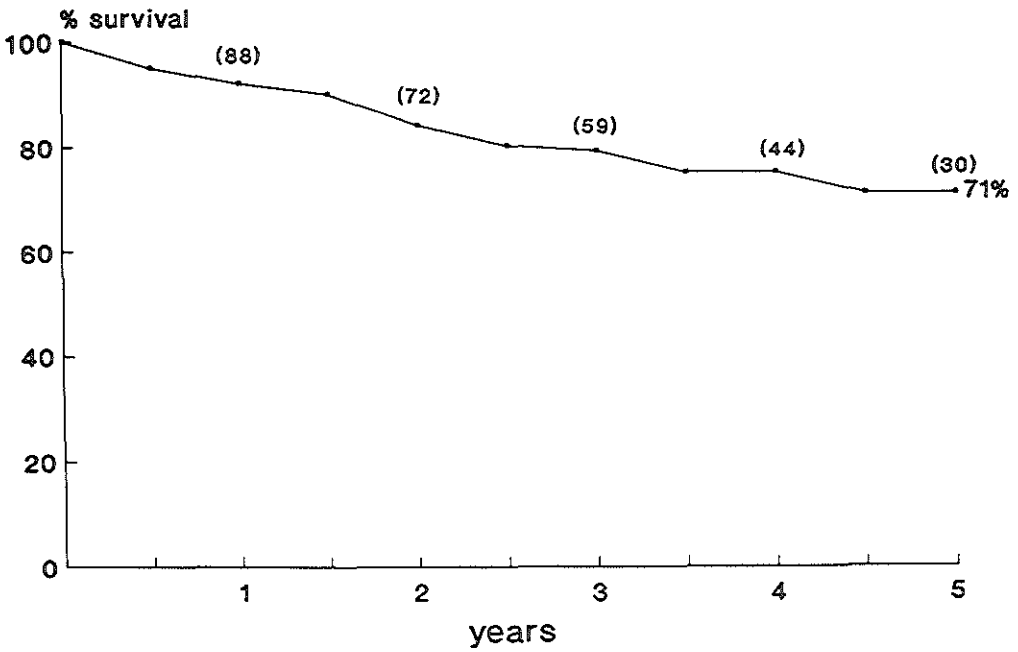

Figure 1. Survival of 98 patients with HBsAg-positive cirrhosis. Curve obtained by 6-month interpolation of the Kaplan-Meier estimates. Numbers along the curve denote the number of patients at risk.

HBeAg seroconversions occurred after treatment with α-interferon. No difference in survival was found for patients with a spontaneous or therapy induced HBeAg seroconversion.

After multivariate analysis, ascites, serum bilirubin level and age remained as factors that were independently related to survival. The influence of each of these three variables is illustrated in figures 2a-c. Patients with ascites had a relative death rate (RDR) of 6.0 relative to patients without ascites (95% CI 1.8-19.5); patients with a higher bilirubin level had an RDR of 1.6 relative to those with a 50% lower level (95% CI 1.1-2.3); and older patients had an RDR of 2.4 relative to those who were 10 years younger (95% CI 1.7-3.3). All factors of less prognostic importance, according to the multivariate analysis, correlated with at least two of the three independent indicators of survival.

After the analysis of the total group, we investigated survival for patients with compensated and decompensated liver disease (figure 3). Decompensation was defined as the presence of ascites and/or jaundice (serum bilirubin of more than 34 μmol/l) and/or encephalopathy

and/or a history of variceal bleeding, at entry into the study. Decompensated liver disease was diagnosed in 21 patients. Fourteen of these patients died within three years, the other seven patients were still alive after 0.8 to 5.9 years of follow-up. The survival rate for patients with decompensated cirrhosis was 70% at 1 year, 35% at 3 years and 14% at 5 years (95% CI 48-93%, 12-58% and 0-31%, respectively). For the 77 patients with compensated cirrhosis the survival rate was still 84% at 5 years (95% CI 76-92%).

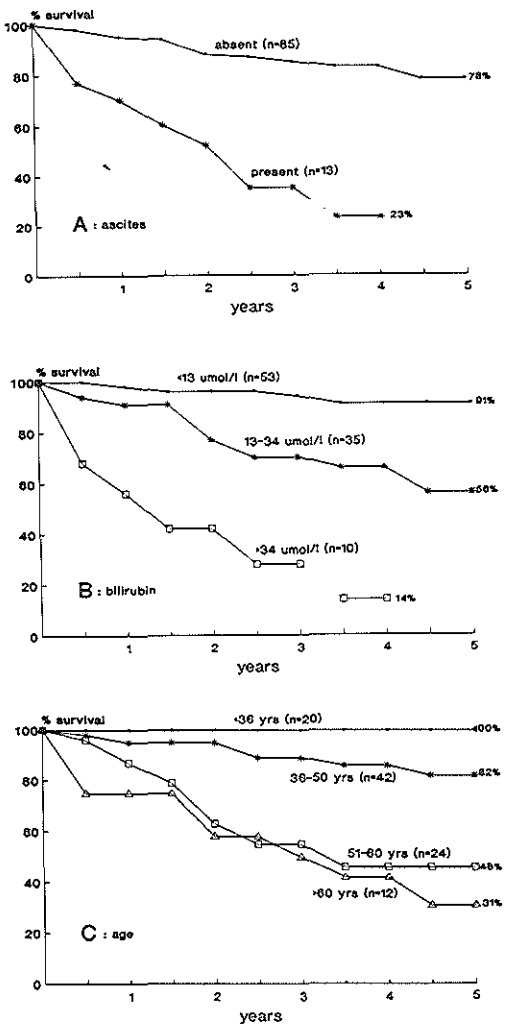

Figure 2. Survival of patients with HBsAg-positive cirrhosis in relation to the presence of ascites (A), the serum bilirubin level (B) and age (C).

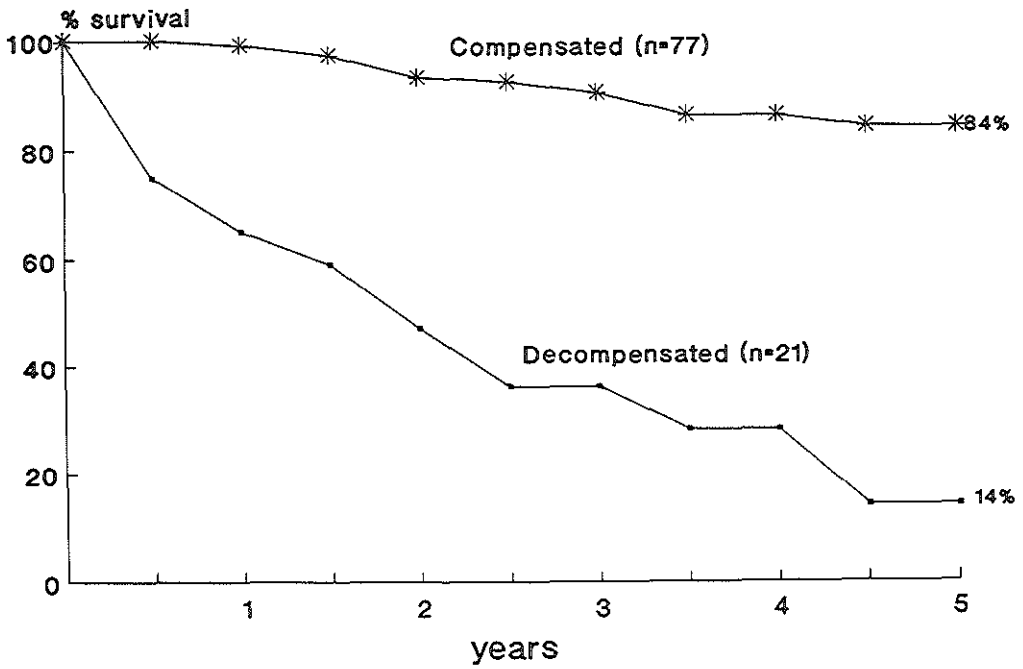

Figure 3. Survival of HBsAg-positive patients with compensated or decompensated cirrhosis.

For the group of patients with compensated cirrhosis, age and HBeAg status at entry were the only factors that significantly influenced the survival rate. The five-year survival probability was 72% for HBeAg-positive patients compared to 97% for HBeAg-negative patients (P=0.03; 95% CI 55-87% and 92-100%, respectively) (figure 4a). After adjustment for age the difference in survival between compensated patients with and without detectable HBeAg at presentation remained significant (P=0.04). The estimated actuarial HBeAg seroconversion rate at 1 and 5 years was 22% and 63%, respectively. For patients who showed clearance of HBeAg from serum, there was a 2.2 fold decrease in death rate (RDR 0.45; 95% CI 0.1-2.1) compared to patients who remained HBeAg positive. The age-adjusted RDR after HBeAg seroconversion was 0.57 (95% CI 0.1-2.6). No difference in survival was found between patients with different HBeAg status in the decompensated group (figure 4b).

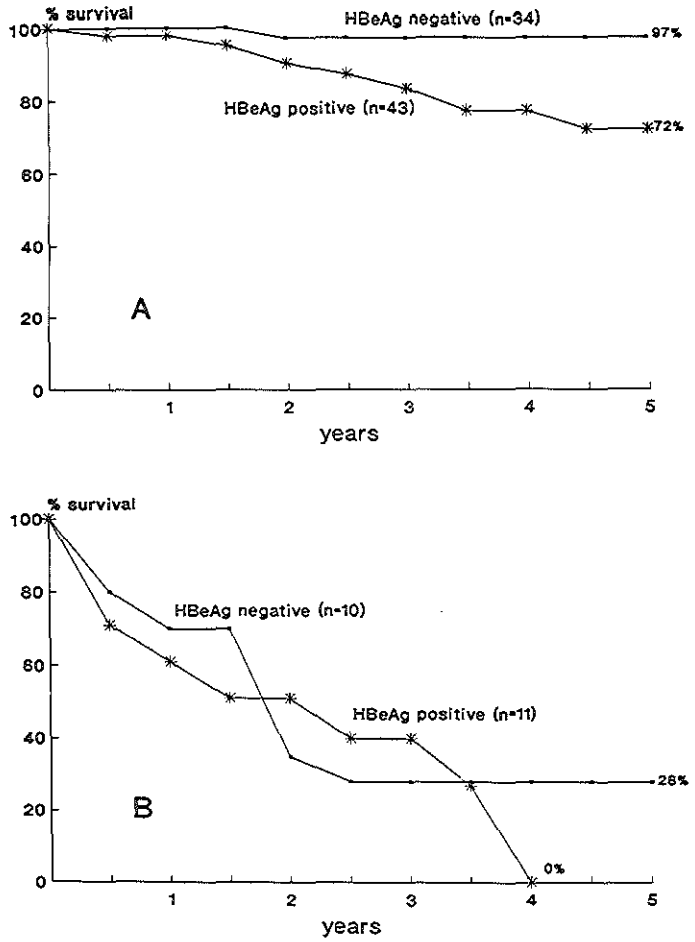

Figure 4. Survival of HBsAg-positive patients with compensated (A) and decompensated (B) cirrhosis in relation to the HBeAg-status at entry.

Discussion

The results of this study show an estimated 71% five-year survival rate after histological diagnosis of HBsAg-positive liver cirrhosis in a predominantly white West-European population. Hepatocellular carcinoma and liver failure with or without variceal bleeding were the main causes of death. Comparable results were obtained in prognostic studies on HBsAg-positive cirrhosis in other geographical areas of the world with estimated five-year survival rates of 55% in the USA, 80% in Taiwan, 66% in Japan and 52% in Italy (13-16). It is

conceivable that both our own survival rate and those of others are somewhat pessimistic since most study centers function as regional hospitals where many tertiairy referrals are treated.

In the present study, all investigated indicators of impaired liver function or portal hypertension - bilirubin, albumin, blood coagulation status, the presence of ascites or encephalopathy and evidence of esophageal varices - were strongly associated with survival, according to the univariate analyses. The incidence of both liver transplantation and concomitant viral infections (hepatits C, delta, HIV) was low and did not influence the outcome of survival in this study. After multivariate analysis age, ascites and total serum bilirubin remained as the most powerful prognostic indicators. A previous study of prognostic factors of HBsAg-positive cirrhosis selected age above 40 years, serum bilirubin above 1.5 mg/dl (25 mmol/l), ascites and spider nevi as independent indicators of mortality (14). Other studies on the prognosis for patients with cirrhosis (viral, alcoholic or cryptogenic) also indicate that the strongest indicators of survival are parameters that relate to the synthetic function of the liver and the presence of portal hypertension (26-29). We separately analyzed the survival rate for patients with compensated and decompensated cirrhosis, since the findings for the whole group may have been influenced by the selected inclusion of patients with an extremely poor prognosis who were referred to our department for liver transplantation or treatment of variceal bleeding. To define decompensation of liver disease we selected four generally accepted criteria known to influence the survival of patients with liver disease (29). For patients with decompensated cirrhosis the five-year survival rate was only 14%. In contrast, patients with compensated cirrhosis exhibited a very good prognosis with a five-year survival rate of 84%. Interestingly, in the compensated group none of the patients (n=30) for whom follow-up was continued beyond the 5th year died in the 5 years thereafter (data not shown).

Active HBV replication is associated with ongoing inflammatory activity and progression of liver disease. HBeAg seroconversion, indicating a transition to viral latency, is usually accompanied by biochemical and histological regression of liver disease activity (30-32). With the availability of antiviral agents, such as α-interferon, cessation of the viral replicating phase can be induced in about one-third of the patients (33,34). However, it is not yet known whether HBeAg seroconversion leads to improved survival for cirrhotic patients (30,35). Some authors state that the severity of the underlying liver histology at the time active viral replication ceases is critical for the final outcome (35). In the present study, in which all patients had similar histopathological diagnoses, patients without detectable HBeAg in the serum had a more favorable prognosis than those with HBeAg. This phenomenon was even more prominent among patients with compensated liver disease for whom there was a

significantly improved life expectancy for HBeAg-negative patients and a strong trend towards better survival after HBeAg seroconversion during follow-up. This significance of the HBeAg status did not disappear after adjustment for age, the only other factor that was indepently related to the survival of patients with compensated cirrhosis. For patients with decompensated cirrhosis, the HBeAg status did not influence the survival rate and it thus seems likely that there is a time point in the course of the disease when HBeAg seroconversion will no longer lead to an improved prognosis.

These results imply that there is a strong indication for antiviral therapy in patients with HBeAg-positive compensated liver cirrhosis, whereas liver transplantation will rarely be indicated in view of the very good prognosis for this group. However, if hepatic decompensation occurs, liver transplantation appears to be the major therapeutic option to reduce the risk of death. The exact timing of surgery as well as measures to suppress viral replication to reduce the risk of reinfection of the graft remain to be determined.

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CHAPTER 3

ANTIVIRAL EFFECT OF PROLONGED INTERMITTENT LYMPHOBLASTOID ALPHA-INTERFERON TREATMENT IN CHRONIC HEPATITIS B.

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Abstract

In a European multicenter study 40 patients with HBeAg-positive chronic hepatitis B virus (HBV) infection were treated with 5 megaunits of lymphoblastoid α-interferon daily according to the following regimen: a 4-week primer course, 4 weeks of rest and a second course lasting 16 to 30 weeks. After 52 weeks of follow-up, a response (HBeAg seroconversion and HBV-DNA negativity) was observed in 22 patients (55%). HBsAg seroconversion occurred in 5 patients (12.5%). One patient exhibited a relapse for serum HBeAg and HBV-DNA after cessation of treatment. According to a response prediction model, the observed response rate was not related to the selection of patients likely to respond.

The initial interferon course induced a reduction of the serum HBV-DNA and HBeAg levels of 87% and 18%, respectively, leading to a significantly lower level of viral replication activity at the start of the second longterm course compared to baseline.

After 24 weeks of follow-up (week 16 of the second course), 19 (48%) patients exhibited a response, 13 (32%) a partial response (HBeAg <50% of initial level or HBV-DNA negative) and 8 (20%) no response. For 8 of the 13 partial responders treatment was stopped at week 24 and viral replication rebounded to pretreatment values. In the last 5 partial responders prolongation of therapy up to week 38 led to a definite response and HBsAg seroconversion in 3 of the 5 patients.

The results of this study suggest that both a short primer course and prolongation of therapy may help to enhance the response rate of α-interferon therapy for chronic hepatitis type B.

Introduction

In patients with chronic hepatitis B virus (HBV) infection active viral replication is usually associated with continuing inflammatory activity and progression of liver disease. Transition to viral latency, as indicated by hepatitis B e antigen (HBeAg) seroconversion and clearance of serum HBV-DNA, is accompanied by biochemical and histological regression of liver disease activity (1-3). With the availability of α-interferon (IFN), termination of the viral replicating phase can be induced in about 30 to 40% of the patients (4-6). Usually α-IFN is given in a single continuous course.

From a pilot study comparing the antiviral effect of α-IFN, acyclovir and a combination of α-IFN and acyclovir, it was concluded that combination therapy appears to be a highly promising treatment for chronic hepatitis B (7). Since more recent controlled studies have shown that acyclovir does not enhance the therapeutic effect of α-IFN alone (8), the results of the pilot study could also be interpreted as indicating that a second course of α-IFN following short-term primer α-IFN therapy is more effective than a single course. Therefore we investigated the effect of intermittent α-IFN treatment on termination of hepatitis B viral replication, measured by HBeAg seroconversion and HBV-DNA clearance from serum.

Methods

Patients and Treatment

Forty-four patients from 6 European hospitals were enrolled in the study after central evaluation of their eligibility. At entry all patients had been seropositive for hepatitis B surface antigen (HBsAg) and HBeAg for at least 6 months. Histological evidence of chronic hepatitis was present in all patients who underwent a liver biopsy (n=43). Patients were excluded on grounds of the following criteria: age under 18 or over 70 years; presence of hepatitis delta antibodies in serum; antiviral or immune modulatory therapies in the preceding 6 months; history or presence of decompensated liver disease (ascites, encephalopathy, variceal hemorrhage); pregnancy; impaired immunity, including seropositivity for human immunodeficiency virus with T4 cells $< 400/mm^3$; inadequate levels of platelets ($< 70 \times 10^9/l$) or white blood cells ($< 3 \times 10^9/l$); recent drug or alcohol abuse; presence of significant other disease that might interfere with the study. The study was approved by the ethical committees of all participating centers and written informed consent was obtained from each patient who entered the study.

Lymphoblastoid α-IFN (Wellferon, Wellcome, Beckenham, U.K.) was given at a dose of

5 megaunits daily according to the following regimen: a 4-week primer course, a 4-week rest period and a second α-IFN course lasting 16 weeks; for 5 patients from Rotterdam the duration of the second course was prolonged up to 30 weeks. Patients were taught to self-administer α-IFN subcutaneously. During the first 4 days of α-IFN therapy indomethacin or paracetamol was given to suppress early side-effects. α-IFN treatment was discontinued in the event of absence of HBeAg on two successive occasions or intolerable side-effects. Follow-up began at the start of therapy and was continued for 52 weeks.

Clinical and Laboratory Evaluation

Patients were seen, and if indicated examined, at the outpatient clinic every 2 to 4 weeks during treatment and every 4 to 8 weeks during the period thereafter. Laboratory assessment was performed every 2 weeks during the treatment period and every 4 to 6 weeks after discontinuation of therapy. On these occasions routine hematological studies were performed and serum markers of viral replication (HBeAg and HBV-DNA) and aspartate aminotransferase (AST) activity were measured. Every 3 months additional biochemical and virological measurements including the prothrombin time and the levels of alanine aminotransferase, albumin, bilirubin, HBsAg, and antibodies against HBsAg as well as HBeAg were performed. Liver biopsies were taken within 6 months of entry to the study for histological assessment.

All virological parameters were determined centrally at the Rotterdam hepatitis laboratory. HBsAg was assessed using a commercial radioimmunoassay kit (Abbott, Ill., USA). HBeAg was measured quantitatively using a radioimmunoassay (Abbott, Ill., USA). For quantification a P/N ratio (counts of patient sample/counts of negative control sample) was determined for each patient in a fixed serum dilution. HBeAg seroconversion was defined as a P/N ratio under 2.1 for undiluted serum from 2 consecutive blood samples. HBV-DNA was measured by a liquid hybridization assay using an iodine-125 probe (Abbott, Ill., USA). Antibodies to hepatitis C virus were determined, retrospectively, by enzyme immunoassay (Abbott, Ill., USA); positive results were confirmed by a recombinant immunoblot assay (Ortho Diagnostics Systems, USA). Routine serobiochemical tests were performed using automated techniques (Coulter, Technicon, NY, USA).

The criteria for response to treatment were: HBeAg seroconversion and serum HBV-DNA negativity. The criteria for partial response to treatment were: a decrease in serum HBeAg of 50% or more of the initial level or serum HBV-DNA negativity with sustained HBeAg positivity after 24 weeks of follow-up.

To analyze whether the response rate was related to treatment modification or selection of patients, the actual response was compared with a predicted response that was obtained

with a response model developed by Brook et al. (9). The prediction of response was based on pretreatment AST levels, the presence of a history of acute hepatitis and HIV-antibody status.

Statistics

Differences in dichotomous and other discrete variables were analyzed by the Fisher exact and Chi-square tests, respectively. For continuous variables medians were used because the results lacked normal distribution. The two-sample Wilcoxon rank sum test was used to analyze unpaired observations and the Wilcoxon signed rank test to analyze paired observations.

Results

Four of the 44 patients were withdrawn from the study. Two patients were found to be HBeAg negative at the start or within one week of treatment while pre-entry assessments were positive. Two patients were lost to follow-up: one withdrew from treatment after 2 weeks for reasons unrelated to the study protocol and one patient failed to comply with the protocol after cessation of therapy.

Patient characteristics at entry are shown in table 1. Of the 40 patients who were analyzed, one was serum HBV-DNA negative on entry to the study.

Response

A response to treatment (HBeAg seroconversion and serum HBV-DNA negativity) was observed in 22 of the 40 patients (55%; 95% confidence interval (CI) 40-70%). Five patients (12.5%; 95% CI 4-27%) became negative for HBsAg. Sustained AST normalization occurred in 16 out of 19 responders with elevated AST levels on entry to the study. Figure 1 shows the timing of elimination of viral parameters and sustained AST normalization in the response group. All but one patient exhibited the response while on α-IFN therapy. A characteristic sequence in clearance of HBV-DNA and HBeAg and then normalization of AST levels was observed for the majority of the responders. One responder showed a relapse for serum HBeAg, HBV-DNA and AST 20 weeks after cessation of therapy. Of the 18 nonresponders, 5 exhibited normalization of AST levels and 6 became - transiently - serum HBV-DNA negative.

Differences in characteristics between responders and nonresponders are shown in table 1. The pretreatment serum HBV-DNA level was lower and the AST level higher in the response group. Also the duration of HBsAg positivity was shorter and the presence of

Table 1. Patient characteristics at entry into the study.

