STUDIES ON TREATMENT OF CHRONIC HEPATITIS B, C AND D

ONDERZOEK NAAR DE BEHANDELING VAN CHRONISCHE HEPATITIS B, C EN D

proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam,
op gezag van de rector magnificus
Prof. dr. C.J. Rijnvos
en volgens besluit van het college van Dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 6 november 1991 om 15.15 uur

door

Lucas Berk

geboren te Delft

Promotiecommissie

Promotor:	Prof. dr. S.W. Schalm
Overige leden:	Prof. dr. N. Masurel Prof. dr. J. Huisman Drs. M. van Blankenstein

voor Gerty en Lisan
voor mijn ouders

Met dank aan:

Roche Nederland B.V.

voor het financieren van de drukkosten van dit proefschrift.

CONTENTS

	List of abbreviations	
Chapter 1	Introduction	1
1.1	History	3
1.2.1	History of hepatitis B virology	3
1.2.2	HBV replication cycle	6
1.2.3	Treatment strategy for chronic hepatitis B	6
1.2.4	Treatment of chronic hepatitis B	7
1.3.1	History of hepatitis C virology	9
1.3.2	Treatment strategy for chronic hepatitis C	9
1.3.3	Treatment of chronic hepatitis C	11
1.4.1	History of hepatitis D virology	12
1.4.2	Treatment strategy for chronic hepatitis D	12
1.4.3	Treatment of chronic hepatitis D	12
1.5	Aims of the study	14
Chapter 2	Failure of acyclovir to enhance the antiviral effect of alpha-lymphoblastoid interferon on HBe-seroconversion in chronic hepatitis B.	19
Chapter 3	Complementary HBeAg monitoring during antiviral therapy for chronic hepatitis B.	33
Chapter 4	Modulation of alpha-interferon effects by indomethacin and paracetamol in chronic hepatitis B.	45
Chapter 5	Zidovudine inhibits hepatitis B virus replication.	55
Chapter 6	Beneficial effects of Phyllanthus Amarus for chronic hepatitis B, not confirmed.	67
Chapter 7	Alpha-lymphoblastoid interferon and acyclovir for chronic hepatitis Delta.	75
Chapter 8	Severe chronic active hepatitis (autoimmune type) mimicked by coinfection of hepatitis C and human immunodeficiency virus.	87
Chapter 9	Rifampicin for chronic hepatitis C with incomplete response to alpha-interferon.	97
Chapter 10	Discussion.	105
Chapter 11	Summary Acknowledgements Curriculum vitae	111

LIST OF ABBREVIATIONS

ACV	acyclovir
ALT	alanine aminotransferase
anti-HBc	antibody to hepatitis B core antigen
anti-HBe	antibody to hepatitis B e antigen
anti-HBs	antibody to hepatitis B surface antigen
anti-preS1	antibody to hepatitis B pre-S1 antigen
anti-preS2	antibody to hepatitis B pre-S2 antigen
ARA-A	adenine arabinoside
ARA-AMP	adenine arabinoside monophosphate
AST	aspartate aminotransferase
AZT	azido thymidine
AZT TP	azido thymidine trihosphate
cpm	counts per minute
DNA	deoxyribonucleic acid
DNAp	deoxyribonucleic acid polymerase
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B virus e antigen
HBsAg	hepatitis B virus surface antigen
HBxAg	hepatitis B virus x antigen
HCV	hepatitis C virus
HDV	hepatitis D virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IFN	interferon
Kb	kilobase
RIA	radioimmunoassay
RNA	ribonucleic acid
RNAp	ribonucleic acid polymerase

CHAPTER 1

INTRODUCTION

1.1 History

Tsji Pa, physician to the Chinese emperor Hoang Ti (2674-2575 B.C.), described the syndrome of jaundice with fatigue, arthralgia and malaise as related to diseases of the liver. At that time the treatment varied from administering herbs to restoring the yin-yang balance with acupuncture (1).

Two thousand years later Hippocrates described the same syndrome and differentiated liver disease due to the abuse of wine, a fulminant form of hepatitis and a third form that rendered the patient immune after recovery. His therapy consisted of melikraton (made from honey) and venapuncture (2).

In the same era liver disease in India was treated with herbs among which Phyllanthus amarus; this plant contains alkaloids, galactosamines and flavonoids, which are substances that have an antihepatotoxic and possibly an antiviral activity (3,4,5).