		Total Group n=40	Response n=22	Nonresponse n=18
Age* (yr)		38.5 (18-67)	42 (27-67)	37 (18-56)
Male/Female		32/8	17/5	15/3
Homo/Heterosexual		9/31	3/19	6/12
HBsAg duration* (mo)		27 (6-132)	14 (6-132)	33 (12-96)
History acute hepatitis		2	1	1
Histology:	CPH	8	2	6
	CAH	20	12	8
	cirrhosis	11	8	3
Anti-HCV positive		1	1	0
HIV-status:	negative	38	21	17
	unknown	2	1	1
HBeAg* (P/N ratio)		12.4 (2.2-18.8)	13.4 (2.5-18.8)	11.9 (2.2-16.4)
HBV-DNA* (pg/ml)		102 (1-1001)	79 (1-730)	127.5 (3-1001)
HBV-DNA	< 50 pg/ml	11	8	3
	51-100	9	6	3
	101-300	13	5	8
	> 300	7	3	4
AST* (U/l)		57.5 (15-475)	66.5 (15-475)	51.5 (16-113)
AST	< 30 U/l	7	3	4
	31-60	14	7	7
	61-100	11	5	6
	> 100	8	7	1

* median (range)

AST normal < 30 U/l; HBV-DNA cut-off: 1.5 pg/ml; HBeAg cut-off: P/N ratio 2.1

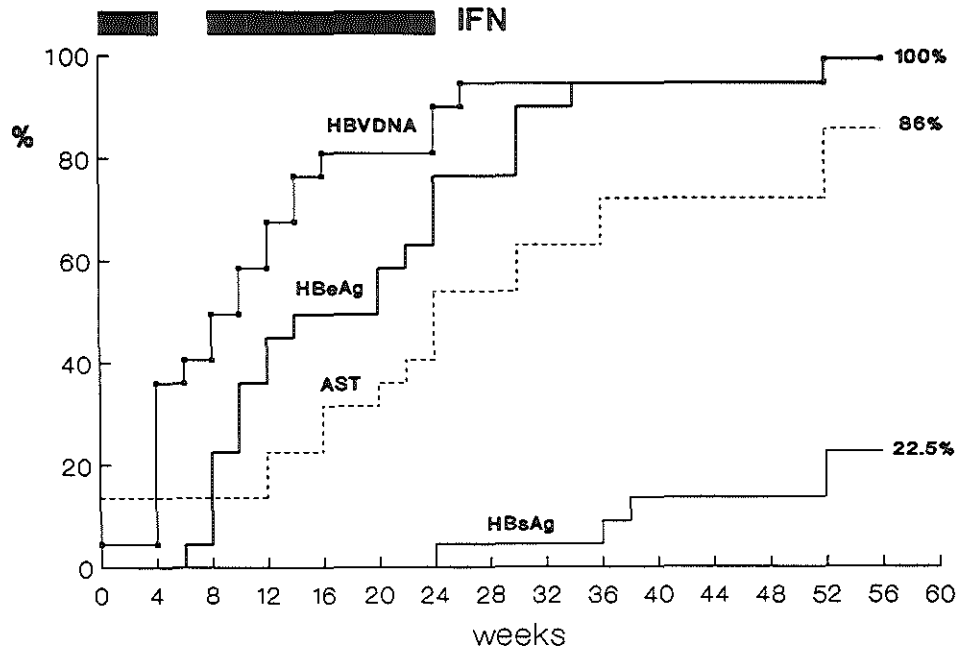

Figure 1. Cumulative percentage of clearance of serum HBV-DNA, HBeAg seroconversion, normalization of serum AST and HBsAg seroconversion for patients who responded to therapy (n=22).

cirrhosis was more prominent in responders. However, no statistical difference between responders and nonresponders was found for any of these parameters.

According to the Brook model, a response was predicted for 14 of the 40 patients (35%; 95% CI 20-50%), with a positive predictive value of 79% and a negative predictive value of 58%. Compared to this prediction the actual response rate of 55% was significantly higher (P = 0.03).

Intermittent Treatment

Changes in serum AST, HBV-DNA and HBeAg values are shown in figure 2. The primer α-IFN course reduced HBV-DNA and HBeAg levels by 87 and 18 percent, respectively, but the decrease did not continue during the 4 weeks without therapy. Nevertheless, at the start of the second α-IFN course, the serum HBeAg and HBV-DNA levels were significantly lower compared to baseline values. No difference was found in the AST levels at the start of the two courses; however both courses seemed to induce a peak of AST values that often preceeds a response to therapy.

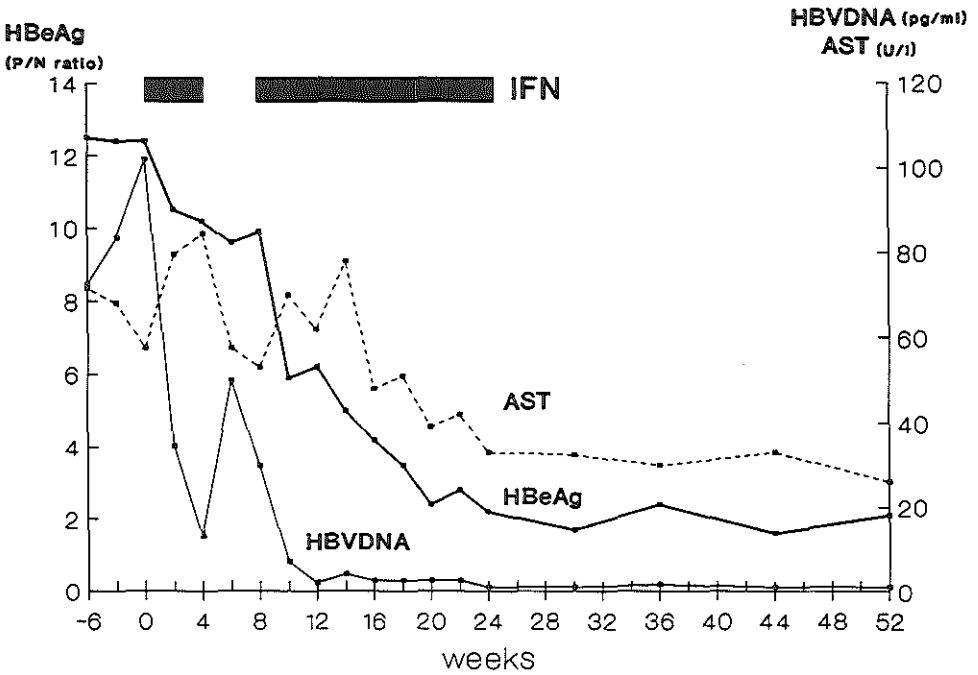

Figure 2. Median levels of HBeAg, HBV-DNA and AST in serum (n=40).

Prolonged Treatment

After 24 weeks of follow-up (week 16 of the second course), 19 (48%; 95% CI 32-63%) patients exhibited a response, 13 (32%; 95% CI 18-47%) a partial response and 8 (20%; 95% CI 8-32%) a definite nonresponse. For 8 of the 13 partial responders, treatment was stopped at 24 weeks. After cessation of therapy the serum HBeAg and HBV-DNA levels in these patients rebounded to baseline levels (figure 3). For the last 5 partial responders α-IFN therapy was prolonged up to week 38. Three of these 5 exhibited an additional response during prolonged therapy (figure 3), and all 3 showed HBsAg seroconversion shortly after the response.

Side Effects of Interferon

During the first week of the initial α-IFN course, a transient flu-like syndrome with fever, chills and myalgia was observed in nearly all of the patients. At the beginning of the second α-IFN course, the majority again reported these symptoms but to a lesser intensity. The predominant adverse effects after the first days of therapy were fatigue (73%),

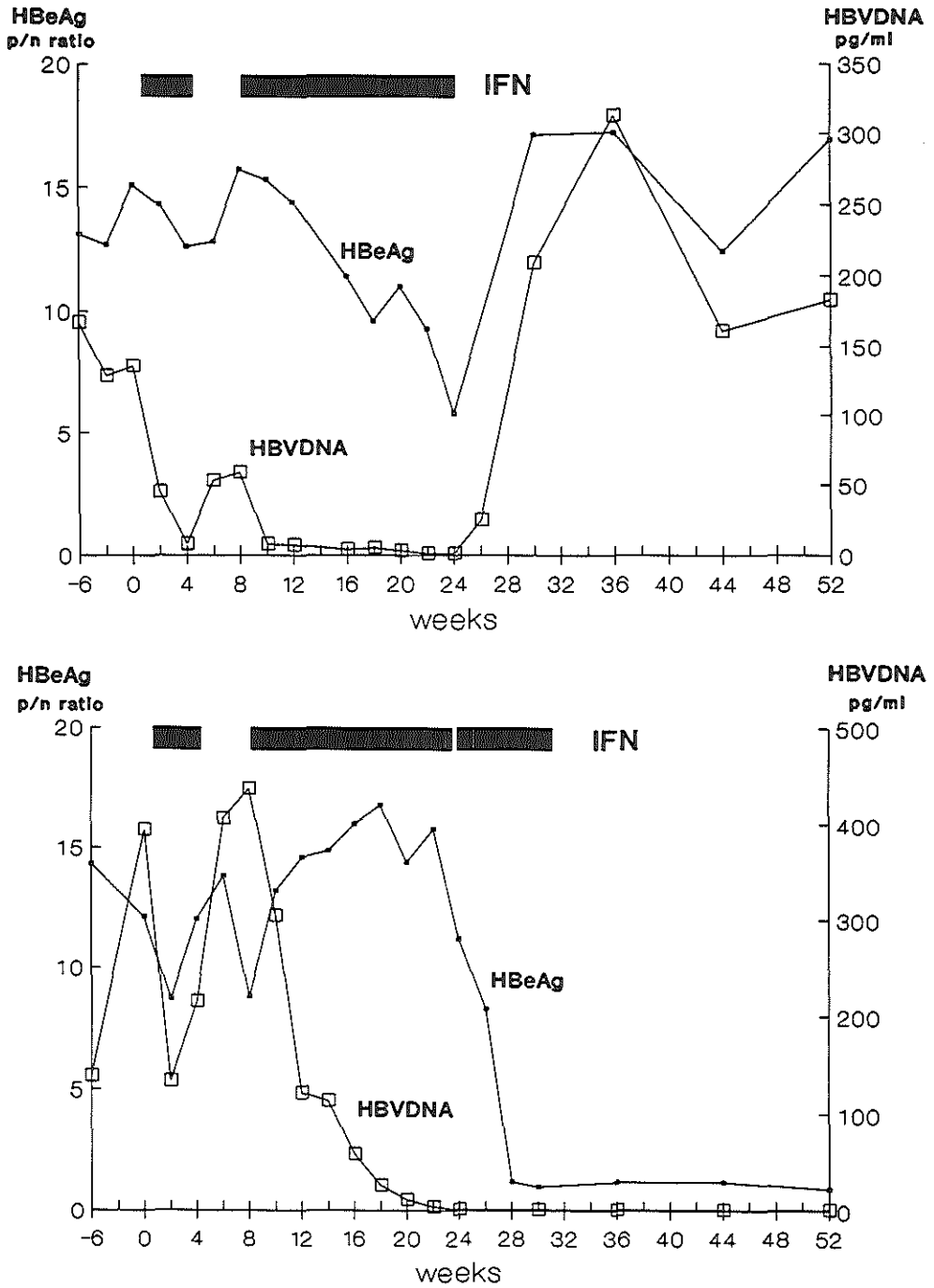

Figure 3. Serum HBeAg and HBV-DNA levels in a partial responder treated until 24 weeks (protocol therapy) and in a partial responder treated until 32 weeks (prolonged therapy) who exhibited an additional response.

myalgia (60%), anorexia (43%), irritability (30%) and hair loss (15%). Two patients developed an acute psychosis: the first, who had just finished therapy, required intensive psychiatric care for 2 weeks; the second, who had been treated with α-IFN for 3 weeks, fell and fractured the acetabulum. Another patient had a generalized seizure after 4 and 11 weeks; he was put on anticonvulsive medication and α-IFN was tapered to 1.25 megaunits daily. Side effects led to dose reduction in 11 subjects (28%). The reasons for dose reduction were fatigue in 6 cases, thrombocytopenia in 2, neurotoxicity in 2 and leukopenia in 1. Four (36%) out of these 11 patients exhibited a response.

Discussion

In recent years several strategies have been tested to enhance the efficacy of α-IFN therapy for chronic hepatitis B. None of these strategies - including additional therapy with agents such as gamma-interferon, prednisone, acyclovir or adeninide arabinoside (5,8,10-12) - has proved to be more beneficial than a standard α-IFN course of 12-16 weeks.

In this uncontrolled pilot study we investigated the effect of prolonged intermittent α-IFN therapy in 40 patients. Fifty-five percent of the patients responded with HBeAg seroconversion and loss of serum HBV-DNA. HBsAg seroconversion occurred in 12.5% of all treated patients and in almost 25% of those who responded. A majority of the responders demonstrated a characteristic sequence in loss of serum HBV-DNA and HBeAg followed by normalization of the AST value. HBeAg seroconversion was often heralded by a rise in transaminase activity and loss of HBV-DNA. Still, in 6 patients HBV-DNA disappeared from the serum but quickly reappeared after discontinuation of therapy, indicating that persistent absence of HBeAg is the best indicator for termination of the viral replicating phase.

Although several differences in pretreatment characteristics of responders versus nonresponders were observed, none of them was found to be significant. The predicted response rate, based on a combination of pretreatment factors (9), was significantly lower (35%) than the actual response rate (55%). This result suggests that the high response rate was not caused by selection of patients likely to respond but by the treatment modification.

It is difficult to determine the contribution of intermittent therapy to the high response rate obtained in this uncontrolled study. The rationale for the initial 4-week course was to decrease viral replication and increase inflammatory activity before the start of the longterm course, thereby enhancing the possibilty of response to therapy. At the start of

the second α-IFN course, viral replication, as monitored by serum HBV-DNA and HBeAg levels, was indeed significantly lower compared to baseline values. In addition, both α-IFN courses induced transient elevation of aminotransferase levels. Therefore both courses could have induced a response in some patients and the double transaminase peak could thus be indicative of early and late responders. These interesting observations certainly justify further evaluation of intermittent treatment in a controlled setting.

Close on-line monitoring of the effects of α-IFN on viral replication revealed that in several patients HBeAg and HBV-DNA serum values decreased continuously and were almost negative when treatment was stopped (week 24) at which point a relapse occurred. Therefore, we decided to offer prolonged treatment to the remaining patients who approximated a response at the end of the scheduled therapy (partial response). For 3 of the 5 partial responders this approach led to a definite response and subsequently to HBsAg seroconversion. Of the 2 patients who did not respond to prolonged treatment one had extremely high initial serum HBV-DNA levels (>1000 pg/ml), while both patients had near normal AST levels at entry. These features - low serum aminotransferase and high HBV-DNA levels - have been suggested to interfere with the response to α-IFN treatment (4,5,9,13). Our findings indicate that prolonging α-IFN therapy may increase the HBeAg and HBsAg seroconversion rates in the subset of patients that partially respond to standard interferon treatment. To select patients eligible for treatment prolongation, close monitoring of quantified levels of HBeAg and HBV-DNA is essential.

From the literature it is not clear whether prolongation of therapy beyond 4 months yields a higher response rate. Prolonged therapy can further decrease HBV replication which may trigger the cellular immune response leading to the hepatocytotoxic reaction that eradicates viral replication (14). In most parts of the world the standard duration of therapy is considered to be 12-16 weeks, usually leading to a response rate of 30-40% (4-6,15). α-IFN has been given for 24 weeks or longer in a few - mainly Mediterranean - studies (response rates 26% to 70%) (16-18). A controlled trial comparing 12 to 24 weeks of treatment failed to demonstrate any beneficial effect of the prolonged therapy (19). However, the outcome of that study was markedly influenced by a lack of compliance (8 of the 20 patients withdrew from the longer course). In the present study none of the patients had to withdraw from therapy because of side effects, irrespective of the length of treatment. Both the intermittent treatment schedule and intensive patient monitoring with mental support may have contributed to the good compliance. Nevertheless, 3 of our patients had major neuro-psychiatric side effects (psychosis, seizures). Future studies might elucidate whether these serious side effects relate to dose and/or duration of the α-IFN treatment.

In summary, prolonged intermittent α-IFN therapy resulted in HBeAg seroconversion and serum HBV-DNA negativity in 55% and in HBsAg seroconversion in 12.5% of the patients. The high rate of induced viral latency was probably related firstly to the short initial α-IFN course that reduced viral replication significantly before the start of the second longterm course and secondly to prolongation of therapy in partial responders which induced additional HBeAg seroconversion.

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CHAPTER 4

ALPHA-INTERFERON AND ZIDOVUDINE COMBINATION THERAPY FOR CHRONIC HEPATITIS TYPE B. RESULTS OF A RANDOMIZED PLACEBO-CONTROLLED TRIAL.

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Abstract

Alpha-interferon (α-IFN) therapy leads to HBeAg seroconversion in only one-third of the patients with chronic hepatitis B. In an attempt to increase the seroconversion rate we investigated the combination of α-IFN and zidovudine in a subset of patients with a presumably low response rate for α-IFN monotherapy. In a double-blind controlled trial 24 HBeAg-positive patients were randomized to receive lymphoblastoid α-IFN in doses increasing to 5 MU s.c. daily, combined with zidovudine given orally in doses increasing from 500 to 1000 mg or placebo daily for 16 weeks. Treatment effects were monitored by quantitative assessment of HBV-DNA, HBeAg and HBV-DNA polymerase. Six months after termination of therapy 1/12 (8%; 95% CI 2-39%) patients treated with α-IFN plus zidovudine and 2/12 (17%; 95% CI 2-48%) patients from the control group exhibited a response (HBeAg seroconversion). All patients remained HBsAg positive. The only responder of the α-IFN-zidovudine group relapsed after cessation of therapy so that none of the zidovudine-treated patients were HBeAg negative at the end of follow-up. No significant difference in AST or any of the virological markers was observed between the 2 groups during the entire course of the study. Adverse effects (anemia, leukopenia) necessitated a reduction in the dose of zidovudine in 50% and α-IFN in 42% of the patients treated with α-IFN plus zidovudine; in the control group these rates were 0% for placebo and 8% for α-IFN. In conclusion, the antiviral effect of α-IFN in chronic hepatitis B was not enhanced by additional zidovudine treatment. The combination therapy induced considerable side effects leading to dose reduction for both zidovudine and α-IFN. For combination therapy with α-IFN, oral nucleoside analogues with a more potent antiviral effect and less toxicity than zidovudine should be developed.

Introduction

Alpha-interferon (α-IFN) is currently the most effective therapy for chronic hepatitis B virus (HBV) infection. Nevertheless only one-third of the patients respond to this treatment with virological and biochemical remission of the disease (1-3). One of the ways to enhance the response rate for α-IFN is combination therapy with nucleoside analogues. Zidovudine (3'-azido-3'-deoxythymidine, AZT) has proven to be a potent inhibitor of the reverse transcriptase activity of the human immunodeficiency virus (HIV) (4,5). Since reverse transcriptase activity also plays an important role in the HBV replication cycle, zidovudine would appear to be a promising candidate for combination therapy with α-IFN which attacks the virus via other steps in the HBV replication cycle and via the cellular immune system.

After both in vitro and in vivo studies demonstrated that zidovudine caused a marked decrease in HBV replication, as monitored by HBV-DNA polymerase (6), we initiated a randomized controlled trial to compare the antiviral effect of α-IFN alone with that of the combination of α-IFN and zidovudine.

Patients and Methods

Twenty-four patients were randomized to receive α-IFN therapy combined with either zidovudine or placebo. The inclusion criteria were serum hepatitis B surface antigen (HBsAg) positivity for at least 12 months, histological evidence of chronic hepatitis and the presence of serum hepatitis B e antigen (HBeAg) for at least 6 months. Since the toxicity profile of zidovudine was not completely known at the time of the design of the study we were only permitted to treat patients who exhibited at least one of the following characteristics: previous nonresponse to α-IFN, anti-HIV positivity and/or the presence of cirrhosis. Patients were excluded for the following reasons: age below 18 or above 65 years; presence of antibodies against the hepatitis delta virus in serum; low hemoglobin level ($<$ 7 mmol/l), thrombocyte count ($<$ 100 x 10^9/l) or leukocyte count ($<$ 3 x 10^9/l); anti-HIV seropositivity with a CD4 cell count below 400 per ml; decompensated liver disease (ascites, albumin $<$ 30 g/l, encephalopathy, history of variceal bleeding); pregnancy; recent drug or alcohol abuse; antiviral or immunosuppressive therapy in the 6 months prior to enrollment; presence of other significant diseases which might interfere with completion of the study.

Lymphoblastoid α-interferon (Wellferon, Wellcome, Beckenham, UK) was given subcutaneously to all patients according to an increasing dose schedule of 1.5 million units daily

for 4 weeks, 3 million units daily for 8 weeks and 5 million units daily for 4 weeks. Patients were carefully instructed to self-administer the α-IFN. Simultaneously with the 16-week α-IFN course, zidovudine or placebo (Wellcome, Beckenham, UK) was administered orally in a dose of 250 mg twice daily for the first 8 weeks and 500 mg twice daily for the last 8 weeks.

All patients were followed in the hepatology outpatient clinic of our hospital which has a tertiary referral function. Twenty-nine consecutive patients were eligible for the study; five were excluded during the pretreatment 6-week screening period: two decided not to participate, one HIV-infected patient exhibited a drop in the CD4 cell count under 400/ml, one patient cleared serum HBeAg and in another the HBeAg level dropped to borderline positivity.

For randomization a serial number that corresponded to a set of bottles containing either zidovudine or placebo capsules was randomly assigned to each consecutive patient who started therapy. Investigators and patients were unaware whether zidovudine or placebo was given. To exclude any indication of the dose of zidovudine, all patients were given 2 capsules twice daily during the entire treatment period. The randomization code was prepared by the Wellcome Foundation (Beckenham,UK), kept in a sealed envelope by the hospital pharmacist and opened after the last patient had completed therapy. All randomized patients gave written informed consent before participation. The study was approved by the hospital committee of medical ethics.

Patients were seen by a physician in the hospital at monthly intervals and followed for 6 months after cessation of therapy. Blood samples were obtained weekly during therapy and monthly in the period thereafter. HBeAg, HBV-DNA polymerase, aspartate aminotransferase (AST), hemoglobin and leukocytes were assessed in all samples. The HBV-DNA level was determined monthly. Additional routine hematological, biochemical and virological tests were performed at entry and at the termination of both therapy and follow-up. Patients were asked to undergo a liver biopsy within 6 months prior to the start of therapy and at the end of follow-up. The biopsies were examined under code by a single experienced pathologist who was unaware of the chronological order of the biopsies and the design of the trial. Histological scoring was based on the histological activity index, as described by Knodell (7).

The hematological, chemical and virological measurements were each performed in a single laboratory. HBsAg and antibodies against HBsAg, HBeAg, hepatitis C and hepatitis delta virus were assessed by enzyme immunoassays (Abbott, Ill, USA); positive results for hepatitis C antibodies were confirmed by a recombinant immunoblot assay (Ortho Diagnostic Systems, USA). HBeAg, HBV-DNA polymerase and HBV-DNA were

determined quantitatively. The HBeAg level was expressed as a P/N ratio (counts of patient sample/counts of negative control sample) using a radioimmunoassay kit (Abbott, Ill, USA). For each patient a fixed serum dilution was used throughout the study. HBeAg seroconversion was defined as a P/N ratio below 2.1 for undiluted serum from 2 consecutive blood samples. The HBV-DNA polymerase activity was measured as described by Fang, using a modification of the thymidine-methyl-5'-triphosphate (3HdTTP) elution technique (8). HBV-DNA was assessed by a solution hybridization assay which utilizes a ^{125}I labelled probe (cut-off 1.7 pg/ml; Abbott, Ill, USA). All hematological and biochemical markers were assayed by means of automated techniques (Technicon, NY, USA).
The criteria for response to therapy were HBeAg seroconversion with serum HBV-DNA and HBV-DNA polymerase negativity. All other patients were defined as nonresponders. Where data lacked a normal distribution, medians were used to denote continuous variables. The Wilcoxon rank sum and signed rank tests were used for comparison of unpaired and paired observations, respectively. Dichotomous variables were analyzed with the Fisher exact test. Differences with a P-value below 0.05 were considered to be significant (two sided testing).

Results

The patient characteristics for the two treatment groups were well matched at the start of therapy (table 1). All patients had detectable serum HBV-DNA and HBV-DNA polymerase levels, except for two (one in each treatment group) who were HBV-DNA negative on enrollment in the study; both patients were included in the study because they had stable, positive HBeAg levels. Co-infection with hepatitis C was not used as an exclusion criterion since the anti-HCV test was not available at the initiation of the study; retrospective screening revealed that one patient was anti-HCV positive.
All randomized patients completed the entire follow-up period of the study. A response was found for 1 (8%;95% confidence interval 2-39%) patient treated with α-IFN plus zidovudine and 2 (17%;95% confidence interval 2-48%) treated with α-IFN plus placebo. The zidovidine-treated patient exhibited a relapse for HBeAg, HBV-DNA and HBV-DNA polymerase after therapy was stopped. All patients remained seropositive for HBsAg. The timing of response and other virological events for the investigated groups is illustrated in figure 1. Clearance of serum HBV-DNA and HBV-DNA polymerase but continued presence of HBeAg was found for 2 and 4 patients, respectively, of the α-IFN-zidovudine group and 1 and 2 patients, respectively, of the control group. In most cases these markers reappeared after cessation of treatment.

Table 1. Patient characteristics at the start of therapy.

	α-interferon zidovudine (n=12)	α-interferon placebo (n=12)
Male/Female	1/1	11/1
Age* (yr)	5 (25-64)	34.5 (23-58)
Caucasian/Oriental	1/1	12/0
Hetero/Homosexual	0/2	6/6
Duration HBsAg positivity* (yr)	4.8 (1.3-9.9)	4.1 (1.0-8.0)
History acute hepatitis yes/no	1/11	1/11
Previous α-IFN therapy yes/no	9/3	7/5
Anti-HIV neg/pos/unknown	11/1/0	9/2/1
Anti-HCV neg/pos	11/1	12/0
HBV-DNA* (pg/ml)	108 (1-505)	85 (1-819)
HBeAg* (P/N ratio)	11.5 (4.4-18.4)	9.6 (3.4-17.8)
HBV-DNA polymerase* (P/N ratio)	15.5 (2-290)	18.5 (2-510)
AST* (U/l; normal<30)	44 (20-109)	46 (22-180)
AST elevated/normal	9/3	10/2
CPH/CAH/Cirrhosis	4/5/3	4/3/5

*: median (range)

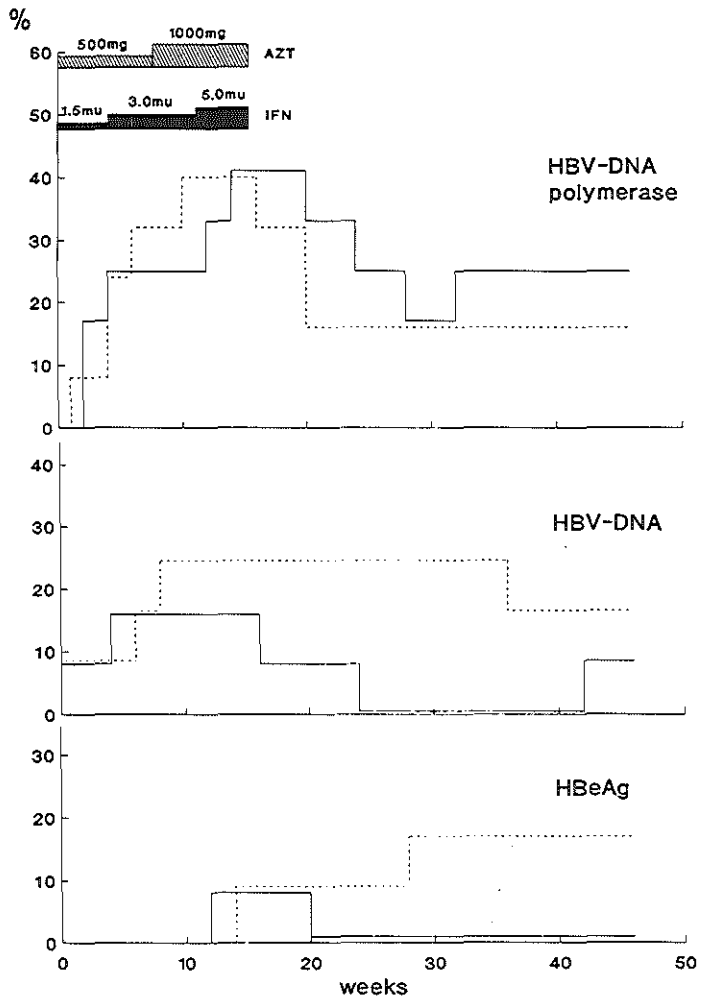

Figure 1. Percentage of patients treated with α-IFN in combination with zidovudine (——; n=12) or placebo (– – – ; n=12) who exhibited clearance and/or reactivation of HBeAg, HBV-DNA and HBV-DNA polymerase during follow-up.

During the treatment period the median decrease in HBV-DNA was 71% and 67%, HBeAg 29% and 20%, and HBV-DNA polymerase 62% and 71% for the α-IFN-zidovudine and α-IFN-placebo groups, respectively; these results indicate a significant decrease in these viral markers for both groups (figure 2). However, after therapy all 3 parameters rebounded to pretreatment levels among the majority of the patients, leaving no significant difference between the initial virological levels and those at the end of the observation period. Of the 19 patients with an elevated AST level at entry only the 2 permanent responders and 1 nonresponder treated with α-IFN and zidovudine exhibited sustained normalization of AST. In 2 patients from each treatment group - all nonresponders - the AST level rose to more than twice the initial value during therapy. Comparison of the two therapy groups did not show a significant difference in AST or any of the quantified virological markers during the entire course of the study (figure 2).

The results of histological scoring are shown in table 2. One patient from each therapy group refused to undergo the second biopsy. A decrease in the histology activity index was found for 3 (27%) patients treated with zidovudine and 4 (36%) treated with placebo, including the 2 persistent responders. No significant difference was found either within or between the two groups in initial and posttreatment histological scores.

Adverse effects of α-IFN and/or zidovudine therapy were observed in the majority of the patients (table 3). Despite the low initial dose of α-IFN most patients experienced flu-like disease with fever, fatigue and myalgia, primarily during the first 2 weeks of treatment. The predominant side effect later in the course of therapy was fatigue. Almost all adverse effects were more common among patients treated with α-IFN and zidovudine. In particular anemia and leukopenia were pronounced in this group; in 2 patients the anemia was symptomatic and necessitated blood transfusions (hemoglobin < 5.0 mmol/l). One (HIV-positive) patient from the α-IFN-placebo group experienced epileptic insults in the 10th and 16th weeks of therapy; after the first seizure α-IFN was continued because no clear relationship between therapy and insult was suspected (9). After the end of the observation period hepatocellular carcinoma was diagnosed in one patient while another died of a variceal hemorrhage. Both patients had received α-IFN plus zidovudine; however a direct relation between these serious events and the treatment regimen appears unlikely. For a total of 9 patients the dose of zidovudine and/or α-IFN had to be reduced (table 3): in 4 cases zidovudine was reduced, all because of anemia; in 3 α-IFN was reduced, all because of leukopenia; and in 2 both zidovudine and α-IFN were reduced because of leukopenia with anemia or thrombocytopenia. The time of dose reduction ranged from 8 to 14 (median 12) weeks of therapy for zidovudine and from 8 to 13 weeks (median 12) for α-interferon; after the dose reduction zidovudine was discontinued in 4

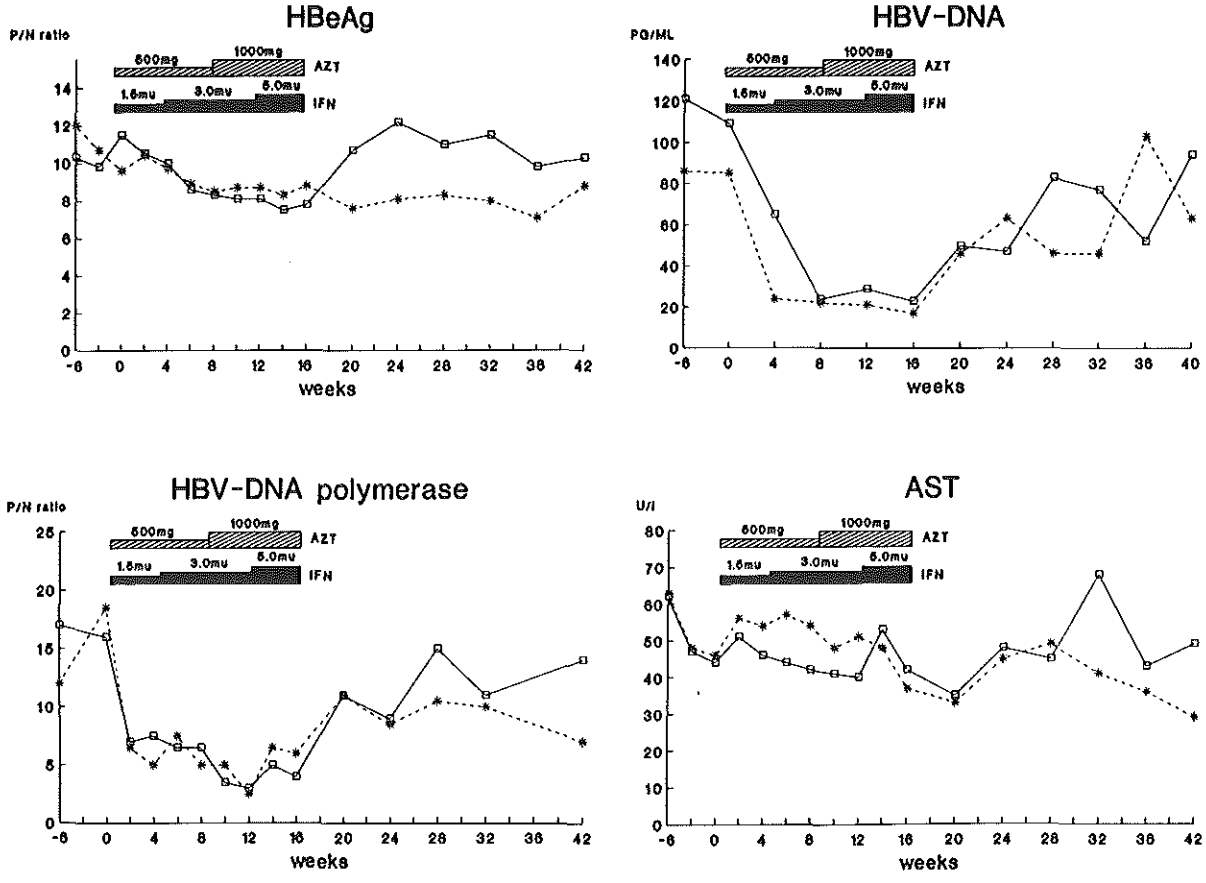

Figure 2. Median levels of HBeAg, HBV-DNA, HBV-DNA polymerase and aspartate aminotransferase (AST) for chronic hepatitis B patients treated with α-IFN in combination with zidovudine (□——□; n=12) or placebo (------*; n=12).*

Table 2. Histological scoring of serial liver biopsy specimens before treatment and at the end of follow-up. Results are denoted as mean ± S.D.; both groups consisted of 11 patients.

	α-interferon zidovudine		α-interferon placebo	
	pretreatment	end follow-up	pretreatment	end follow-up
Piecemeal necrosis	2.0 ± 1.2	2.3 ± 1.3	2.2 ± 1.4	1.8 ± 1.1
Focal necrosis	0.9 ± 0.8	1.2 ± 0.6	1.1 ± 0.7	1.2 ± 1.2
Portal inflammation	2.5 ± 0.9	2.3 ± 1.0	2.4 ± 0.9	2.0 ± 1.1
Fibrosis	2.2 ± 1.3	2.7 ± 1.5	2.7 ± 1.2	3.2 ± 0.9
Total score	7.7 ± 3.1	8.3 ± 3.4	7.8 ± 3.8	8.1 ± 3.7

patients and α-IFN therapy in none due to persisting adverse effects. Reduction of the α-IFN dose was associated with a diminished decrease in HBV-DNA and HBeAg (median decline of 40% for HBV-DNA and 17% for HBeAg after α-IFN dose reduction versus 71% (P=0.23) and 32% (P=0.06), respectively, for those on full dose α-IFN) during therapy. The percentage decrease in HBV-DNA, HBeAg and HBV-DNA polymerase for those who underwent zidovudine dose reduction was similar to that found for patients on full dose therapy.

Discussion

Many studies have indicated that α-IFN can induce a transition from active viral replication to virus latency in 30 to 40% of the patients with chronic hepatitis B (1-3). One of the methods used in the search for more effective therapy is to combine α-IFN with antiviral agents that act on different steps of the replication cycle of HBV.

In the present randomized placebo-controlled trial of α-IFN and zidovudine combination therapy we could not find an additional antiviral effect of zidovudine. The HBeAg seroconversion response was low both among patients treated with α-IFN plus zidovudine (8%) and those receiving α-IFN plus placebo (17%). All responders remained positive for HBsAg and for HBV-DNA measured by the polymerase chain reaction. Quantitative

Table 3. Side effects and consequential dose reductions of α-interferon and/or zidovudine therapy.

	α-interferon zidovudine (n=12)	α-interferon placebo (n=12)
Clinical:		
Fatigue	10 (83%)	8 (67%)
Flu-like symptoms	9 (75%)	8 (67%)
Apathy/Depression	5 (42%)	4 (33%)
Weight loss (>5%)	5 (42%)	1 (8%)
Gastrointestinal symptoms	4 (33%)	1 (8%)
Dizziness	3 (25%)	0 (0%)
Laboratory:		
Anemia (Hb < 7.0 mmol/l)	6 (50%)	0 (0%)
Leukocytopenia (< $3.0*10^9$/l)	9 (75%)	4 (33%)
Thrombocytopenia (< $70*10^9$/l)	2 (17%)	2 (17%)
Dose reductions due to side effects:		
α-interferon	5 (42%)	1 (8%)
zidovudine/placebo	6 (50%)	0 (0%)

analysis of the virological parameters revealed a significant decrease in HBeAg, HBV-DNA and HBV-DNA polymerase during therapy in both the α-IFN plus zidovudine and the α-IFN monotherapy group. However, no significant difference in these markers was observed between the two groups.

There are two notable explanations for the overall low response in this study. Firstly, all patients included were either cirrhotic, anti-HIV positive and/or previous nonresponders to α-IFN therapy; in particular the latter two characteristics are known to reduce the response rate for α-IFN therapy (10-12). Secondly, the α-IFN dose of 1.5 to 3 MU daily during the first 3 months of therapy might have been suboptimal. Several recent studies have demonstrated that doses of at least 30 MU per week, administered either daily or thrice weekly, lead to higher HBeAg seroconversion rates than low-dose regimens (1,13). In spite of the low number of HBeAg seroconversion and the relatively small number of patients investigated, the comparable response rates and the similarity of the curves for all quantified virological markers for the two investigated groups are highly suggestive of a lack of additional HBV inhibitory effect of zidovudine.

The rationale to study combination therapy consisting of zidovudine with α-IFN is that these compounds interfere in the HBV replication cycle at different sites. Zidovudine inhibits the DNA polymerase associated reverse transcriptase activity while α-IFN - in addition to its immunomodulatory effects - interferes primarily with viral protein synthesis. Furthermore zidovudine can be administered orally and therefore allows monitoring on an outpatient basis. The lack of an additional HBV inhibitory effect of zidovudine, as found in the present study, does not agree with previous in vitro studies in which a clear inhibitory effect of zidovudine-triphosphate on HBV-DNA polymerase activity was found nor with in vivo studies in which zidovudine therapy (800-1200 mg per day) resulted in a reduction of HBV-DNA and HBV-DNA polymerase in immunocompetent chronic HBV-infected patients (6,14). More in accordance with the results of the present study is the fact that in the hepatitis B duck model 1.5-15 mg/kg zidovudine per day failed to induce a suppression of DHBV replication (15). Other recent studies in which HBV patients were treated with zidovudine either with or without α-IFN also could not show a beneficial effect of zidovudine on the HBV infection (16-19). Unlike our trial these studies were uncontrolled and only included patients with a concurrent symptomatic HIV infection.

The design of the study, i.e. to start with a low dose of α-IFN that was gradually increased, was chosen to avoid a dangerous inflammatory exacerbation in patients with cirrhosis (20) and also to reduce the risk of severe bone marrow depression in those treated with α-IFN and zidovudine. Our concern about side effects in the combination group was confirmed by the dose-limiting toxicities that occurred predominantly in the last month of

therapy when high doses of α-IFN and zidovudine were given. The severe anemia and leukopenia which only occurred in the α-IFN-zidovudine group indicates that a synergistic bone marrow-suppressing effect of α-IFN and zidovudine is probable (21). Similar adverse effects of α-IFN-zidovudine combination therapy were recently observed in HIV-infected patients with Kaposi sarcoma. The maximum tolerated dose for these patients was approximately 800 mg zidovudine and 5 MU α-IFN per day (22). In our patients additional toxic effects may have been generated by a delay in the biotransformation and clearance of zidovudine due to limited hepatic function (23).

So far, combination of α-IFN with other antiviral agents has induced considerable side effects and has not proven to be more effective than α-IFN alone (24-26). Nevertheless, nucleoside analogues and α-IFN interfere with HBV replication through different inhibitory mechanisms and in theory their combination could thus lead to a synergistic antiviral effect. On the other hand since nucleoside analogues may affect the immune system, they could potentially reduce the important immunoregulatory properties of α-IFN. Moreover, it is essential to avoid the problem - as encountered in the present study - of a combination therapy that is so toxic that the dose of not only the nucleoside analogue but also α-IFN has to be reduced.

In summary, we could not find an additional antiviral effect when zidovudine was given in combination with α-IFN therapy to chronic HBeAg-positive HBV-infected patients. Patients undergoing the combination therapy exhibited more dose-limiting side effects of both zidovudine and α-IFN than those on α-IFN monotherapy. In subsequent clinical studies to elucidate the antiviral effect of combination therapy, α-IFN should only be combined with antiviral agents which have demonstrated optimal efficacy and very low toxicity in HBV replication models.

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CHAPTER 5

REPEATED COURSES OF ALPHA-INTERFERON FOR TREATMENT OF CHRONIC HEPATITIS TYPE B.

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Abstract

In chronic hepatitis B transition from active replication to viral latency (HBeAg seroconversion) usually leads to remission of the disease. Alpha-interferon (IFN) therapy induces HBeAg seroconversion in about one-third of the patients, thus leaving the majority of patients with persistent disease.

Eighteen chronic hepatitis B patients who did not respond (HBeAg seroconversion and clearance of HBV-DNA) to an initial 16-week course of α-IFN subsequently received α-IFN again after at least 6 months of no therapy. The repeated therapy consisted of 1.5 - 5 MU lymphoblastoid α-IFN daily for 16 weeks. Treatment effects were monitored by quantitative measurement of HBeAg and HBV-DNA. To analyze whether the results were related to patient characteristics known to affect the response to initial treatment, a predicted response rate, based on pretreatment factors, was determined.

After a follow-up of 52 weeks, 2 of the 18 patients (11%) had responded to therapy. Two additional patients became HBV-DNA negative with sustained HBeAg positivity. All patients remained HBsAg positive. According to the pretreatment parameters, a response was predicted for 9 of the 18 patients (50%). This predicted response rate was significantly higher than the actual response rate ($P=0.03$).

In conclusion, this pilot study with moderate dosages of α-IFN suggests that the HBeAg seroconversion rate after repeated α-IFN treatment is low for previous nonresponders and probably is not related to important clinical characteristics that influence the response to initial α-IFN treatment. A large controlled trial with higher doses of α-IFN is desirable to further evaluate the benefits of retreatment.

Introduction

In chronic hepatitis B virus (HBV) infections, termination of the viral replicating phase, as indicated by HBeAg seroconversion, is accompanied by pronounced clinical, biochemical and histological regression of liver disease activity (1,2). Alpha-interferon (IFN) therapy usually results in HBeAg seroconversion in 30-40% of the patients (3,4). This finding implies that α-IFN treatment is not successful in more than half of the cases and that these patients are left with persisting disease activity. The best therapeutic approach for these patients is still not known.

From various reports it appears that patient characteristics, such as immunocompetence and the degree of both inflammatory activity and viral replication, can influence the response to α-IFN therapy (3,5).

To determine the effect of repeated courses of α-IFN we studied the results of α-IFN retreatment in patients who did not respond with HBeAg seroconversion to previous courses of α-IFN. Furthermore, we investigated the predictive value of clinical pretreatment factors for response to α-IFN therapy.

Patients and Methods

Twenty courses of α-IFN retreatment were given to 18 chronic hepatitis B patients who did not respond to previous therapy. Only patients who had received an initial course of lymphoblastoid α-IFN (Wellferon,Wellcome,Beckenham,U.K.) for at least 16 weeks in a dosage of more than 20 MU per week were included in the study. The repeated α-IFN course started after a therapy-free period of at least 6 months. All patients were seropositive for HBeAg and HBV-DNA in the 6 months preceding the start of the repeated therapy. Liver biopsies were taken within a 3-month period prior to repeated therapy. For the 2 patients who received two courses of α-IFN retreatment only the last course was analyzed.

During the repeated course lymphoblastoid α-IFN was given subcutaneously for a period of 4 months in an increasing daily dose of 1.5 MU (4 weeks), 3 MU (8 weeks) and 5 MU (4 weeks). Follow-up ended 52 weeks after the start of this course of therapy.

Treatment effects were monitored by quantitative measurement of HBV-DNA, HBeAg and aspartate aminotransferase (AST) in serum obtained weekly during therapy and monthly in the follow-up period. Every 3 months additional biochemical and virological measurements, including the prothrombin time, HBsAg and the levels of alanine aminotransferase, albumin and bilirubin, were performed. A response was defined as the occurrence of

HBeAg seroconversion and clearance of serum HBV-DNA.

HBsAg and HBeAg were assessed by means of commercial radioimmunoassay kits (Abbott, Ill., USA). For quantification of HBeAg a P/N ratio (counts of patient sample/counts of negative control sample) was determined for each patient using a fixed serum dilution. HBeAg seroconversion was defined as a P/N ratio below 2.1 for undiluted serum from 2 successive blood samples. HBV-DNA was measured by a liquid hybridization assay using an iodine-125 probe (Abbott, Ill., USA).

To analyze whether the results were related to patient characteristics of value for predicting the response to initial treatment, a predicted response rate - based on pretreatment AST level, anti-HIV status and history of acute hepatitis - was determined for the repeated α-IFN courses (5). This prediction was compared to the actual response rate for the repeated α-IFN therapy. A positive response was predicted for the following pretreatment states: anti-HIV negative, history of acute hepatitis and an AST level above 45 U/l or anti-HIV negative, no history of acute hepatitis and an AST level above 85 U/l.

The Fisher exact test was used for statistical analysis.

Table 1. Patient characteristics at the start of the repeated α-IFN course (n=18).

Male/Female	15/3
Age[1] (yr)	38 (25-64)
Hetero/Homosexual	13/5
Caucasian/Oriental	17/1
Known duration of infection[1] (months)	60 (24-108)
Interval between start of α-IFN therapies[1] (months)	21.5 (13-52)
CPH/CAH/Cirrhosis/Unknown	4/8/5/1
HBV-DNA[1] (pg/ml)	109 (5-819)
AST[1,2] (U/l)	45 (19-475)
History of Acute Hepatitis[2]	2/16
Anti-HIV positive[2]	0/18

1: median (range);

2: response predictive factor

Results

Patient characteristics at the start of the repeated course are shown in table 1. Histological evidence of chronic hepatitis was found for all patients who underwent a liver biopsy (n=17). AST levels were elevated in 16 of the 18 patients. None of the patients were seropositive for antibodies to hepatitis C or hepatitis delta virus. One patient was of Chinese origin, one Turkish; all the others were West-European caucasians.

At the end of follow-up 2 of the 18 patients (11%) had responded to therapy (figure 1). The responses occurred at week 10 and 14 of treatment. Two patients became negative only for HBV-DNA, one of them relapsed to HBV-DNA positivity after cessation of therapy. All patients remained HBsAg positive.

Figure 2 shows the median level of HBV-DNA and AST. During therapy the HBV-DNA and HBeAg levels decreased by 80% and 31%, respectively. There was more than a 50%

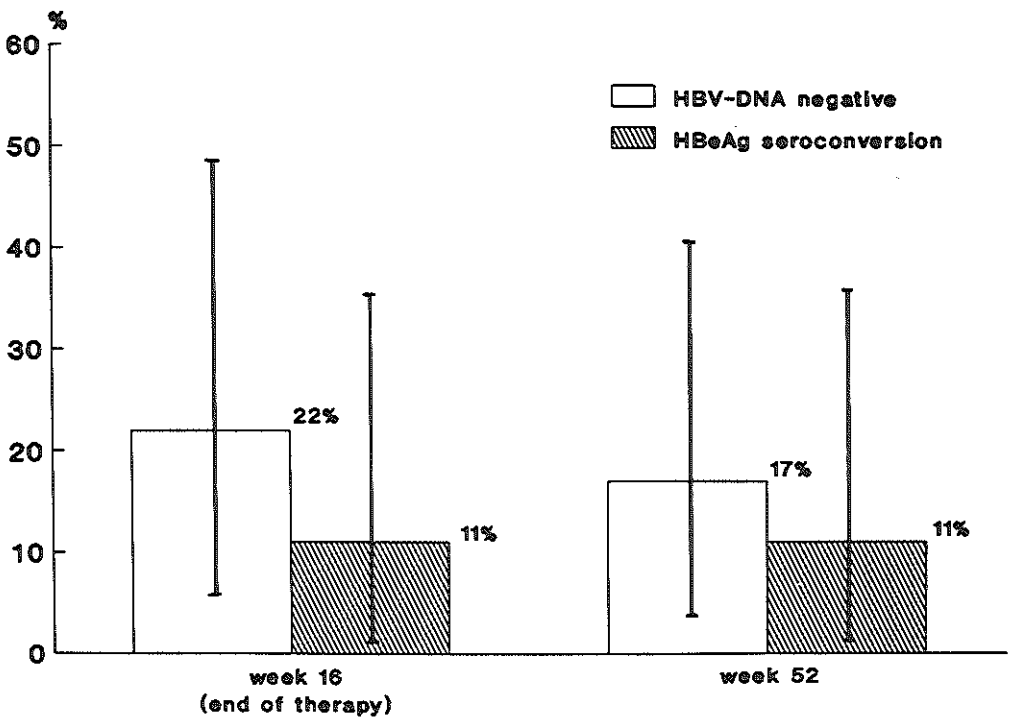

Figure 1. Percentage of patients who exhibited clearance of HBV-DNA and HBeAg at the end of the repeated α-IFN course and at the end of follow-up (n=18). The vertical lines denote the 95% confidence interval.

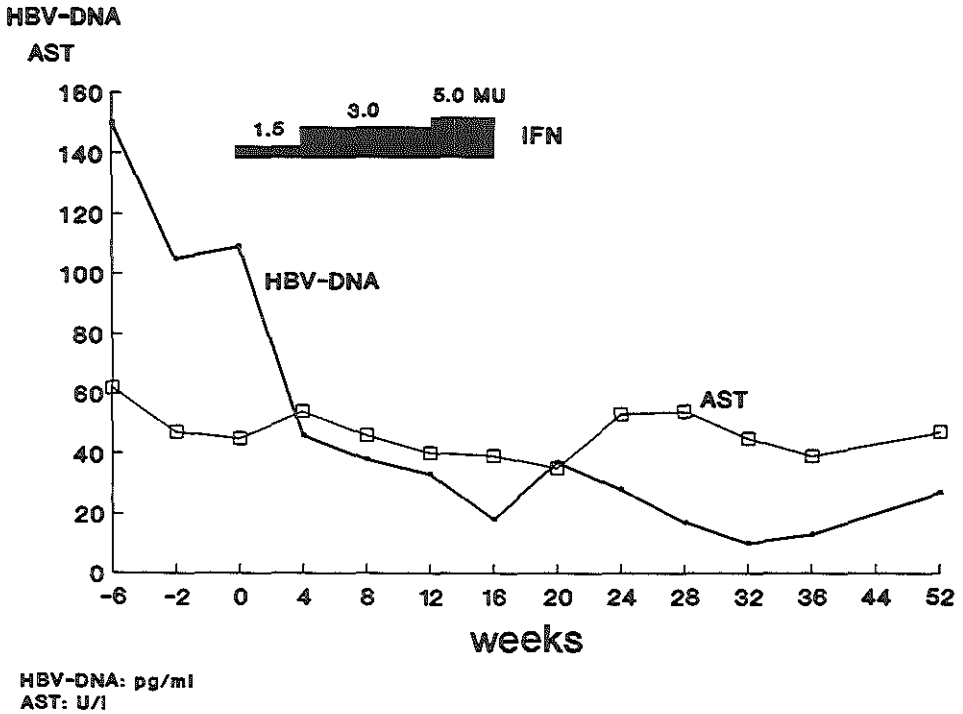

Figure 2. Median HBV-DNA and AST levels during treatment and follow-up of the repeated α-IFN course (n=18).

reduction of the HBV-DNA level in all 18 patients and of the HBeAg level in 5 of the 18. The AST level showed only minor fluctuations without evidence of a clear peak that might be indicative of the immunological response which can terminate the HBV replicating phase. Only 2 of the 18 patients - both nonresponders - exhibited a more than 2-fold rise in AST levels during α-IFN treatment.

On the basis of the pretreatment characteristics, a response was predicted for 9 of the 18 patients (50%); the predicted response rate was significantly higher than the actual response rate for α-IFN retreatment (P=0.03). Both responders belonged to the group of patients for whom a response was predicted.

One of the 2 patients who received a total of 3 α-IFN courses exhibited a response in the 10th week of the second course, which consisted of intermittent α-IFN therapy (figure 3). However, 12 weeks after the therapy was discontinued he suffered a relapse: both the HBeAg and HBV-DNA became positive with a sudden rise in aminotransferase levels. His last course of therapy which started 6 months after the relapse was unsuccessful. The AST

level for this patient was 3 to 4 times higher at the start of his second course as compared to the other - nonresponsive - courses.

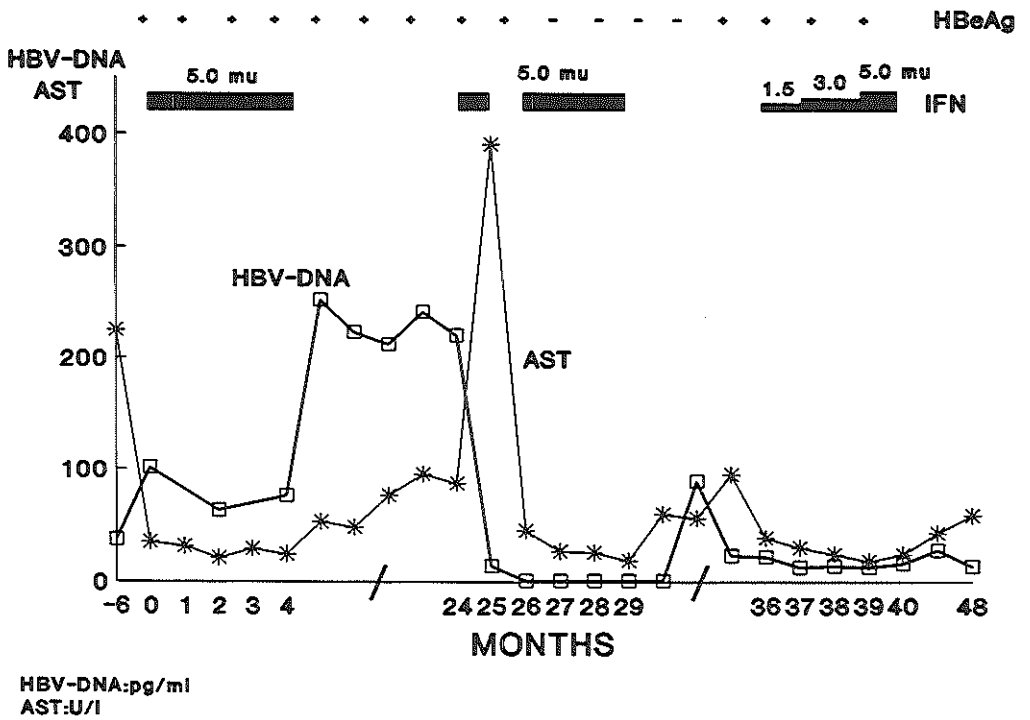

Figure 3. HBeAg status and levels of HBV-DNA and AST for a patient who received 3 courses of α-IFN and exhibited a response and relapse during the second course.

Discussion

The effects of α-IFN retreatment in chronic HBV-infected patients have not yet been studied in detail. In this pilot study the HBeAg seroconversion rate for repeated α-IFN treatment of previous nonresponders appears to be low (11%). The type of interferon used and the duration of the successive therapeutic courses were comparable for each patient. According to the prediction model described by Brook (5), a response was predicted for 9/18 (50%) patients. This figure was significantly higher than the actual response rate for retreatment. According to the same prediction model, none of the 18 patients were expected to respond to the first course of treatment. Thus, there was no difference between the actual and the predicted response rates for initial therapy (figure 4).

In all patients there was a clear decline in HBV-DNA levels during retreatment, sugges-

ting the presence of a direct inhibitory effect of α-IFN on HBV replication. However, the virus-specific immunological response, which is usually reflected by a rise in inflammatory activity (AST) and might be the most prominent effect of α-IFN therapy, appeared to be absent in most of the patients. It thus seems important to focus on the immunoregulatory properties of α-IFN to unravel the problem of nonresponse. α-IFN leads to an increased expression of HLA type I protein on the hepatocyte surface, thereby facilitating recognition and lysis of virus-infected cells by the cellular immune system. This α-IFN-induced immunological response is accompanied by a rise in serum tumor necrosis factor α, interleukin-1β and the CD4/CD8 lymphocyte ratio; it is unknown, however, whether these phenomena themselves exert a specific antiviral action or whether they only coincide with other more important steps in the cascade of cytokine activities. Unfortunately, the outcome of α-IFN therapy cannot be predicted by the pretreatment CD4/CD8 lymphocyte ratio, natural killer cell activity or lymphocyte proliferative response (6,7).

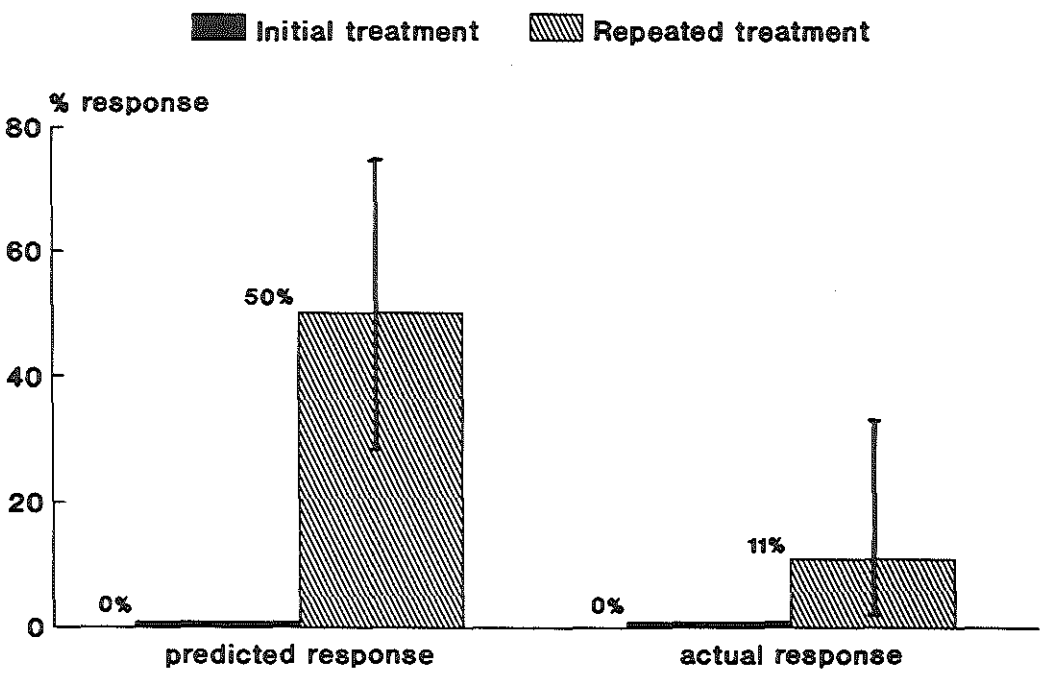

Figure 4. The predicted and actual response rates for the initial and repeated α-IFN course. The vertical lines denote the 95% confidence interval.

Other factors that might influence the response rate are the number of cellular IFN receptors and the level of antibodies to α-IFN. The IFN receptor expression appears to drop quickly after α-IFN treatment is started (8). This inhibitory effect might be compensated by an increase in the IFN receptor binding affinity (9). Nevertheless, patients with different patterns of response to α-IFN treatment do not seem to differ in the expression of IFN receptors on peripheral blood mononuclear cells. Antibodies to α-IFN were not present in any of the patients in this study (data not shown). The question whether α-IFN antibodies indeed influence the α-IFN response rate is still a subject of debate (10,11). Maybe these antibodies play an important role in the low responsiveness of patients from oriental areas (12).

It is unclear whether longer α-IFN retreatment with higher dosages would be more effective than the therapeutic regimen used in the present study. Longer courses of therapy looks promising for patients who partially respond to a first course of α-IFN (13) and might be efficacious for those who previously did not respond to the standard (16 week) α-IFN therapy. Combination of antiviral agents is also an option but the results of α-IFN combined with acyclovir, adenine arabinoside monophosphate or prednison have been disappointing for patients undergoing initial treatment (3,14,15). Regimens combining α-IFN with γ-interferon or interleukin-2 are also not superior to α-IFN alone (16,17). However from a theoretical viewpoint the combination of different cytokines appears to be attractive and this alternative certainly deserves further evaluation (6). Another approach for repeated therapy is to follow the patient regularly until the time when the aminotransferase levels have increased (preferably $>$ 100 U/l) or the HBV-DNA levels are low (preferably $<$ 50 pg/ml). Starting retreatment at that moment might result in enhanced response rates (18). Nevertheless, in our experience only 2 of the 9 patients with promising pretreatment factors responded to α-IFN retreatment. Thus, the predictive value of clinical pretreatment factors derived from first α-IFN courses (anti-HIV status, history of acute hepatitis and aminotransferase levels) was not confirmed for repeated α-IFN therapy in this study.

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CHAPTER 6

QUANTITATIVE ASSESSMENT OF HEPATITIS B VIRUS DNA IN CHRONIC HEPATITIS B: COMPARISON OF TWO SOLUTION HYBRIDIZATION ASSAYS

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Abstract

We compared two solution hybridization assays, the AffiProbe assay (Orion Corporation) and the Abbott HBV-DNA assay (Abbott), for quantitative measurement of hepatitis B virus (HBV) DNA in serum samples obtained from chronic HBsAg carriers. Forty transversally collected (group 1) and 83 serially collected (group 2) serum samples from chronic hepatitis B patients were tested with both assays. The serial serum samples were obtained from 6 patients who underwent α-interferon therapy with different outcomes (nonresponse, HBeAg seroconversion, HBeAg and HBsAg seroconversion).

In group 1 we found a good correlation ($r=0.91$; $P<0.001$) between the HBV-DNA results of the two assays. Two samples (5%) were HBV-DNA positive according to the Abbott but negative according to the AffiProbe assay; for all other samples the HBV-DNA status corresponded. In group 2 the assays gave colinear HBV-DNA results during follow-up of 5 of the 6 patients (correlation for the total group: $r=0.90$; $P<0.001$). Nevertheless, in both groups the AffiProbe assay yielded about 5-10 times higher HBV-DNA levels than the Abbott HBV-DNA assay ($P<0.001$). These discordant results were most probably due to standardization differences of the positive control samples of the two test systems. This observation underlines the need for international standardization of HBV-DNA and uniform reference panels.

Introduction

Advances in hepatitis B virus (HBV) DNA detection methods based on molecular hybridization techniques have opened the way for reliable quantification of HBV replication (1,2,3). As a result HBV-DNA has become an important parameter of the effect of antiviral therapy (4). Assays presently used to detect other markers of viral replication, such as hepatitis B e antigen (HBeAg) or the expression of hepatitis B core antigen (HBcAg) in liver cells, are difficult to quantify and therefore less suitable for treatment monitoring. Furthermore, the link between viral replication and HBeAg has been questioned since the discovery of HBV pre-core mutants that replicate without producing HBeAg (5).

HBV-DNA detection by dot-blot hybridization is widely used in research laboratories but is not yet applied in routine diagnostic laboratories. A similar problem exists for the quantification of HBV-DNA after amplification by polymerase chain reaction (PCR) (6,7). Hybridization in solution, which has been found to be suitable for HBV-DNA detection in serum, was recently incorporated in commercial test systems. The Abbott HBV-DNA assay (Abbott Laboratories, Chicago, Ill., USA) which is based on the separation of hybrids and free probe by column chromatography, appears to give satisfactory quantitative results (8,9). Using sera obtained primarily from chronic hepatitis B patients undergoing α-interferon therapy we compared the HBV-DNA results of this assay with those of another solution hybridization assay (AffiProbe assay, Orion Corporation, Helsinki, Finland) which depends on affinity-based hybrid collection (10,11)

Materials and Methods

Patients and Serum Samples

Two groups of sera were selected from chronic hepatitis B surface antigen (HBsAg) positive patients: Forty serum samples (group 1) were collected transversally from 36 patients. These sera were classified on the basis of the HBV-DNA level measured by the Abbott HBV-DNA assay as follows: below 1.7 pg/ml (under the cut-off; group 1A), 1.7 to 50 pg/ml (group 1B), 50 to 100 pg/ml (group 1C) and above 100 pg/ml (group 1D). Each of these subgroups included 10 sera. All samples were negative for antibodies against the human immunodeficiency virus; antibodies against hepatitis C and D virus were detected in 1 and 4 of the serum samples, respectively. All sera which tested HBV-DNA negative in the Abbott HBV-DNA assay were also negative for HBeAg; all other sera were HBeAg positive.

Eighty-three serum samples (group 2) were collected serially from 6 patients treated with α-interferon and followed for a period of 1 year. Sera were obtained every 4 weeks during α-interferon therapy and every 4-16 weeks in the period thereafter. All patients received 5 mega-units lymphoblastoid α-interferon (Wellferon, Wellcome, Beckenham, UK) per day according to the same protocol: a 4-week primer course, 4 weeks without therapy and a second α-interferon course lasting 16 weeks (12). All patients were HBeAg positive and had elevated serum aspartate aminotransferase levels at the start of therapy. None exhibited antibodies against hepatitis C or D virus or against the human immunodeficiency virus. Two patients remained HBeAg positive, 2 patients exhibited HBeAg seroconversion and 2 patients exhibited both HBeAg and HBsAg seroconversion during follow-up. One of the patients who only seroconverted for HBeAg showed HBeAg reactivation after cessation of therapy.

Affinity-based Hybridization Assay (AffiProbe Assay)

The AffiProbe assay is based on sandwich hybridization in solution followed by affinity-based hybrid collection (10,11). Target HBV-DNA is allowed to hybridize with a ^{35}S-labelled detector probe and a biotinylated capture DNA. The hybrids formed are then collected onto an affinity matrix coated with streptavidin, from which they are eluted for measurement by scintillation counting. Detectable label is bound to the matrix if the correct target is present in the reaction.

The concentration of the positive control was 1.67×10^9 HBV-DNA molecules (5840 pg/ml; cloned double stranded DNA) per ml. The diluent for the test samples was used as negative control. Thirty μl of the control samples were tested in duplicate and the mean cpm values were used. The cpm values for the negative control and the 1/100, 1/10 and 1/1 dilutions of the positive control sample (equivalent to 5×10^5, 5×10^6 and 5×10^7 DNA molecules, respectively) were plotted logarithmically for construction of the standard curve. The cut-off value - if at least 40 cpm and 1.5 times the cpm for the negative control - was set as the cpm value for the 1/100 dilution of the positive control. The manufacturer allows the use of 50 to 500 μl sample volumes; we chose a 100 μl sample volume which is standard for the Abbott HBV-DNA assay. The detection level for the AffiProbe assay using 100 μl of sample was 17.2 pg/ml.

Column-based Solution Hybridization Assay (Abbott HBV-DNA Assay)

The Abbott HBV-DNA assay was performed according to the instructions of the manufacturer (Abbott, Chicago, Ill, USA) (8). Briefly, 3 separate reagents were added to 100 μl of serum for solubilization and denaturation of the nucleic acids. A ^{125}I-labelled HBV-

DNA probe was then added to the samples for overnight hybridization. Free probe was separated from the ^{125}I-labelled hybrids by passage over a prepacked column. After elution of the hybrids from the column radioactivity was measured in a gamma counter. The amount of HBV-DNA, expressed as pg/ml, was calculated according to the following formula:

$$\frac{\text{sample counts - mean negative control counts}}{\text{mean positive control counts - negative control counts}} \times 103$$

The concentration of the positive control was 3 x 10^7 genomes/ml (103 $\pm$ 10 pg/ml; native HBV-DNA). The detection level of the assay was 1.7 pg of HBV-DNA per ml.

Polymerase Chain Reaction

Serum samples were subjected to HBV-DNA extraction and amplified by means of a polymerase chain reaction (PCR) as described previously (13,14). Two oligonucleotide primer sets that were specific for the surface and core regions of the HBV-DNA genome were used. Absence of HBV-DNA for both primer sets was considered a negative result.

Serological Assays

HBsAg and HBeAg were measured by solid phase radioimmunoassays (Abbott, Chicago, Ill., USA). Aspartate aminotransferase levels were determined with a sequential multiple autoanalyzer (Technicon Instruments Corp., Tarrytown, NY, USA).

Statistics

Pearson's correlation coefficient (r) was calculated to assess the correlation between the HBV-DNA levels obtained with the two assays. For the serial samples we also calculated the correlation coefficient for each individual patient and compared these values to 0, using the Wilcoxon signed-rank test. Differences between the HBV-DNA results of the two assays or between the AffiProbe/Abbott HBV-DNA ratio's were analyzed with the Student's t-test after logarithmic transformation of the HBV-DNA value. HBV-DNA results below the cut-off values of the assays were not quantified and therefore not included in the assessment of the correlation or statistical differences. P-values were two-sided, the significance level was 0.05.

Results

The HBV-DNA results of the AffiProbe and Abbott HBV-DNA assay of the samples from group 1 (transversal samples) are plotted in figure 1. Two sera (5%) were HBV-DNA negative according to the AffiProbe assay but positive (2 and 203 pg/ml) according to the Abbott HBV-DNA assay. In all other cases the HBV-DNA status corresponded. Quantitatively, we found a good correlation between the two assays ($r = 0.91$; $P < 0.001$). However, the AffiProbe assay yielded 2.1 to 17.0 (median 7.1; $P < 0.001$) times higher HBV-DNA values than the Abbott HBV-DNA assay. The difference between the two assays was most prominent for the samples with the higher HBV-DNA levels (table 1).
The HBV-DNA results found for group 2 (serial samples) are shown in figure 2. Seven samples (8%) tested positive for HBV-DNA (range 2-37 pg/ml) in the Abbott HBV-DNA

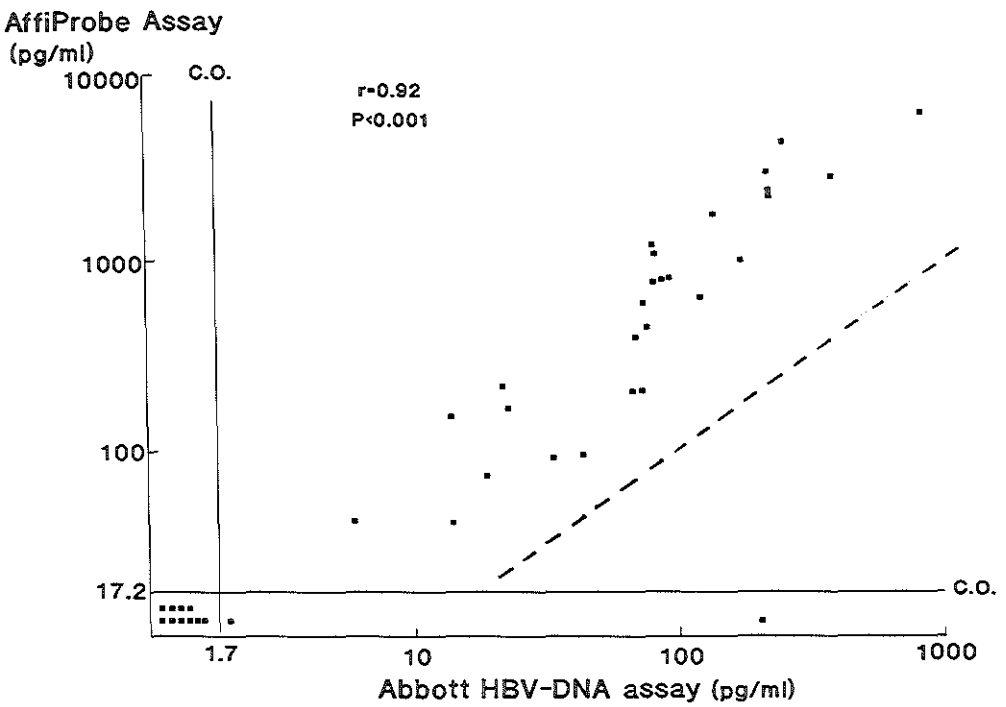


Figure 1. HBV-DNA levels (pg/ml) in 40 transversally collected serum samples (group 1) determined by means of the AffiProbe and Abbott HBV-DNA assays. The samples were obtained from chronic hepatitis B patients (HBeAg positive n=10; HBeAg negative n=30). HBV-DNA results below the cut-off level (C.O.) are not quantified.

Table 1. Results of the assessment of HBV-DNA with the AffiProbe and Abbott HBV-DNA assays in sera obtained from chronic HBV carriers. The sera were classified according to the HBV-DNA level obtained with the Abbott HBV-DNA assay: < 1.7 pg/ml (below the cut-off; group 1A), 1.7 to 50 pg/ml (group 1B), 50 to 100 pg/ml (group 1C) and > 100 pg/ml (group 1D). The cut-off value of the AffiProbe assay was 17.2 pg/ml. Results are expressed in median (range). The ratio AffiProbe/Abbott was only assessed for serum samples with an HBV-DNA level above the cut-off value. N.A.: not applicable.

Group	HBV-DNA (pg/ml)			
	Abbott HBV-DNA Assay	AffiProbe Assay	Ratio Affiprobe/Abbott	
1A (n=10)	< 1.7	< 17.2	N.A.	
1B (n=10)	16.5 (2-44)	79.5 (<17.2-221)	3.7 (2.1-10.4)	P=0.01
1C (n=10)	79 (68-94)	668 (198-1200)	8.1 (2.7-14.8)	
1D (n=10)	221.5 (123-836)	2275 (<17.2-6101)	9.7 (5.1-17.0)	

assay and negative in the AffiProbe assay; one sample was HBV-DNA positive (20 pg/ml) according to the AffiProbe assay and HBV-DNA negative according to the Abbott HBV-DNA assay. As for group 1 we found a good correlation ($r = 0.90$; $P < 0.001$) between the assays with 7.5 (median value) times higher HBV-DNA values obtained with the AffiProbe assay ($P < 0.001$). When the individual patients were analyzed the HBV-DNA values changed during follow-up in a linear fashion for 5 of the 6 patients, irrespective of the pattern of response to α-interferon therapy (figure 3; $r = 0.34$-0.96; $P = 0.03$). Two patients cleared HBV-DNA when measured with the PCR technique: one who only exhibited HBeAg seroconversion reactivated for HBV-DNA and HBeAg, the other who also exhibited HBsAg seroconversion remained HBV-DNA negative.

Since we consistently observed 5-10 times higher HBV-DNA values with the AffiProbe assay compared to the Abbott HBV-DNA assay we tested the HBV-DNA level of the positive control (HBV particles in serum) of the Abbott HBV-DNA assay in the AffiProbe assay. The AffiProbe assay yielded a value that was 6.9 (707 pg/ml) times the HBV-DNA

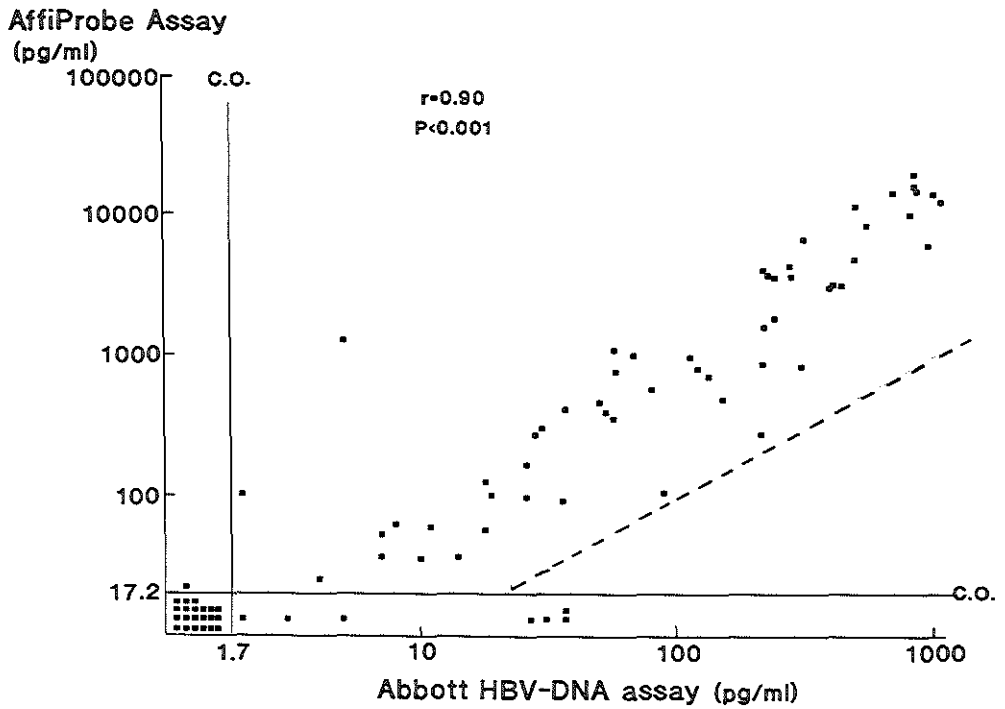


Figure 2. HBV-DNA levels (pg/ml) for 83 serial serum samples (group 2) determined by the AffiProbe and Abbott HBV-DNA assays. The samples were obtained from 6 HBeAg-positive chronic hepatitis B patients who were treated with α-interferon. HBV-DNA results below the cut-off level (C.O.) are not quantified.

concentration that was supposedly present in the Abbott HBV-DNA assay control sample. The Abbott HBV-DNA assay did not detect the cloned HBV-DNA from the AffiProbe control sample.

Discussion

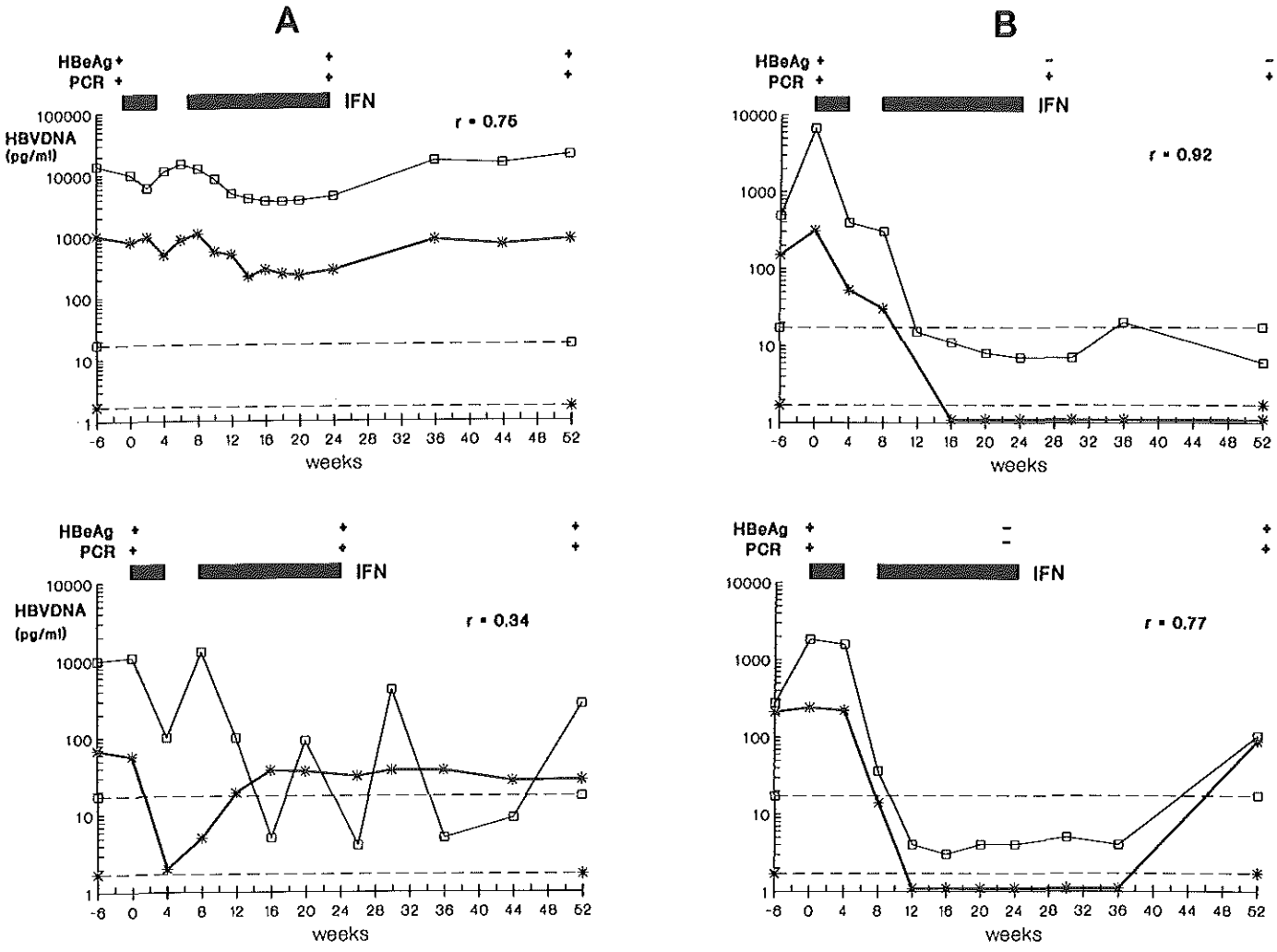
In this study the AffiProbe assay for detection of HBV-DNA was evaluated and compared with the Abbott HBV-DNA assay. The HBV-DNA values obtained with the two assays exhibited a close correlation, for both transversally and longitudinally obtained serum samples. Discrepancies in the (qualitative) HBV-DNA status were rare among the transversal samples. In the group of serial samples differences in the HBV-DNA status were found for two patients, one with unexpectedly large sequential HBV-DNA changes

according to the AffiProbe assay, the other suggesting a minor rebound phenomenon according to the Abbott HBV-DNA assay only.

To obtain optimal comparability we used the same sample volume (100 μl) in each test. According to the manufacturers' instructions this resulted in a 1.7 pg/ml cut-off level for the Abbott HBV-DNA and 17.2 pg/ml for the AffiProbe assay. Since the HBV-DNA values obtained with the AffiProbe assay were generally 5-10 times higher than those of the Abbott HBV-DNA assay - a finding that was also reported by others (G Gerken, personal communication) - the difference in cut-off level did not markedly influence the sensitivity of the AffiProbe as compared with the Abbott HBV-DNA assay. The divergence between the HBV-DNA values of the AffiProbe and Abbott HBV-DNA assay was most notable for sera with high HBV-DNA concentrations.

In practical terms both the AffiProbe and the Abbott HBV-DNA assay appear to be suitable for HBV-DNA monitoring of patients undergoing antiviral therapy. Since both tests are based on nucleic acid hybridization in solution, they ensure easier handling of samples and a faster hybridization rate than solid phase hybridization techniques (3,5). In addition, the results are given in numerical values which allows unequivocal interpretation and circumvents the possible interobserver variation of dot-spot methods. Nevertheless, the assays described in this study could be improved by eliminating the radioactive probes which require safety precautions and limit the shelf-life of the test kits (15,16). In our experience the AffiProbe assay appeared to be more elaborate than the Abbott HBV-DNA assay due to the many working steps involved, including ethanol precipitation and resuspension of DNA, a step that may introduce an experimental error. For example, loss of the HBV-DNA containing pellet after precipitation could potentially lead to false negative results. The instructions of the AffiProbe assay suggest using 50 and 500 μl of serum. In our experience a volume of 100 μl of serum, as was used in the Abbott HBV-DNA assay, resulted in satisfactory assessment of HBV-DNA levels in most samples. However, for precise quantitative measurement of low-range HBV-DNA samples and for therapeutic endpoint determinations, the use of 500 μl of serum appears preferable because of its lower detection level.

The question which of the 2 assays gives the closest approximation of the actual HBV-DNA content of the samples is difficult to answer. Since the AffiProbe assay uses cloned HBV-DNA (circular covalently closed double stranded DNA) as standard which fits in the format of that assay but not in that of the Abbott HBV-DNA assay which uses native HBV-DNA (partially single stranded DNA), positive control samples could not be exchanged. The result for the Abbott control sample tested in the Affiprobe assay was in agreement with the results for the patient samples in which 5-10 times higher HBV-DNA


A
HBeAg +
PCR +
IFN
r = 0.75
HBVDNA
(pg/ml)
100000
10000
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weeks
B
HBeAg +
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r = 0.92
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weeks
HBeAg +
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r = 0.34
HBVDNA
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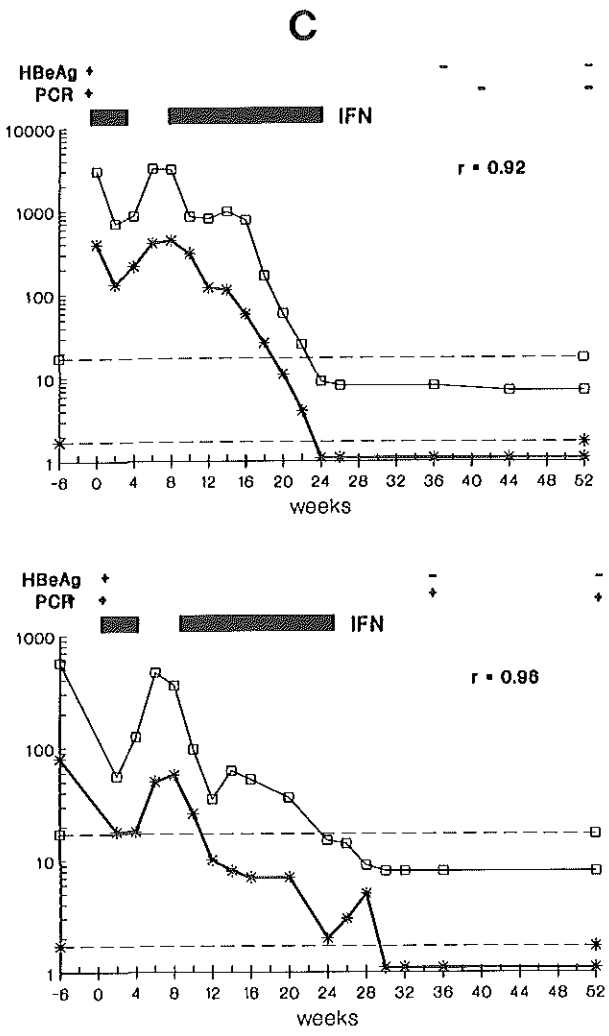

Figure 3. HBV-DNA levels (pg/ml) found by means of the AffiProbe (□———□) and Abbott HBV-DNA (———*) assays for 6 patients (group 2) undergoing α-interferon therapy. Two patients remained HBeAg positive (panel A), 2 patients exhibited HBeAg seroconversion (panel B) and 2 patients exhibited both HBeAg and HBsAg seroconversion (panel C) during follow-up. The cut-off HBV-DNA level was 17.2 pg/ml for the AffiProbe (□-----□) and 1.7 pg/ml for the Abbott HBV-DNA (*-----*) assay. IFN: α-interferon; PCR: HBV-DNA measured with polymerase chain reaction.*

levels were found in the AffiProbe compared with the Abbott assay. This observation reduces the discordant but consistent differences between the two assay systems to standardization differences of the positive control samples. Defining an HBV-DNA standard with native DNA may suffer from differences in the relative composition of structural forms of the viral DNA between the standard virus preparation and the sera of patients. On the other hand, the use of cloned viral HBV-DNA as standard, as was done earlier in dot blot hybridization, may be hampered by the fact that cloned HBV-DNA does not simulate solubilization and hybridization properties of native HBV-DNA in the assays. The differences in the quantitation of HBV-DNA in this study prove that there is a clear need for standardization and assessment of a uniform HBV-DNA reference panel. The use of complete hepatitis B virion appears to be the most realistic option for these reference sera. At present several European centers are trying to achieve this goal in concerted action (17).

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CHAPTER 7

MEASUREMENT OF HBsAg TO MONITOR HEPATITIS B VIRAL REPLICATION IN PATIENTS ON α-INTERFERON THERAPY

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Abstract

For the monitoring of chronic hepatitis B patients undergoing antiviral therapy, measurement of hepatitis B virus (HBV) DNA is widely used in research laboratories but is not yet a routine clinical procedure. We investigated whether quantitative measurement of HBsAg can be used to monitor viral replication in chronic hepatitis B patients undergoing α-interferon therapy. HBsAg was measured quantitatively in serum samples collected serially before and after the HBeAg seroconversion date from 69 patients with HBeAg seroconversion and 17 patients with both HBeAg and HBsAg seroconversion. Serial samples from 39 HBsAg-positive patients without seroconversion (18 HBeAg positive, 21 HBeAg negative) served as controls. From 12 other patients on α-interferon therapy (nonresponse n=6, HBeAg seroconversion n=3, HBeAg and HBsAg seroconversion n=3) monthly samples were collected until 6 months after cessation of therapy. HBsAg was measured quantitatively with an immunometric assay (Amersham, UK) and compared to the levels of HBV-DNA and HBeAg. In 56/69 (81%) patients with only HBeAg seroconversion, the HBsAg level dropped after HBeAg seroconversion; the median HBsAg level was 8.39 μg/ml (range 0.01-57.51) before HBeAg seroconversion compared to 3.53 μg/ml (range 0.002-68.66) after seroconversion ($P<0.001$). No siginificant decrease in HBsAg was found for the control group. For the group of 12 patients treated with α-interferon we found parallel results and a good correlation between the HBsAg level and both the HBV-DNA ($r=0.76$; $P<0.001$) and the HBeAg ($r=0.70$; $P<0.001$) level, irrespective of the response pattern.

Quantified assessment of HBsAg appears promising as a simple and cheap method for monitoring viral replication in chronic hepatitis B. This test deserves to be further evaluated and compared with quantitated HBeAg and HBV DNA assays to determine its usefulness for medical decision-making regarding α-interferon therapy.

Introduction

Chronic hepatitis B is a common liver disease which may progress to cirrhosis with such complications as portal hypertension and hepatocellular carcinoma (1,2). With the availability of effective antiviral agents for chronic hepatitis B, quantitative monitoring of hepatitis B virus (HBV) replication has become an important factor in patient management (3,4). The aim of antiviral therapy is to induce a transition from active HBV replication to viral latency which is usually accompanied by arrest of disease progression (5,6). To monitor HBV replication measurement of HBV-DNA is widely used in research laboratories but not in routine clinical laboratories because of its time-consuming methods and its high cost in material and personnel (7,8). There is a need for a simple and cheap assay to assess the level of HBV replication.

Hepatitis B "surface" antigen (HBsAg) was the first hepatitis B-related antigen discovered and is by definition present in all chronic hepatitis B patients (9). HBsAg testing is therefore part of the routine diagnostic assessments in almost every hospital around the world. We investigated the level of HBsAg in patients who exhibited cessation of viral replication, as indicated by clearance of HBeAg and HBV-DNA. Furthermore we determined whether quantitative measurement of HBsAg can be used as a marker of viral replication in patients undergoing α-interferon therapy.

Patients and Methods

Patients and Treatment

HBsAg was assessed quantitatively in sera collected from 4 different groups of chronic HBsAg carriers (total of 137 patients): Sixty-nine patients exhibited HBeAg seroconversion (group A), 39 after α-interferon therapy (5 MU per day for 4 to 6 months) and 30 spontaneously. Seventeen patients exhibited both HBeAg and HBsAg seroconversion within 6 months (group B), 8 after α-interferon treatment and 9 spontaneously. Thirty-nine HBsAg-positive patients (HBeAg positive n=18; HBeAg negative n=21) without α-interferon therapy and without HBeAg and/or HBsAg seroconversion served as controls (group C). Two serial samples were collected 6 to 12 months before and after the HBeAg seroconversion date; similarly in the control group two sera were collected 12 to 24 months apart. The remaining group (group D) consisted of 12 patients who were followed longitudinally during α-interferon therapy with different outcomes: nonresponse in 6, HBeAg seroconversion in 3 and HBeAg as well as HBsAg seroconversion in 3 patients. α-Interferon was given in a dose of 5 MU daily, in courses

of 1 and 4 months duration, separated by one month of rest (10). Serum samples of these 12 patients were collected every month during therapy and every 1-2 months afterwards (follow-up 1 year). In addition to quantitative HBsAg measurement, HBV-DNA and HBeAg were assessed quantitatively in the samples from group D.
All patients receiving α-interferon therapy were HBeAg and HBV-DNA positive at the start of therapy. HBeAg seroconversion was always accompanied by clearance of serum HBV-DNA. All patients studied were negative for antibodies against the human immunodeficiency virus and hepatitis C and D virus.

Laboratory methods

HBsAg was detected by an enzyme immunoassay (EIA) employing enhanced luminescence (Amerlite HBsAg assay, Amersham, United Kingdom). Serum sample and mouse anti-HBs monoclonal antibody labeled with peroxidase were incubated in microwells coated with a second anti-HBs monoclonal antibody of different specificity. After removal of unbound material the peroxidase activity of the conjugate was measured by an enhanced luminescence reaction (11). The sensitivity of the assay was approximately 0.3 ng/ml of HBsAg (ad and ay). When appropriate, samples were tested undiluted or diluted 1/100 and 1/400 to remain within the detection range of the assay. HBsAg was quantified using a serial dilution curve of a reference sample from the Paul Ehrlich Institute (Langen, Germany) containing both the ad and ay (mixture 1:1) subtypes of HBsAg. Results were expressed in micrograms of HBsAg per ml of serum. HBV-DNA was measured by a liquid-phase hybridization assay using an ^{125}I-labelled HBV-DNA probe (Abbott HBV-DNA assay, Abbott, Chicago, USA). Free probe was separated from the ^{125}I-labelled hybrids by passage over a prepacked column. The HBV-DNA level was expressed in picograms per ml of serum; the cut-off of the assay was 1.7 pg/ml. HBeAg was measured by a radioimmunoassay (Abbott HBeAg test, Abbott, Chicago, USA). For quantification the P/N ratio (counts patient sample/counts negative control sample) was determined. For each patient a fixed serum dilution (1/1, 1/5, 1/25, 1/125 or 1/625) was maintained. HBeAg seroconversion was defined as a P/N ratio below 2.1 for undiluted serum.

Statistics

Medians were used because the HBsAg results lacked a normal distribution (also after logarithmic transformation). The two-sample Wilcoxon rank sum test was used to analyze unpaired observations and the Wilcoxon signed rank test to analyze paired observations. Spearman's correlation coefficient (r) was calculated to assess the correlation between the levels of HBsAg and HBV-DNA or HBeAg. P-values were two-sided, the significance

level was 0.05.

Results

The levels of HBsAg before and after seroconversion for HBeAg and HBsAg are illustrated in table 1. The HBsAg level decreased in 56 of the 69 patients who exhibited only HBeAg seroconversion (group A; median decrease of 59%; $P<0.001$). The median decrease in HBsAg was 59% for patients with α-interferon-induced HBeAg seroconversion and 55% for those with spontaneous HBeAg seroconversion. The difference between the HBsAg levels before and after HBeAg seroconversion was significant for both groups. The median HBsAg level in the initial samples was higher for the patients who underwent both HBeAg and HBsAg seroconversion (group B) - independent of α-interferon therapy - in comparison to those who only exhibited HBeAg seroconversion (group A); these differences were not statistically significant.

For both the HBeAg-positive and HBeAg-negative control group (group C) we did not

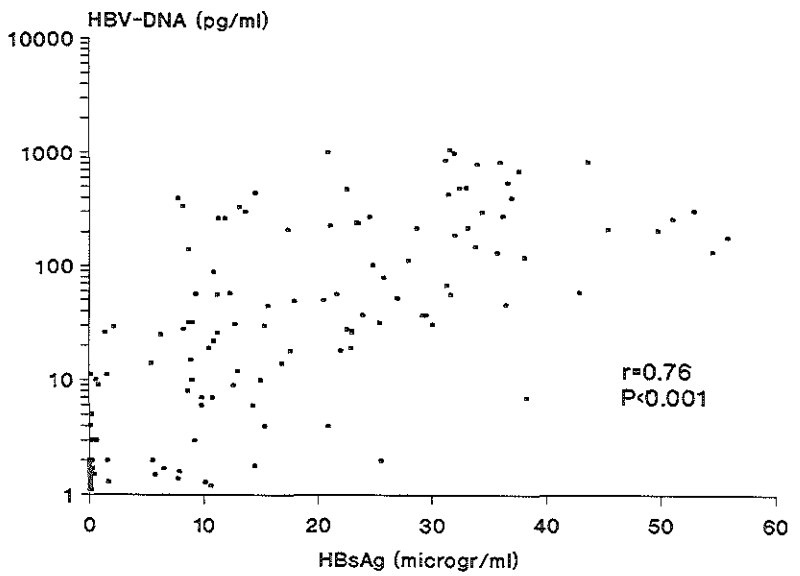

Figure 1. HBV-DNA and HBsAg levels in 148 serum samples obtained from 12 chronic hepatitis B patients (group D) treated with α-interferon.

Table 1. Median HBsAg level (range) before and after HBeAg seroconversion or HBeAg and HBsAg seroconversion.

		n	HBsAg level (μg/ml)		P value
			month 0	month 12	
A					
HBeAg seroconversion :	IFN treatment	39	8.39 (0.01-57.51)	3.53 (0.002-68.66)	< 0.001
	No treatment	30	8.43 (0.02-52.12)	3.63 (0.002-65.99)	0.02
	Total	69	8.39 (0.01-57.51)	3.53 (0.002-68.66)	< 0.001
B					
HBeAg and HBsAg seroconversion :	IFN treatment	8	32.58 (4.12-42.31)	< 0.001*	N.A.
	No treatment	9	12.04 (0.02-61.69)	< 0.001*	N.A.
	Total	17	13.09 (0.02-61.69)	< 0.001*	N.A.
C					
HBeAg-positive controls :		18	25.55 (1.51-75.65)	21.70 (0.03-79.43)	0.87
HBeAg-negative controls :		21	4.96 (0.13-17.12)	6.01 (0.01-17.30)	0.18

* cut-off HBsAg assay: 0.3 ng/ml
N.A.: not applicable

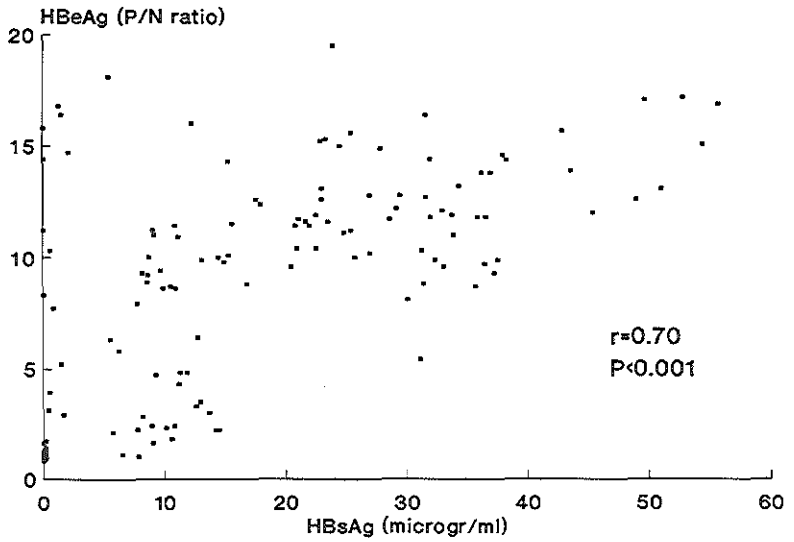

Figure 2. HBeAg and HBsAg levels in 148 serum samples obtained from 12 chronic hepatitis B patients (group D) treated with α-interferon.

find significant differences between the HBsAg level in the initial and follow-up serum samples (table 1). The baseline HBsAg level for the 21 HBeAg-negative controls was lower compared to the HBsAg results of all other subgroups ($P<0.05$).

A total of 148 serum samples were obtained from the 12 patients who were followed longitudinally during α-interferon therapy (group D). For this group we found co-linear results and a good correlation between the HBsAg level and both the HBV-DNA ($r=0.76$; $P<0.001$) and the HBeAg ($r=0.70$; $P<0.001$) level (figure 1 and 2). No difference in the correlation coefficients was found between patients with different response patterns (nonrespone n=6, HBeAg seroconversion n=3, HBeAg and HBsAg seroconversion n=3; figure 3).

Discussion

In the present study we assessed the quantitative HBsAg level in relation to loss of HBV replication and evaluated the usefulness of HBsAg for monitoring antiviral therapy. As in

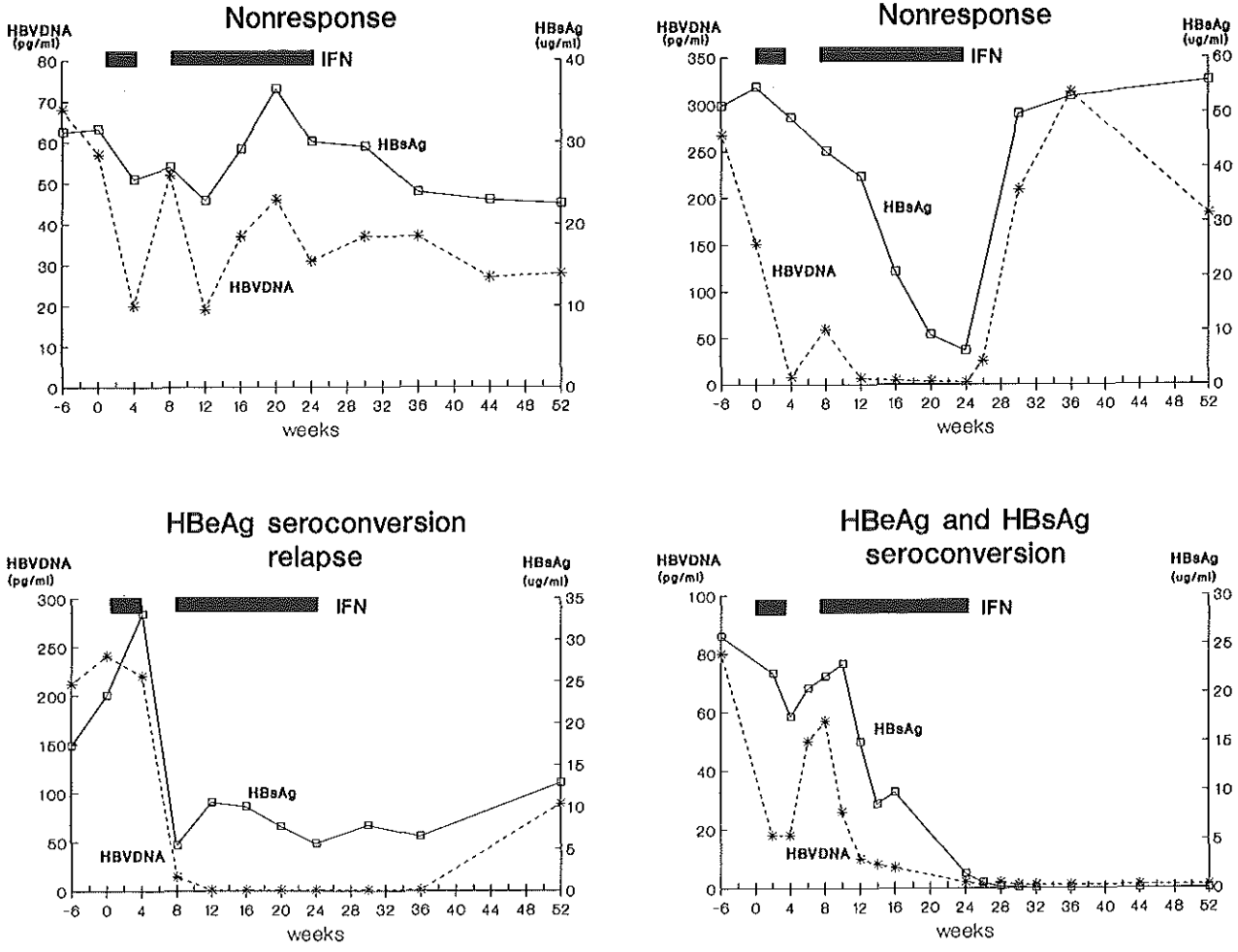

Figure 3. HBV-DNA and HBsAg levels in 4 chronic hepatitis B patients with a different response to α-interferon therapy.

previous studies, we found that the transition from HBV replication to viral latency, as indicated by HBeAg seroconversion, is accompanied by a significant decrease in the HBsAg level (12-14). Other s gene encoded proteins, such as pre-S1 Ag and pre-S2 Ag, have also been shown to correlate with the degree of HBV replication (15).

Patients who underwent HBeAg seroconversion (no HBsAg seroconversion; group A) during α-interferon therapy exhibited a similar decrease in HBsAg level as patients who seroconverted spontaneously, suggesting that therapy-induced and spontaneous HBeAg seroconversion result in comparable reductions of the virus load. Since we included only a few patients who did not respond to α-interferon we cannot judge whether HBsAg assessment prior to therapy could help to predict the outcome of this treatment. Eight patients exhibited both HBeAg and HBsAg seroconversion after α-interferon treatment (group B). The initial HBsAg level found for these patients was similar to that for those who exhibited only HBeAg seroconversion (group A) during therapy, indicating that the amount of HBsAg in serum before therapy is not predictive for the occurrence of HBsAg seroconversion after α-interferon therapy.

For patients who were followed closely during α-interferon therapy (group D) we found a good correlation between the HBsAg and the HBeAg or HBV-DNA levels. In most patients the HBV-DNA level dropped first, followed by the HBeAg and HBsAg levels. A similar sequence was described by Zoulim and Hess in previous studies (4,16). Interestingly, we found co-linear results for the HBsAg, HBeAg and HBV-DNA levels, irrespective of the response (nonresponse, HBeAg seroconversion, HBeAg and HBsAg seroconversion) to therapy. The interval between the start of the decrease in the HBV-DNA and HBsAg levels was short which suggests that the HBsAg level reflects changes in viral replication in a direct way.

With the availability of effective antiviral agents, quantified parameters of HBV replication have become important for patient management and the development of new treatment regimens. One of the reasons why HBsAg is as yet rarely quantified in studies involving antiviral therapy could be that the range of this antigen can only be covered by using several dilution steps in assays presently available for qualitative measurement. Using the HBsAg Amerlite kit we were able to obtain reliable quantification in 3 dilution steps. More than 80% of the samples could be measured reliably after a 1/100 dilution step. A second possible reason for the lack of interest in measuring the level of HBsAg in patients treated with α-interferon could be the obvious fact that HBsAg does not disappear completely from serum after cessation of viral replication as detected by HBeAg seroconversion. Although the HBsAg level decreased in the majority of our patients who exhibited HBeAg seroconversion it remained high in some. Therefore we could not define

an HBsAg cut-off level which corresponds to disappearance of HBeAg and which could be used as endpoint determination for α-interferon therapy. On the other hand, quantitative HBsAg assessment could indicate in most cases a significant decrease in viral replication during therapy like HBeAg and HBV-DNA measurement. In comparison to quantitative assessment of HBeAg and HBV-DNA, HBsAg quantitation is less laborious and cheaper, especially when performed by assays designed for quantitative measurement (Amerlite assay, Abbott IMX). For the many laboratories which do not have the ability to measure HBV-DNA or HBeAg quantitatively, HBsAg measurement might be a good alternative to assess changes in HBV replication. In addition to HBsAg measurement a simple qualitative HBeAg/anti-HBe assay indicating seroconversion is most reliable for endpoint determination of α-interferon therapy. Further studies should elucidate whether the HBsAg level can also be used as a virus marker for patients with hepatitis D or for those who carry HBV mutants with a deletion in the pre-core region of the genome which impedes HBeAg production (17,18). For these patients, who often have active disease without the presence of HBeAg, the effect of treatment is currently monitored by assessment of aminotransferase levels.

In conclusion we found that transition from HBV replication to viral latency, as indicated by HBeAg seroconversion, is accompanied by a signifcant decrease in the HBsAg level and that the quantified HBsAg level correlates well with the quantified HBeAg and HBV-DNA levels. Therefore quantitative measurement of HBsAg appears promising as a simple and cheap method for monitoring HBV replication and deserves further evaluation of its usefulness for medical decision-making regarding α-interferon therapy.

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CHAPTER 8

FATAL DECOMPENSATION OF CHRONIC VIRAL HEPATITIS ASSOCIATED WITH ALPHA-INTERFERON TREATMENT

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(adapted version of this chapter)

Abstract

As a result of an inquiry among 16 European hospitals, in which 2,490 chronic viral hepatitis patients have been treated with α-interferon, we report on 9 patients with cirrhosis who exhibited a fatal decompensation of their liver disease during or shortly after α-interferon therapy. Seven patients had chronic hepatitis B, 1 chronic hepatitis C and 1 a co-infection of hepatitis B and C. Four of the 9 patients had no evidence of decompensated liver disease previously (Child classification A at the start of therapy) and 5 exhibited signs of decompensation in the past (Child classification A (n=1), B (n=3) and C (n=1) at the start of therapy). The pattern of decompensation during therapy included ascites in 9, encephalopathy in 7 and jaundice in 6 patients. The cause of death was liver failure in 6 and variceal bleeding in 3 cases. Particularly in patients with previously compensated liver disease the relation between the fatal decompensation and the α-interferon therapy appears highly probable. A mechanism which could explain most of the observed deaths in this study is that α-interferon induces an exacerbation of inflammatory activity, leading to a fatal destruction of the limited residual capacity of the liver in patients with cirrhosis.

The present study suggests that α-interferon can induce a severe aggravation of the liver disease and that it is important to closely monitor patients with cirrhosis undergoing this therapy. A very careful attitude with regard to α-interferon is necessary for patients with decompensated cirrhosis who should only be treated on a clear indication by experienced hepatologists.

Introduction

Currently, alpha-interferon is the most promising therapy for chronic viral hepatitis. A sustained response can be obtained in approximately 30% of the patients with chronic hepatitis B or C (1,2). Also in patients with cirrhosis α-interferon may improve the outcome of disease and obviate the need for liver transplantation (3). A response in chronic hepatitis B, as defined by loss of viral replication (hepatitis B e antigen seroconversion), is usually accompanied by a transient rise in aminotransferases (4). This inflammatory exacerbation ensues the risk of a hepatic decompensation in cirrhotic patients. We describe 9 patients with viral hepatitis related cirrhosis who died from decompensated liver disease during or shortly after α-interferon treatment, and discuss the possible contributing role of α-interferon.

Case report

A 44-year old male with hepatitis B e antigen positive chronic hepatitis B was started on 5 megaunits of recombinant α-interferon per day at the Rotterdam University Hospital. His liver disease was not detected until 6 months previously when he complained of ankle edema and ascites after a long car drive from Morocco to the Netherlands. Liver biopsy showed a macronodular cirrhosis with active hepatitis and hepatitis B core antigen expression in 30% of the hepatocytes; endoscopic examination showed medium-sized esophageal varices. At the start of therapy his liver disease appeared to be compensated, both the ascites and edema were undetectable with diuretic therapy. During the first 8 weeks of treatment he only complained of chills and a slight fatigue, symptoms that can be attributed to the α-interferon therapy. In this period the aspartate aminotransferase level doubled while no substantial change was found for the serum hepatitis B virus DNA level (figure 1). In the 11th week of treatment the patient complained of jaundice, drowsiness and reappearance of the ascites. He was admitted to hospital and the α-interferon therapy was discontinued. The aspartate aminotransferase and bilirubin level had risen to 830 U/l (normal <30) and 93 μmol/l (normal <14), respectively. The ammonia level was 115 μmol/l (normal <30) and a grade IIb encephalopathy was found on spectral analysis of the electroencephalogram (5). He was started on lactulose and 7 liters of ascitic fluid were drained. There were no signs of bacterial peritonitis. No markers of any hepatotrophic virus causing a superinfection were detected in serum or liver tissue. During the exacerbation the serum hepatitis B virus DNA level dropped to 11 pg/ml (cut off 1.7 pg/ml; Genostics, Abbott, USA), however no hepatitis B e antigen seroconversion occurred.

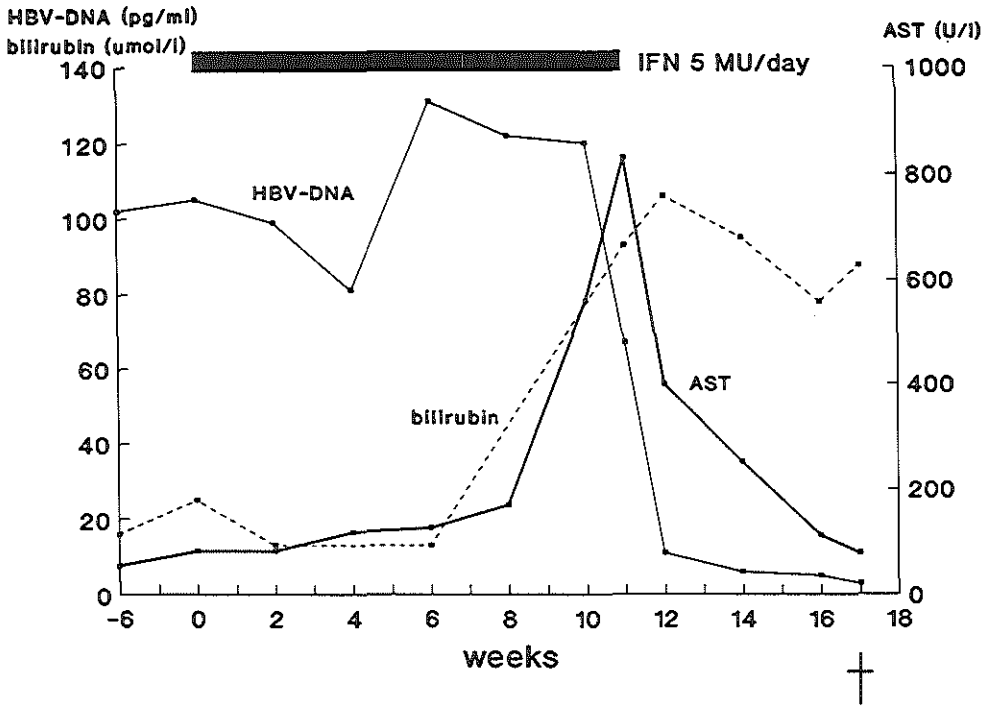

Figure 1. Level of serum hepatitis B virus DNA (pg/ml), aspartate aminotransferase (u/l) and bilirubin (μmol/l) in a chronic hepatitis B patient (case 5) who exhibited a fatal decompensation of liver disease during α-interferon therapy.

After 2 more weeks during which the condition of the patient improved (bilirubin and aspartate aminotransferase level decreased) he suddenly developed a variceal hemorrhage and died.

Survey

To review the frequency and clinical aspects of α-interferon related fatal decompensation of liver disease a questionnaire was sent out to 19 European centres with a large experience in α-interferon therapy for chronic viral hepatitis. Sixteen hospitals from 9 countries responded. In these centres a total number of 2,490 chronic viral hepatitis patients had been treated with α-interferon. Only cases in which the patient exhibited an aggravation of liver disease with fatal outcome during or within 2 months after discontinuation of α-interferon were included. Eight more cases from 5 different hospitals were reported. From

Table 1a. Characteristics of previously compensated chronic viral hepatitis patients who exhibted fatal liver failure associated with α-interferon therapy.

GROUP A	age	sex	type of hepatitis	previous decompensation	dose/duration IFN	type of IFN	signs of decomp. during IFN	last results before death AST (U/l)	bilirubin (μmol/l)	albumin (g/l)	HBeAg seroconv.	cause of death
Case 1[a]	62	male	B	No	5 MU t.i.w. 12 weeks	recombinant α-2b	ascites jaundice encephalopathy	244	648	43	Yes	liver failure
Case 2	41	male	B (HBe-mutant)	No	3 MU t.i.w. 3 weeks	recombinant α-2b	ascites jaundice encephalopathy	302	680	28	N.A.	liver failure
Case 3	60	female	C	No	10 MU t.i.w. 11 weeks	recombinant α-2b	ascites jaundice encephalopathy	76	410	28	N.A.	liver failure
Case 4	62	male	B	No	5 MU daily 8 weeks (after steroid withdrawal)	recombinant α-2b	ascites jaundice	394	226	26	No	variceal bleed

a: reference nr 13.
N.A.: not applicable; IFN: α-interferon; HBeAg: hepatitis B e antigen; AST: aspartate aminotransferase

Table 1b. Characteristics of previously decompensated chronic viral hepatitis patients who exhibted fatal liver failure associated with α-interferon therapy.

GROUP B	age	sex	type of hepatitis	previous decompensation	dose/duration IFN	type of IFN	signs of decomp. during IFN	last results before death AST (U/l)	bilirubin (μmol/l)	albumin (g/l)	HBeAg seroconv.	cause of death
Case 5[a]	44	male	B	Yes (ascites) (edema)	5 MU daily 11 weeks	recombinant α-2a	ascites jaundice encephalopathy	79	88	27	No	variceal bleed
Case 6[b]	44	male	B and C	Yes (ascites) (jaundice)	3 MU t.i.w. 10 weeks	lymphoblastoid	ascites jaundice encephalopathy	289	272	28	N.A.	liver failure H-R syndr. peritonitis
Case 7	70	male	B	Yes (ascites) (variceal bleed) (encephalopathy)	2 MU t.i.w. 24 weeks	recombinant α-2b	ascites encephalopathy	9	111	34	Yes	liver failure
Case 8[b]	36	male	B	Yes (ascites)	2 MU t.i.w. 16 weeks	recombinant α-2b	ascites peritonitis	16	52	27	Yes	variceal bleed
Case 9[b]	53	male	B	Yes (ascites) (HCC)	2 MU t.i.w. 3 weeks	recombinant α-2b	ascites jaundice encephalopathy	500	171	29	No	liver failure

a: presented above
b: patients on waiting list for liver transplantation
N.A.: not applicable; HCC: hepatocellular carcinoma; H-R syndr.: hepato-renal syndrome; IFN:α-interferon; HBeAg: hepatitis B e antigen; AST: aspartate aminotransferse

the total of 9 patients 4 had no evidence of decompensated liver disease (presence of ascites, jaundice, encephalopathy or variceal bleeding) before α-interferon therapy was started (group A; table 1a) and 5 patients had signs of decompensated liver disease in the past (group B; table 1b). From group B, 1 patient (case 5) appeared to be recompensated at the start of treatment while 3 other patients (case 6, 8 and 9) were on the waiting list for hepatic transplantation. Case 9 was the only patient with evidence for hepatocellular carcinoma. According to the Child-Pugh criteria (6) case 1-5 had class A, case 6, 8 and 9 class B, and case 7 class C cirrhosis at the start of the α-interferon therapy.

At the time of death the median age was 53 years. Seven patients had chronic hepatitis B of whom 1 (case 2) carried a mutant type of virus with a deletion in the pre-core region of the genome which impedes hepatitis B e antigen production (7). One patient had chronic hepatitis C and one had a co-infection of chronic hepatitis B (hepatitis B e antigen negative) and C. No serum markers that indicate a viral superimposed infection of the liver appeared during the period of decompensation in any of the patients. Furthermore none had been exposed to hepatotoxic drugs or other possible causes of liver injury. Pretreatment histologic examination showed chronic active hepatitis with cirrhosis in all cases. The weekly dosage of α-interferon in group A ranged from 9 to 35 MU; in group B, 1 patient received a dose of 35 MU/week and 3 less than 10 MU/week. In 7 of the 9 patients α-interferon was administered thrice weekly. Three of the 6 hepatitis B e antigen positive hepatitis B patients exhibited an hepatitis B e antigen seroconversion during therapy. The hepatitis C and the hepatitis B mutant infected patients (case 2, 3 and 6) did not respond to the α-interferon therapy with normalization of aminotransferase values. The clinical complications occurred within the first 3 months of therapy in 7 of the 9 patients. In the other 2 patients (case 7 and 8) the scheduled α-interferon course was completed with clearance of hepatitis B virus DNA but clinical deterioration developed 2 and 8 weeks after discontinuation of treatment. The following signs of decompensation were present after start of therapy: ascites in 9 patients (previously present in 5), encephalopathy in 7 (previously present in 1) and jaundice in 6 (previously present in 1). Spontaneous bacterial peritonitis was found in 2 patients (case 6 and 8; previously present in 1). The reported eventual cause of death was liver failure in 6 and hypovolemic shock due to variceal bleeding in 3 patients.

Discussion

We describe 9 chronic viral hepatitis patients who underwent α-interferon therapy with fatal outcome. All patients had cirrhosis and 5 of the 9 had signs of decompensated liver

disease previously. It must be emphasized that the patients described were selected from a large population treated with α-interferon. Nevertheless these cases suggest that α-interferon treatment can induce a dangerous aggravation of the liver disease and that a cautious attitude is warranted for treatment of cirrhotic patients.

Spontaneous exacerbations of chronic viral hepatitis have been reported to cause severe hepatic injury that may result in fatal liver failure (8,9,10). Withdrawal of immunosuppressive or cytotoxic therapy can induce similar complications in patients with chronic viral hepatitis (11,12). A first example of fatal exacerbation induced by α-interferon therapy, was recently reported by Marcellin et al. (13; case 1 of this study).

The relation between liver failure and α-interferon appeared probable in the patients who decompensated during therapy and never had an episode of decompensation before the start of treatment (group A). In case 4 the withdrawal of prednison could have been an additional impetus for the occurence of the fatal exacerbation. The death of the 4 patients who already exhibited signs of decompensated liver disease at the start of treatment (case 6-9 from group B) might have been secondary to a spontaneous progression of the disease and can thus not unequivocally be linked to the α-interferon therapy.

In the literature several explanations can be found for an association between α-interferon and fatal liver injury. In chronic hepatitis B α-interferon facilitates lysis of virus-infected cells by the cellular immune system which leads to an increase of inflammatory activity that could be lethal in a liver with limited residual capacity (4,9,14). A second possibility may be that after clearance of hepatitis B e antigen, whether or not α-interferon induced, coexisting precore mutant hepatitis B viruses are selected (15). These mutants have been implicated in fatal exacerbations (16,17). Other recently postulated reasons for liver failure secondary to α-interferon therapy are a direct hepatocellular toxic effect of α-interferon and α-interferon induced autoimmune-mediated chronic active hepatitis (18,19). In our study none of the patients had evidence of autoimmune chronic active hepatitis during α-interferon treatment.

The clinical pattern of deterioration in our patients mostly involved appearance of ascites, jaundice and encephalopathy that progressed even after α-interferon therapy was stopped. A relative late discontinuation of therapy in some of the patients could be mentioned as the reason for the unfavourable outcome. In any case it is clear that careful monitoring of cirrhotic patients who develop signs of decompensation under α-interferon therapy is essential.

Despite the serious adverse effects that can result from α-interferon in decompensated cirrhosis it is - in our opinion - justified to keep α-interferon in consideration as a therapy for these patients. Firstly, because a response with transition to viral latency can lead to a

marked regression of liver disease and probably prolonged survival (3,20). Secondly, for those cases in which liver transplantation remains the only option, transition to viral latency reduces the risk of reinfection of the graft (21). It is important, however, that the α-interferon therapy in decompensated patients is monitored closely by an experienced hepatologist, preferably in a centre with a liver transplantation program. Since 5 of the 9 patients described received an α-interferon dose under 10 MU per week, α-interferon related fatal decompensation is not restricted to high dose courses. Nonetheless, from a practical viewpoint it seems prudent to start with a low dose of α-interferon and carefully adjust the dose to the clinical, biochemical and virological condition of the patient. If severe decompensation occurs, possibilities to alter the course of the disease are early discontinuation of α-interferon treatment and in case of severe inflammatory activity a corticosteroid rescue course (22).

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CHAPTER 9

SEIZURES ASSOCIATED WITH LOW DOSE α-INTERFERON TREATMENT OF CHRONIC HEPATITIS B

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Introduction

The predominant neuropsychiatric side effects associated with α-interferon (IFN) treatment for chronic viral hepatitis are lethargy, lack of concentration, depression, emotional lability and confusion (1). We observed generalized seizures during low dose lymphoblastoid α-IFN treatment in 3 patients with HBeAg-positive chronic hepatitis B. Neither of the patients had a history of neurologic disease and we propose that seizures should be added to the list of neurotoxic effects of IFN treatment for patients with chronic viral hepatitis.

Case Reports

Case 1

A 24-year-old homosexual man with asymptomatic HIV-positive (CDC II) chronic hepatitis B, participating in a trial which evaluated combination therapy of α-IFN and retrovir, was started on 1.5 MU α-IFN daily; the dosage was increased to 3 MU after 4 weeks. After 10 weeks of treatment he had a generalized seizure, lasting 5 minutes. After recovery neurologic examination, cerebral CT-scan and analysis of cerebrospinal fluid were normal. Neuropsychological assessment revealed no signs of HIV-encephalopathy. Electroencephalogram showed a diffuse slow wave activity without epileptiform disturbances. He was started on phenytoin. IFN was continued because no clear relation between the seizure and IFN treatment was suspected. Six weeks later he had a second generalized seizure, 4 days after discontinuation of phenytoin therapy. He was given carbamazepin; the IFN course was completed a few days later. No neurologic sequelae have developed until now, 7 months later.

Case 2

A 49-year-old man with chronic hepatitis B entered a study of prolonged intermittent IFN treatment. He started with 5 MU of α-IFN daily. Four days after the first IFN-course of 4 weeks he had a generalized seizure. Neurologic examination, electroencephalogram and cerebral CT-scan, performed a few days later were normal. He was given diazepam. IFN was reinstituted 4 weeks later and in the third week of this course the patient again experienced a generalized seizure. He was started on carbamazepin; IFN was tapered to 1.25 MU daily and discontinued 17 weeks later. No seizures or other neurological problems have occured until now, 1 year later.

Case 3

A 28-year-old man suffering from chronic renal failure due to hepatitis B related membranoproliferative glomerulonephritis was treated with 3 MU of α-IFN thrice weekly. After 2 weeks of IFN treatment he developed a Moraxella peritonitis secondary to peritoneal dialysis. He was treated with gentamycin and rifampicin; IFN was continued. One week later he had a generalized seizure, lasting 10 minutes. The patient was afebrile and signs of peritonitis or meningitis were absent. There was no evidence of hypertensive encephalopathy. Diazepam was given; IFN was discontinued. The next day neurologic examination, electroencephalogram and cerebral CT-scan were normal; lumbar puncture was not performed in view of his normal temperature and rapid recovery. The patient was discharged from the hospital elsewhere, 5 days later. No further seizures have been observed until now, 8 months later.

Discussion

The 3 patients described had seizures which appeared to be related to IFN treatment. Apart from the electroencephalographic findings in the first patient which could have been induced by IFN (2), neurologic investigations were normal and at follow-up no signs or symptoms of a cerebral disorder have developed. Seizures have not been reported previously as a side effect of IFN treatment in chronic viral hepatitis. In cancer patients, receiving high doses (10-30 MU/m^2) of recombinant α-α-IFN, seizures were occasionally documented (3,4). Unlike these studies, we used low doses of lymphoblastoid α-IFN and the seizures we observed were not accompanied by other neuropsychiatric symptoms.

The mechanism by which IFN can induce seizures remains unclear. Passage of IFN through the blood-brain barrier was reported in animals (5), however, in humans IFN does hardly cross the blood-brain barrier and no correlations between neurotoxicity and IFN levels in cerebrospinal fluid could be made. In vitro, IFN is known to enhance the excitability of neurons (6). IFN is also known to stimulate endorphin-like activity suggesting that interference of IFN with the neuroendocrinic system is possible (7). Thus it is questionable whether IFN itself or other induced substances are responsible for the neurotoxic effects.

Now that IFN will be used more extensively in chronic viral hepatitis, it is important to warn physicians that even low doses of IFN may induce seizures, irrespective of the presence of other signs of neurotoxicity. In our experience, combination of adequate anticonvulsive medication during continued IFN treatment can be succesful in preventing further seizures.

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CHAPTER 10

SUICIDE ASSOCIATED WITH ALPHA-INTERFERON THERAPY FOR CHRONIC VIRAL HEPATITIS

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Abstract

We report on 2 attempted suicides and 1 completed suicide during or shortly after alpha-interferon (IFN) therapy for chronic viral hepatitis. While on therapy all 3 patients developed a psychiatric disorder leading to their suicidal behaviour. In a survey among fifteen European hospitals 3 cases of attempted and 2 of completed suicide during α-IFN therapy for chronic viral hepatitis, were additionally reported. None of the patients had a psychiatric history. α-IFN is known to lead to neuropsychiatric symptoms and our observations strongly suggest that these mental disorders could lead to suicidal behaviour. Therefore it is important that physicians, patients and their family are informed about the potential risk of the emotional and psychiatric disturbances that can occur during α-IFN therapy.

Introduction

Alpha-interferon (IFN) has become the major therapeutic modality for chronic viral hepatitis. The spectrum of side effects is one of the main drawbacks of this treatment (1). Neuropsychiatric toxicity, including cognitive dysfunction, irritability, change in personality and emotional lability, give rise to predominantly interpersonal problems, discontinuation of work and domestic discord (2,3). Other more serious IFN-induced signs of neurotoxicity include delirium, depression and seizures (2-4). These complications are thought to be reversible with dose reduction or cessation of therapy. We report on 3 patients who attempted to commit suicide during or immediately after treatment with α-IFN for chronic viral hepatitis.

Patients

Patient 1 was a 49-year-old marketing manager, married and father of three children. He had cirrhosis due to chronic hepatitis B with fatigue as major symptom. He had no psychiatric history. Serum HBeAg and HBV-DNA were positive and he was treated with 5 million units lymphoblastoid α-IFN daily. During the first months of therapy he complained of anorexia, extreme fatigue and depressive feelings, symptoms that could be attributed to α-IFN therapy. Just before cessation (week 12) of the succesful (HBeAg seroconversion) α-IFN course his behaviour changed completely. He was noted to be overactive, irritable and aggressive. He did not sleep anymore and thought he had contracted AIDS. One week later, a compulsary admission to the Psychiatry Department was necessary because of suicidal ideation and violent behaviour against his wife. Mental status examination elicited a manic psychosis characterized by agitation, dysphoria and persecutory delusions. There were no gross cognitive dysfunctions. One day after admission he attempted to swallow a dishwashing brush to deliberately lacerate his esophageal varices. Because of this suicidal behaviour and because of aggressive outburst towards staff personnel he had to be isolated. He was treated with 5-10 mg haloperidol daily, recovered completely and could be discharged from hospital within 4 weeks. Until now, three years later, he has been without any psychiatric symptoms.

Patient 2 was a 40-year-old housewife, married and mother of three children. She had contracted hepatitis C two years earlier, after receiving blood transfusions during a partial hepatectomy after a motorbike accident. Since that time she had suffered severe fatigue. She had a history of depressive feelings which occurred as a result of marital problems at

the age of 36 years, but she hadn't been in psychiatric treatment. Her aminotransferase values were about 5 times the upper limit of normal; on liver biopsy she had chronic active hepatitis without evidence of cirrhosis. For the chronic hepatitis C infection (serum anti-HCV and HCV-RNA positive) she received 6 million units recombinant α-IFN thrice weekly. During therapy she became increasingly withdrawn and depressed despite the fact that she responded to therapy with normalisation of aminotransferase values. Furthermore, she was very disappointed and angry that she could not get enough domestic help to relieve her from her daily household work. After 14 weeks of therapy she attempted suicide by taking an overdose of diazepam (40-60 mg). She was admitted to hospital and α-IFN was discontinued immediately. On admission she was somnolent but not disoriented. One day later she became agitated and insisted on going home. On mental status examination she exhibited no severe psychopathology, particularly no suicidal ideation and she was therefore discharged from the hospital. Subsequently she entered psychotherapy and her emotional problems disappeared.

Patient 3 was a 62-year-old chronic hepatitis B patient, married and father of two children. He had retired from his job as autopsy assistent 1 year before. At this job he had been infected with the hepatitis B virus 2.5 years previously. He had no psychiatric history. He was serum HBeAg and HBV-DNA positive, and had a mild chronic active hepatitis on histologic examination. He received 5 million units recombinant α-IFN daily for 4 and 16 weeks, separated by a 4-week interval without therapy. At the start of therapy he had no complaints. During treatment he complained of fatigue, myalgia, hair loss and lack of concentration. These side-effects of α-IFN did not seem to progress during the course of therapy. After 19 weeks of treatment he unexpectedly committed suicide by jumping before a train. When we - together with his family - retrospectively analysed his treatment course, it appeared that about 1 week before his death he had lost all interest for his usual daily activities and had become increasingly apathetic, withdrawn and reticent, symptoms suggestive of a depression. He had not overtly expressed suicidal thoughts. Nevertheless, his family was convinced that the suicide was related to the mental condition induced by α-IFN treatment.

Discussion

These 3 patients who became suicidal during or directly after α-IFN therapy were part of 215 patients with chronic viral hepatitis who have been treated with α-IFN (in courses of 3 to 6 months duration) at our department since 1985. At the start of α-IFN therapy all 3

patients lived a relatively normal life and did not have apparent psychiatric problems or a psychiatric history. Furthermore, they had no history of alcohol or drug abuse, were anti-HIV negative and had compensated liver disease with a good short-term prognosis. During α-IFN therapy they developed various mental disorders, in particular affective syndromes (emotional lability, apathy, depression and mania), that resulted in suicide attempts. The 2 patients who survived exhibited a complete psychiatric recovery after discontinuation of α-IFN.

To obtain more information on the frequency of this serious complication during α-IFN therapy we sent out a questionnaire to 19 European centres with large experience in α-IFN treatment of chronic viral hepatitis (participants of the European community sponsored concerted action on viral hepatitis: EUROHEP (5)). We asked about suicide attempts during - or within one month of discontinuation of - α-IFN therapy for chronic viral hepatitis. Fifteen hospitals from 10 countries responded. Among a total of 2,575 patients 2 completed and 3 attempted suicides were reported. One patient had chronic hepatitis B, 2 hepatitis C and 2 hepatitis D. Four of the 5 patients received at least 15 million units α-IFN per week (range 9-26 million units). The duration of therapy varied from 1.5 to 12 months (median 6 months). None of the 5 patients had decompensated liver disease and although 3 of the 5 contracted hepatitis by intravenous drug abuse none had an apparent psychiatric history and none was anti-HIV positive. Previously, Renault et al. described 2 α-IFN-treated patients with delirium who had developed suicidal ideation; one of them injected α-IFN intravenously in an attempt to commit suicide (3).

Since suicide appears to be an uncommon event during treatment with α-IFN, one might question whether this complication is indeed associated with α-IFN therapy. We could not find reports on the incidence of suicide attempts for untreated patients with chronic viral hepatitis. The combined incidence of suicide and suicide attempts over a period of 6 months in the general Dutch population, matched for age and sex to patients described in this paper is estimated to be 0.2 - 0.6‰ (6,7). The observed incidence of suicide attempts for our population treated with α-IFN was 13.9‰. The fact that none of our patients had serious psychiatric problems prior to therapy, that suicide has also been encoutered among α-IFN-treated patients in other hepatology clinics around the world and that several mental disorders (depression, psychosis and delirium) which could lead to suicidal behaviour are acknowledged as side effects of α-IFN therapy (2,3), argue in favor of a relation between suicide and α-IFN therapy.

The exact role of IFN in the origin of the wide range of organic mental disorders is as yet unknown. Although passage of IFN through the blood-brain barrier is limited, psychiatric symptoms and EEG abnormalities during IFN therapy have been described in detail

(2,3,8). IFN or one of its metabolites could be directly neurotoxic but may also induce other substances which interfere with neuronal excitation or neuroendocrine regulation (9,10).

Although we cannot prove directly that α-IFN induces psychiatric disturbances which lead to suicidal behaviour, this complication is so important and dangerous that physicians should be aware of the potential risk. Since it is difficult to predict which of the patients who exhibit common and reversible side-effects such as fatigue and mood changes are at risk of becoming suicidal, both the patient and the family should be informed to recognize signs of cognitive dysfunction, psychosis, affective disturbances and suicidality. If these signs occur immediate discontinuation of α-IFN therapy and psychiatric treatment are indicated, in which case the prognosis is good (3). Furthermore, physicians should be reluctant to start patients with a psychiatric history on an α-IFN treatment programme without psychiatric consultation beforehand.

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CHAPTER 11

DISCUSSION: HOW TO OPTIMIZE ALPHA-INTERFERON THERAPY FOR CHRONIC HEPATITIS B

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Introduction

Chronic hepatitis B is a disease which can lead to cirrhosis and serious complications such as portal hypertension, hepatic failure and hepatocellular carcinoma (1,2,3). For many years chronic HBV infections, like other forms of hepatitis, were classified according to the histologic degree of inflammatory activity as chronic persistent or chronic active hepatitis (4). Since patients can move from one category to the other and progression to cirrhosis is seen both for patients with chronic persistent and chronic active hepatitis, the prognostic value of this classification is presumably low (5,6). Symptoms during chronic HBV infections are also unreliable indicators of prognosis since many patients do not have any symptoms other than fatigue until the moment they exhibit decompensated liver disease. In addition to the results of the liver biopsy, classification of chronic hepatitis B should include information on the serum aminotransferase level and markers of viral replication (7,8). The latter two parameters are important for assessment of the prognosis and pivotal for determination of the necessity and the monitoring of antiviral therapy (table 1) (9,10).

Table 1. Indications for α-interferon (IFN) therapy for chronic hepatitis B patients.

HBeAg	HBV-DNA*	Aminotransferase	IFN	Remarks
positive	positive	elevated	yes	active chronic HBV infection
positive	positive	normal	no yes	monitor for rise in aminotransf. if biopsy shows active hepatitis if patient's wish
negative	positive	elevated	yes	pre-core mutant
negative	negative	elevated	no yes	exclude other liver disease if HDV or HCV positive
negative	negative	normal	no	

* detected with conventional hybridization techniques

Until recently no effective therapy was available for chronic hepatitis B. Although corticosteroid therapy can temporarily reduce the inflammatory activity, HBV replication usually increases resulting in a detrimental rather than a beneficial effect in the long run (11,12). Longitudinal studies on the natural history of chronic HBV infection have shown that active viral replication, indicated by serum positivity for HBeAg and HBV-DNA, is associated with continuing inflammatory activity and with deterioration of liver disease.

Termination of viral replication with HBeAg and HBV-DNA clearance (HBeAg seroconversion) is usually accompanied by clinical, biochemical and histological remission of liver disease activity, and presumably with a better quality of life (13,14). α-IFN can induce HBeAg seroconversion in 30 to 40 percent of the patients with chronic hepatitis B (15-19). In more than 85% of the cases this response is sustained and more than half of the patients become HBsAg negative after long-term follow-up (20). Until now it has not been clear whether successful α-IFN therapy reduces the risk for hepatocellular carcinoma.

Modification of the Alpha-Interferon Treatment Schedule

Standard Treatment

In randomized controlled trials both recombinant and human lymphoblastoid α-IFN have been shown to be effective in inducing HBeAg seroconversion in chronic hepatitis B patients. Many studies on α-IFN treatment in HBV infections, however, are characterized by a considerable heterogeneity in patient population and response rate, contain few patients and should therefore be interpreted with caution. Perrillo et al. (15) reported on a large randomized controlled trial of recombinant α-IFN treatment: HBeAg seroconversion with histologic improvement was achieved in 38% of the patients who received the standard treatment of 5 mega U α-interferon daily for 16 weeks compared to 7% of the untreated controls. In the USA and in northwestern Europe the standard duration of α-IFN therapy is now considered to be 12 to 16 weeks. The most effective dosage of α-IFN for chronic hepatitis B is probably 30 MU per week. Regimens with α-IFN dosages under 15 MU per week have been shown to lack efficacy while doses above 30 MU weekly are too toxic for the vast majority of patients (15). There does not appear to be a difference in response rate between daily dosages and a schedule of 3 administrations per week.

Prolonged Treatment

In several trials from southern Europe α-IFN therapy was given for approximately 24 weeks (19,21). One of these studies suggests but does not prove an additional effect of prolonged treatment schedules. In the UK a comparison between 12 and 24 weeks of lymphoblastoid α-IFN treatment could not demonstrate a beneficial effect of the prolonged course (22). The results of this study were, however, markedly influenced by a lack of compliance (8 of the 20 patients withdrew from the longer course) mainly due to side-effects of α-IFN. Results of a pilot study performed in our institute suggest that extending α-IFN treatment for up to 32 weeks might enhance the HBeAg seroconversion and HBsAg

seroconversion rates (23). Interestingly, none of the patients in this study had to discontinue α-IFN therapy because of intolerance. On the grounds of these results a group of European hospitals (Eurohep; 24) initiated a large randomized trial to evaluate whether prolongation of α-IFN therapy for chronic hepatitis B is effective in enhancing the rate of HBeAg seroconversion in patients who did not respond to standard α-IFN treatment of 16 weeks duration.

Intermittent Treatment

Intermittent therapy is a rather new approach to increase the response rate for α-IFN treatment (23). We conducted a pilot study consisting of a one-month priming course of α-IFN, one month of rest and a second course lasting 4 months. The rationale for the initial month of therapy was to decrease viral replication and induce a short immunologic rebound before the start of the second long-term course, thereby enhancing the possibility of successful therapy. Forty-eight percent of these patients exhibited HBeAg seroconversion. The level of HBV replication, as monitored by quantitative HBV-DNA and HBeAg assessment at the start of the second course, was significantly lower than at baseline. Furthermore we observed a double flare of the median aminotransferase values, indicating that both courses could have induced a response in some of the patients. In our opinion these interesting results justify evaluation of intermittent α-IFN therapy in a controlled setting.

Combination Treatment with Alpha-Interferon

Alpha-Interferon and other Immunemodulating Agents

A withdrawal course of corticosteroids before α-IFN therapy may combine enhanced immunity with suppression of HBV replication. Several controlled trials which evaluated the benefit of pretreatment with prednisone (tapered from 45-60 to 15-20 mg per day in 6 weeks followed by 30-35 MU α-IFN per week for 16 weeks) did not show an additional effect of the priming steroid course (15,25,26). Nevertheless, in two trials the HBeAg seroconversion rate of the prednisone-α-IFN schedule was markedly higher for the subgroup of patients with low aminotransferase levels at baseline. This finding suggests that patients with mild inflammation may benefit from this combination therapy. The benefit of combining α-IFN with another immunomodifying agent, levamisol, is questionable as a recent study has demonstrated that this combination resulted in a lower response rate than α-IFN monotherapy (27). Simultaneous administration of α-IFN with other cytokines such as γ-interferon or interleukin-2 appears to be an attractive option since

expression of not only HLA type I but also HLA type II antigens on the hepatocyte membrane is stimulated (28). This could lead to an additional impetus for the attack of virus-infected hepatocytes by the cellular immune system. However, the results of several studies of these combination therapies have so far been disappointing (29,30).

Alpha-Interferon and Nucleoside Analogues

In addition to α-IFN, which exerts its antiviral effect primarily by stimulation of cytotoxic T cells and inhibition of HBV protein synthesis, nucleoside analogues interfere at a different site in the HBV replication cycle inhibiting HBV DNA polymerase, a crucial enzyme for viral DNA synthesis. ARA-AMP, acyclovir and zidovudine are at this moment the most extensively investigated agents in HBV infected humans. Garcia and colleagues investigated the alternating administration of ARA-AMP and α-IFN in comparison to ARA-AMP and placebo (31). No effect of ARA-AMP alone or the combination could be demonstrated. About 25% of the patients receiving ARA-AMP suffered from painful paresthesia necessitating dose reduction. The combination of α-IFN with other nucleoside analogues like acyclovir, descylovir or zidovudine was extensively studied in our institute. As a result of the positive findings of preliminary studies on the combination of α-IFN with acyclovir derivatives (32,33), we initiated a large randomized controlled trial comparing patients on combined α-IFN (5 MU daily for 16 weeks) and acyclovir (2 g daily iv for 4 weeks) therapy with untreated controls (34). Only 25% of the treated patients responded with HBeAg seroconversion as opposed to 14% of the controls. Similarly, we conducted a controlled trial comparing the antiviral effect of α-IFN and zidovudine with that of α-IFN and placebo after both in vitro and in vivo pilot studies showed that zidovudine caused a decrease in HBV replication (35-37). α-IFN and zidovudine were given for 16 weeks in doses increasing to 5 MU and 1000 mg daily, respectively. Again we were disappointed as there was no significant difference in the response rate between the 2 groups and quantitative analyses of viral markers indicated that no additional virus-inhibiting effect was obtained by adding zidovudine to the α-IFN therapy. Furthermore, in the combination group bone marrow toxicity was prominent, leading to reduction of the dose of both α-IFN and zidovudine in more than 40% of the cases.

The conclusion to be derived from these results is that as yet no combination therapy appears more effective than α-IFN alone and that several combinations give rise to an increase in dose-limiting toxicity. Therefore in future studies α-IFN should only be co-administered with agents which combine a potent antiviral effect with a very low toxicity profile.

Response Prediction of Alpha-Interferon Therapy

Since only one-third of the chronic hepatitis B patients respond to α-IFN therapy and since α-IFN is a costly drug associated with marked side effects, it is important to know what the chances of a succes are before the start of α-IFN therapy. The most important variables which have been found to influence the response to α-IFN are listed in table 2 (15,16,18,23,38,39). In almost all studies on factors predictive for response, a high aminotransferase level and a low level of viral replication (HBV-DNA) appeared to enhance the response rate. Because the spontaneous HBeAg seroconversion rate is much higher in this type of patient than in other untreated patients it is presumed that the immune system is already in an activated state. For this reason some investigators question the usefulness of α-IFN for patients with such a profile. From our experience α-IFN therapy may be beneficial for this group because the response rate with α-IFN is higher than that for controls matched for the level of HBV replication and inflammatory activity.

Table 2. Factors with predictive value for the occurrence of HBeAg seroconversion in chronic hepatitis B patients treated with α-interferon.

Pretreatment	During treatment
High aminotransferase level	Rise or peak of aminotransferases
Low HBV-DNA level	HBV-DNA negativity
HIV antibodies absent	Rise of IgM anti-HBc
History of acute symptomatic hepatitis	
Short duration of HBsAg positivity	
Non-oriental race	
Absence of immunosuppressive therapy	
Inflammatory activity on liver biopsy	

Brook and associates applied univariate and logistic regression analysis to pretreatment variables of 114 chronic hepatitis B patients treated with α-IFN (40). They found the levels of HBV-DNA and aspartate aminotransferase as well as the history of acute hepatitis to be independently related to response. Using these same variables plus the HIV status they constructed a model which could predict a response to therapy with a sensiti-

vity and specificty of about 80%. Although the accuracy of this model could be improved, it does help physicians to estimate the chances of response and possibly also the best time for starting therapy. Furthermore, this model helps investigators to determine whether a certain response rate in an uncontrolled population is caused by selection of the patients or by modification of the α-IFN therapy.

So far all studies on response prediction have been applied on patients who had not been treated with α-IFN previously. Evaluation of the results of α-IFN retreatment of a group of 18 patients who did not respond to an initial 16-week α-IFN course revealed that after 1 year of follow-up only 11% of the patients had responded to the second α-IFN course (41). This low response rate did not seem to be related to important clinical characteristics that influence the response to initial α-IFN therapy, suggesting that previous nonresponse in itself or other (unknown) factors could play an important role in response prediction. A large controlled trial which is in progress will further elucidate the benefit of α-IFN retreatment.

Quantitative Monitoring of HBV Replication

HBV-DNA, HBV-DNA polymerase and HBeAg are the most frequently used parameters of HBV replication. Until recently these markers were only assessed qualitatively or semiquantitatively. The availability of successful antiviral therapy has led to the need for quantitative monitoring of viral replication. Close monitoring of quantified HBV markers during an α-IFN course gives the physician important information on the therapeutic effect which in turn supports the process of medical-decision making and determines modification of the treatment schedule. For example, on the basis of a decline or stabilization of the HBV-DNA level one could decide to continue or stop therapy, respectively (23).

HBV-DNA and HBV-DNA polymerase

During successful α-IFN therapy the usual sequence of the disappearance of viral markers in serum is HBV-DNA polymerase, HBV-DNA and HBeAg. HBV-DNA and HBV-DNA polymerase are widely used in research laboratories but not yet in routine diagnostics (42-44). Since HBV-DNA polymerase activity is a marker which can only be detected by laborious methods that yield results with a rather low sensitivity for loss of viral replication, it is increasingly being replaced by HBV-DNA (45). HBV-DNA provides a more direct indication of the presence of infectious viral particles and has therefore become an important parameter of the effect of α-IFN therapy. Dot spot or liquid phase hybridization methods can be applied to measure HBV-DNA, yielding a sensitivity of about 10^4 to 10^5

genomes per ml (46,47). In a comparison of two commercially available HBV-DNA assays we found a good correlation between the HBV-DNA concentrations (48). However, the actual agreement of the results was low because the HBV-DNA level between the assays consistently differed by a factor 5 to 10. These discordant results were most probably due to differences in standardization of the positive control samples, indicating the need for a uniform HBV-DNA reference panel (49). In addition to conventional techniques HBV-DNA can also be assessed by means of the polymerase chain reaction (PCR) (50-52). By in vitro amplification of target DNA, levels below 10 genomes can be detected. The clinical relevance of this extremely sensitive method is as yet unknown because many HBsAg-positive carriers with inactive liver disease are still HBV-DNA positive by PCR. Longitudinal studies have demonstrated that a negativate PCR assessement of HBV-DNA often approximates the moment of HBsAg seroconversion (20).

Hepatitis B related Antigens

Of the HBV antigens present in serum HBeAg probably reflects the level of HBV replication most directly. For patients infected with wild type HBV the dynamics of the HBeAg level correlate closely with those of the HBV-DNA and HBV-DNA polymerase levels (23,53). HBeAg seroconversion is an important event which indicates a transition from HBV replication to viral latency. Unlike HBV-DNA negativity determined by dot-blot or solution hybridization methods, which can be followed by a relapse, HBeAg seroconversion is almost always sustained (20,23). Therefore, quantitative HBeAg assessment could be used both for monitoring and endpoint determination of antiviral therapy in patients carrying wild type HBV (53). At present a standard test for quantitative measurement of HBeAg is being developed (L. Mimms, personal communication) (54). Patients carrying a mutant type of HBV with a deletion in the pre-core region of the genome cannot produce HBeAg and often have active viral replication in the absence HBeAg (55,56). Although the efficacy of α-IFN against this HBV variant is still a subject of debate (57,58), obviously HBV-DNA and not HBeAg is the viral marker of choice for monitoring treatment of these patients.

Other viral antigens which can be detected in serum are the s gene encoded proteins HBsAg, pre-S1 Ag and pre-S2 Ag. All 3 markers have been found to correlate with the degree of HBV replication (59-61). Patients who were followed during α-IFN therapy exhibited a good correlation between the HBsAg and the HBeAg or HBV-DNA level (62). Since more than 90% of the patients who respond (HBeAg and HBV-DNA negative) to α-IFN remain HBsAg positive, the HBsAg cannot serve as an assessment for the endpoint of α-IFN therapy. Furthermore, the wide range of HBsAg concentrations necessitate the use

of dilution steps in the quantitative measurement of this antigen. Nevertheless, for those laboratories without the possibility to measure HBV-DNA or HBeAg quantitatively, HBsAg measurement might be an alternative for assessment of changes in HBV replication. HBsAg could also be a marker of interest for patients with hepatitis D or pre-core mutants who often have active disease without the presence of HBeAg.

Decompensated Liver Disease and Alpha-Interferon

As in other forms of liver disease, indicators of impaired liver function (bilirubin, albumin, coagulation factors) and portal hypertension (ascites and variceal bleeding) are related to the survival of patients with HBsAg-positive cirrhosis (63-65). In addition to these variables the HBeAg status also seems to be a factor that influences life expectancy of cirrhotic HBV patients since the event of HBeAg seroconversion is associated with prolonged survival (65). However, if patients exhibit signs of hepatic decompensation the HBeAg status appears to loose its predictive value, suggesting that HBeAg seroconversion in decompensated patients - whether α-IFN-induced or not - will not lead to an improved prognosis. Nevertheless, anecdotal results in the USA indicate that α-IFN-induced arrest of HBV replication in decompensated patients may lead to termination of disease progression, disappearance of symptoms and preservation of the remaining hepatic function (66). During therapy the majority of patients developed serious bacterial infections requiring antibiotic therapy. Moreover, either exacerbation of hepatitis or side effects led to restriction of dose and/or duration of therapy in all cases. In addition to its potential therapeutic effect on the patient's own liver α-IFN may also be beneficial for patients with decompensated disease for which liver transplantation remains the only realistic therapeutic option. In these patients pretransplant transition to viral latency would reduce the risk of infection of the graft (67). Currently a controlled trial to investigate the benefit of low dose α-IFN for patients with decompensated HBV cirrhosis is in progress (R. Perrillo, personal communication).

That α-IFN therapy for patients with (decompensated) cirrhosis is not without risks was illustrated by 9 reported cases of fatal decompensation during or shortly after α-IFN therapy (68,69). All patients had cirrhosis and 5 did not have signs of decompensation at the start of therapy. It is conceivable that the transient exacerbation of hepatitis, which is a sign of α-IFN-facilitated hepatocyte lysis, could be deadly in patients with limited residual liver capacity. One of the 9 patients first received a short-term prednisone course which could have been an additional factor in the occurrence of fatal decompensation (70). One could conclude from these results that physicians should closely monitor α-IFN-treated

patients with cirrhosis; when signs of decompensation occur therapy should be discontinued immediately. Patients with decompensated cirrhosis should only be treated with α-IFN on strict indications and under careful monitoring, preferably in a centre with a liver transplantation programme. It seems prudent to start with low dose α-IFN therapy (1-2 MU thrice weekly) and carefully adapt the dose according to the clinical, biochemical and virological parameters.

Adverse Effects of Alpha-Interferon

The variety of side effects associated with α-IFN therapy is well known. Table 3 lists the most frequently reported side effects in 100 consecutive chronic hepatitis B patients treated with α-IFN (71). Fever, myalgia and other flu-like symptoms are seen in the majority of the patients during the initial days of therapy but can be suppressed by paracetamol or indomethacin therapy. Administration of these drugs does not affect the antiviral or immunomodulating effects of α-IFN (72); indomethacin might even enhance the action of α-IFN (73). After the first week of therapy complaints of fatigue, apathy and anorexia prevail. Fortunately, most adverse effects are reversible with dose reduction or discontinuation of therapy.

Table 3. Side effects during α-interferon therapy in 100 consecutive HBV patients.

	Incidence	Cause of IFN dose reduction
Fatigue	74%	11%
Fever/Malaise	61%	-
Myalgia	58%	4%
Leukopenia ($<3 \times 10^9$/l)	56%	8%
Anorexia	29%	-
Gastrointestinal problems	28%	-
Depression/lethargy	24%	3%
Loss of hair	21%	-
Weight loss (>5%)	18%	-
Trombocytopenia ($<50 \times 10^9$/l)	7%	4%
Neurotoxicity	7%	4%

A serious side effect of α-IFN which is mostly not reversible is autoimmune-mediated thyroid disease (74). Although it appears to be confined mainly to hepatitis C, both hyperthyroidism and hypothyroidism can also occur during α-IFN therapy for HBV-infected patients. Since these diseases can be treated successfully, screening of thyroid hormones before and during therapy is recommended.

Neuropsychiatric Side Effects

A group of underestimated symptoms induced by α-IFN is the spectrum attributable to neuropsychiatric toxicity. In particular lethargy, loss of concentration, depressive feelings, emotional lability and irritability are not always diagnosed or thought to be related to α-IFN therapy (75,76). These symptoms appear rather innocent but they often give rise to domestic problems or temporary discontinuation of work. The best way to reduce these problems is careful explanation that the symptoms are treatment-related and will disappear once therapy has been discontinued. As a second step dose reduction or psychiatric counseling should be considered. More serious forms of neuropsychiatric toxicity include depression, psychosis and seizures (77,78). One of the most dramatic consequences of the serious psychiatric disorders arising from α-IFN therapy is suicidal behavior. Renault et al. described one patient who became suicidal during α-IFN therapy (77). After an extensive survey in 16 European hospitals we found 5 cases of attempted and 3 of completed suicide among a total of about 2,500 chronic viral hepatitis patients on α-IFN therapy (79). Even though it appears to be an uncommon event and we cannot prove directly that the suicides were caused by α-IFN, the fact that none of the patients had a psychiatric history, none had a poor life expectancy and all developed serious psychiatric disorders (depression, psychosis and delirium) during α-IFN therapy argue in favor of a relation between α-IFN therapy and suicide. Therefore, although this complication is rare it is so dangerous that it is important to instruct patients and family to recognize signs suggestive of serious cognitive dysfunction and affective disturbances. If these signs occur psychiatric consultation and/or discontinuation of therapy is warranted.

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SUMMARY

The positive results of α-interferon (IFN) therapy have generated an important change in the therapeutic approach of chronic hepatitis B patients. The studies presented in this thesis are directed to the question how the efficacy of α-IFN therapy for chronic hepatitis B could be improved (chapter 1). In particular we investigated whether patient selection, modification of the treatment schedule and new virological assessments could contribute to answer this question.

For optimal management of the HBV-infected patients it is important to define indications for new treatment modalities as α-IFN and liver transplantation. We followed a cohort of 98 patients with HBsAg-positive cirrhosis and determined which variables influenced the survival (chapter 2). High age, the presence of ascites and a high serum bilirubin level were indepently associated with a short duration survival. For the group with a compensated cirrhosis HBeAg-negative patients exhibited a significantly better prognosis than HBeAg-positive patients. In contrast we could not find a difference in survival in decompensated patients with a different HBeAg status. These findings suggest that there is an indication for antiviral therapy for patients with a compensated hepatitis B cirrhosis and active viral replication (HBeAg positive) whereas liver transplantation appears the major therapeutic option for patients with a decompensated chronic hepatitis B infection.

The standard course of α-IFN therapy for chronic hepatitis B lasts 16 weeks and leads in about one third of the patients to cessation of virus replication. Intermittent α-IFN therapy of 24 weeks duration and prolongation of treatment in patients who already exhibited a partial response (HBV-DNA negative) could enhance the HBeAg serocvonversion rate (chapter 3). Another modification of the standard α-IFN treatment is combination therapy with zidovudine. In a randomized controlled study (chapter 4) we found that this combination was not more effective than α-IFN monotherapy. In patients treated with the combination of α-IFN and zidovudine side effects, in particular anemia and leucopenia, were more prominent than in those treated with α-IFN and placebo. In chapter 5 the benefit of α-IFN retreatment in chronic hepatitis B patients who previously failed to respond was evaluated. A response (HBeAg seroconversion) to therapy occurred in 2 of the 18 (11%) investigated patients. This response rate may be comparable to the spontaneous HBeAg seroconversion incidence and therefore the efficacy of α-IFN retreatment in chronic hepatitis B appears limited. Variables with a known predictive value towards response for initial α-IFN courses (e.g. the level of inflammatory acitvity) did not influence the result of the retreatment.

An important aspect of antiviral therapy in chronic hepatitis B is monitoring of quantitated

viral markers. Using these markers one could inform patients during α-IFN treatment about the effect of the therapy and one could either prolong or stop therapy in case of a partial or no response, respectively. The most important marker reflecting the level of virus replication is the Hepatitis B virus DNA (HBV-DNA). We compared 2 HBV-DNA test systems (Abbott HBV-DNA assay, Abbott and AffiProbe assay, Orion) in sera obtained from patients treated with α-IFN (chapter 6). Although there was a good correlation between the HBV-DNA results of the assays the AffiProbe test yielded systematically 5 to 10 times higher HBV-DNA values than the Abbott assay. These discordant results were most probably due to differences in the standardization of the positive control samples of the test systems indicating the need for a uniform HBV-DNA reference panel. Since HBV-DNA can only be measured quantitatively with time-consuming and costly methods its detection has been restricted to research laboratories so far. For the many hospitals not having the possibility to assess HBV-DNA there might be a cheap and simpel alternative, quantitative HBsAg measurement. The level of HBsAg decreases significantly after cessation of HBV replication (HBeAg seroconversion) and for patients who were followed during α-IFN therapy we found a good correlation between the HBsAg level and both the quantitative HBV-DNA and HBeAg values, irrespective of the result of therapy (chapter 7).

Due to a virus specific immunological response α-IFN can cause a transient exacerbation of inflammatory activity in chronic hepatitis B patients. This exacerbation could lead to a fatal hepatic decompensation in patients with cirrhosis (chapter 8). After a patient in our institute died due to a fatal decompensation during α-IFN therapy we sent out a questionnaire to study the frequency and clinical aspects of this serious complication. Among 2490 patients 8 additional cases with fatal hepatic decompensation during α-IFN therapy were reported. Seizures and suicide attempts are 2 other complications associated with α-IFN therapy of patients with chronic viral hepatitis. In chapters 9 and 10 we described several patients who exhibited these serious neuropsychiatric adverse events of α-IFN treatment.

In the chapter 11 the findings of studies presented in this thesis are discussed within an overview of other recent developments of α-IFN therapy for chronic hepatitis B.

SAMENVATTING

De hoopgevende behandelingsresultaten met α-interferon (IFN) hebben in de laatste jaren geleid tot een belangrijke wijziging in het medisch handelen bij patiënten met chronische hepatitis B. Het onderzoek in deze dissertatie richt zich op de vraag hoe de effectiviteit van α-IFN behandeling bij chronische hepatitis B verbeterd kan worden (hoofdstuk 1). Met name is onderzocht in welke mate de selectie van patiënten, verandering van het behandelingsschema en nieuwe virologische vervolgmethoden hieraan kunnen bijdragen.
Het is voor een goed medisch beleid belangrijk om te weten in welke fase van de leverziekte behandeling met α-IFN geïndiceerd is en wanneer beter overgegaan kan worden op levertransplantatie. Wij onderzochten welke factoren de overleving van een cohort van 98 patiënten met een HBV-gerelateerde levercirrhose beïnvloeden. Hoge leeftijd, de aanwezigheid van ascites en een hoog serum bilirubine-gehalte waren onafhankelijk van andere factoren geassocieerd met een korte overlevingsduur. In de groep met een gecompenseerde cirrhose hadden HBeAg-negatieve patiënten een significant langere overleving dan HBeAg-positieve patiënten. In tegenstelling was er bij de groep van gedecompenseerde patiënten geen verschil in overleving bij patiënten met een verschillende HBeAg status. Deze bevinding suggereert dat er voor patiënten met een gecompenseerde hepatitis B cirrhose met virus replicatie (HBeAg positief) een indicatie bestaat om door middel van antivirale behandeling een viruslatentie te induceren terwijl bij patiënten met een gedecompenseerde leverziekte levertransplantatie waarschijnlijk de belangrijkste therapeutische optie is.
De standaard α-IFN behandeling bij chronische hepatitis B duurt 16 weken en leidt in ongeveer een derde van de patiënten tot een verdwijning van de virusreplicatie. Intermitterende behandeling gedurende 24 weken en een verlenging van de behandeling bij patiënten die al een partiële response (HBV-DNA negatief) vertonen zou mogelijk kunnen leiden tot een verhoging van het aantal HBeAg seroconversies (hoofdstuk 3). Een andere modificatie van de standaard behandeling met α-IFN is combinatie therapie met zidovudine. In een gecontroleerd onderzoek (hoofdstuk 4) werd aangetoond dat deze combinatie niet effectiever is dan α-IFN monotherapie. In de groep behandeld met de combinatie van α-IFN en zidovudine waren er beduidend meer bijwerkingen, met name anemie en leukopenie, dan in de groep die alleen α-IFN kreeg. In hoofdstuk 5 wordt nagegaan of herbehandeling van chronische hepatitis B patiënten die bij een voorgaande behandeling geen response (HBeAg seroconversie) ondergingen effectief is. Van de 18 patiënten waren er slechts 2 (11%) die een response vertoonden. Dit percentage is niet hoger dan het spontane HBeAg seroconversie percentage en de effectiviteit van

herbehandeling met α-IFN lijkt dus beperkt. Factoren die een bewezen predictieve waarde ten aanzien van de response bij een eerste α-IFN therapie hebben (bijvoorbeeld het niveau van ontstekingsactiviteit) bleken de uitkomst van de herbehandeling niet te beinvloeden.
Een belangrijk aspect van de antivirale behandeling bij chronische hepatitis B is het vervolgen van gekwantificeerde virusparameters (hoofdstuk 6 en 7). Bij α-IFN therapie kan men met behulp van deze waarden de patient informeren over het beloop van de behandeling en kan men bepalen of er al dan niet een response aanstaande is zodat de behandeling respectievelijk gecontinueerd of gestopt dient te worden. Een van de belangrijkste parameters die de mate van virusreplicatie weergeven is het hepatitis B virus DNA (HBV-DNA). Wij hebben 2 HBV-DNA testsystemen (HBV-DNA assay, Abbott en AffiProbe assay, Orion) vergeleken in sera van patienten behandeld met α-IFN. Hoewel er een zeer goede correlatie bleek te bestaan tussen de HBV-DNA uitslagen van de 2 testen was er een systematisch 5 tot 10-voudig verschil tussen de uitkomsten. Uit onderzoek naar positieve controle panels van de testen bleek er een verschil in de standaardisatie van de hoeveelheid HBV-DNA in deze panels te zijn. Deze bevinding maakt duidelijk dat er behoefte is aan een uniform HBV-DNA referentiepanel. Omdat het HBV-DNA alleen met behulp van bewerkelijke en kostbare methoden kwantitatief gemeten kan worden is de uitvoering van deze bepaling vooralsnog beperkt tot gespecialiseerde laboratoria. Voor de vele centra die niet de mogelijkheid hebben om HBV-DNA te meten is er wellicht een goedkoop en simpel alternatief, de kwantitatieve HBsAg bepaling. De HBsAg concentratie in serum blijkt significant te dalen na het verdwijnen van de virusreplicatie (HBeAg seroconversie) en bij patienten die vervolgd zijn tijdens behandeling met α-IFN blijkt er onafhankelijk van de uitkomst van de therapie een goede correlatie te zijn tussen het de kwantitatieve HBsAg waarden en zowel de kwantitatieve HBV-DNA als HBeAg waarden.
Bij α-IFN behandeling van chronische hepatitis B patienten kan er een door het immuunsysteem gegenereerde tijdelijke exacerbatie van ontstekingsactiviteit optreden. Deze exacerbatie kan bij patienten met een cirrhose leiden tot een fatale leverdecompensatie (hoofdstuk 8). Naar aanleiding van een dergelijke casus in ons ziekenhuis onderzochten wij met behulp van een enquete de frequentie en klinische aspecten van deze ernstige complicatie van de behandeling. Epilepsie en suicidepogingen zijn 2 andere ernstige complicaties welke gerelateerd lijken te zijn aan α-IFN therapie bij chronische virale hepatitis. In hoofdstuk 9 en 10 zijn enkele cases beschreven die de kliniek van deze ernstige neuropsychiatrische bijwerkingen weergeven.
In de discussie (hoofdstuk 11) worden de bevindingen van de studies uit dit proefschrift geplaatst binnen een overzicht van andere recente ontwikkelingen van antivirale behandeling met α-IFN bij chronische hepatitis B.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 19 mei 1964 te Alkmaar. Hij groeide op in de Achterhoek waar hij achtereenvolgens in Groenlo (Marianum) en Silvolde (Isala College) het V.W.O. bezocht. In 1982 verhuisde hij naar Nijmegen om aan de Katholieke Universiteit geneeskunde te studeren. Het doctoraal examen werd behaald in juli 1986 en het artsexamen in oktober 1989. Van januari tot en met oktober 1988 verrichtte hij onderzoek naar de neurotoxiciteit van cyclosporine bij levertransplantaties (supervisie P.C. de Groen, M.D.) op de afdeling Farmacologie van de Mayo Clinic in Rochester, Minnesota, U.S.A. Na het artsexamen was hij voor een periode van 3 jaar werkzaam op de afdeling Interne Geneeskunde II van het Academisch Ziekenhuis Dijkzigt te Rotterdam (hoofd Prof. J.H.P. Wilson). In deze periode werd onder leiding van Prof. dr. S.W. Schalm gewerkt aan het onderzoek beschreven in dit proefschrift. In januari 1993 werd begonnen met de opleiding tot internist in het Westeinde Ziekenhuis te 's Gravenhage (opleider dr. E.J. Buurke).