In the previous century jaundice was described to a gastroduodenitis with a catarrhal obstruction to bile (Bamberger 1855, Virchow 1865). The first report of a parenterally transmitted outbreak of hepatitis appeared by the end of the 19th century in factory personnel after revaccination against smallpox (Lürman 1885). In 1937 epidemiologic data accumulated to substantiate the existence of an infectious form of hepatitis, with outbreaks in schools, hospitals and other institutions (Sergeant 1937, Lisney 1937, Barber 1937, Cullinan 1939). Until the sixties, the main treatment for hepatitis remained symptomatic with rest, a diet rich in carbohydrates, low in fat and free of alcohol combined with laxatives and ample fluid. For the prevention of liver dystrophy a bovine adrenal extract was advised (6).

Waldenström (1950) described a serious form of chronic hepatitis in young women with liver cirrhosis, plasma cell infiltration of the liver and hypergammaglobulinemia. This disease was associated with high morbidity and mortality, and because of hypergammaglobulinemia and plasmacell infiltration in the liver, immunosuppressive therapy with prednisone seemed obvious (7). The hypothesis of immunologic damage to the liver led to controlled, clinical studies evaluating the effect of immunosuppressive therapy. Treament with prednisone improved liver function and life expectancy in patients compared to untreated control patients (8,9,10). The etiology of this immunologically induced hepatitis was unknown.

1.2.1 history of hepatitis B virology

In 1967 2 forms of viral hepatitis were recognised; one type resembling the classical infectious hepatitis and the other type resembling serum hepatitis (11). After the discovery of australia antigen as the hepatitis B virus surface antigen (HBsAg, Blumberg

1965, 1967), hepatitis B was recognised as serum hepatitis. Thereafter, the hepatitis B virus (HBV) particles were discovered in patient serum (Dane 1970) followed by the core antigen (HBcAg, Almeida 1971), HBV associated DNA polymerase activity (DNAp, Hirschman 1971), the 'e' antigen (HBeAg, Magnius 1972) and HBV DNA (Robinson 1974). With the recognition of a virus as the causative agent for immunological liver damage, a more rational approach of treatment became possible and prednisone treatment appeared to be contra-indicated (12,13,14). The first treatment aiming at the viral origin of chronic hepatitis was leukocyte interferon (IFN). It lead to the disappearance of HBeAg and DNAp activity from serum in patients with chronic HBV infection and to a marked decrease of HBsAg titers (15).

HBV is now characterized as a DNA virus with a small, circular, partly double stranded genome. The genome consists of a long strand (L (-) strand) of about 3200 nucleotides and a short strand (S (+) strand) of 50 - 100 % of the L (-) strand (15). The L (-) strand contains the complete coding capacity of the virus with four major open reading frames (ORF's): S, C, P and X. The L (-) strand RNA transcript codes for four proteins and via reverse transcriptase activity also serves as the virus pregenome, that functions as a template for new HBV DNA genome synthesis (17). ORF S codes for the protein of the viral envelope. HBsAg is a conformational antigen composed of three protein pairs which have a terminal part in common (18). The common part is encoded by the S gene, while a 55 amino acid part upstream is encoded by the preS2 region and a 108-116 amino acid section by the preS1 gene. Each of these three proteins can be glycosylated in its S gene part, so six possible proteins can be found on gel electrophoresis. P24s and GP27s, GP33s and GP36s and GP39s and GP42s contain, respectively, the S gene product, the S and preS2 gene products and both the S, preS2 and preS1 gene products and their glycosylated derivatives. The C gene encodes the nucleocapsid protein and contains a preC and C region. The complete gene product is cleaved on the membrane of the endoplasmic reticulum into a part that is assembled in the nucleocapsid particle and a part that is secreted in the blood (HBeAg) (19). The P gene has the largest ORF and encodes the HBV DNA polymerase that has a duel function; it can synthesize viral DNA by DNA and RNA dependent DNAp activity (16). The X gene product (HBxAg) is a small polypeptide of 145-154 amino acids with trans-activating properties and a close association with HBsAg and HBcAg expression in liver sections (20). These findings and the relationship between HBxAg expression in the nucleus and dysplasia suggest a relation to viral replication and to the pathogenesis of primary hepatocellular carcinoma.

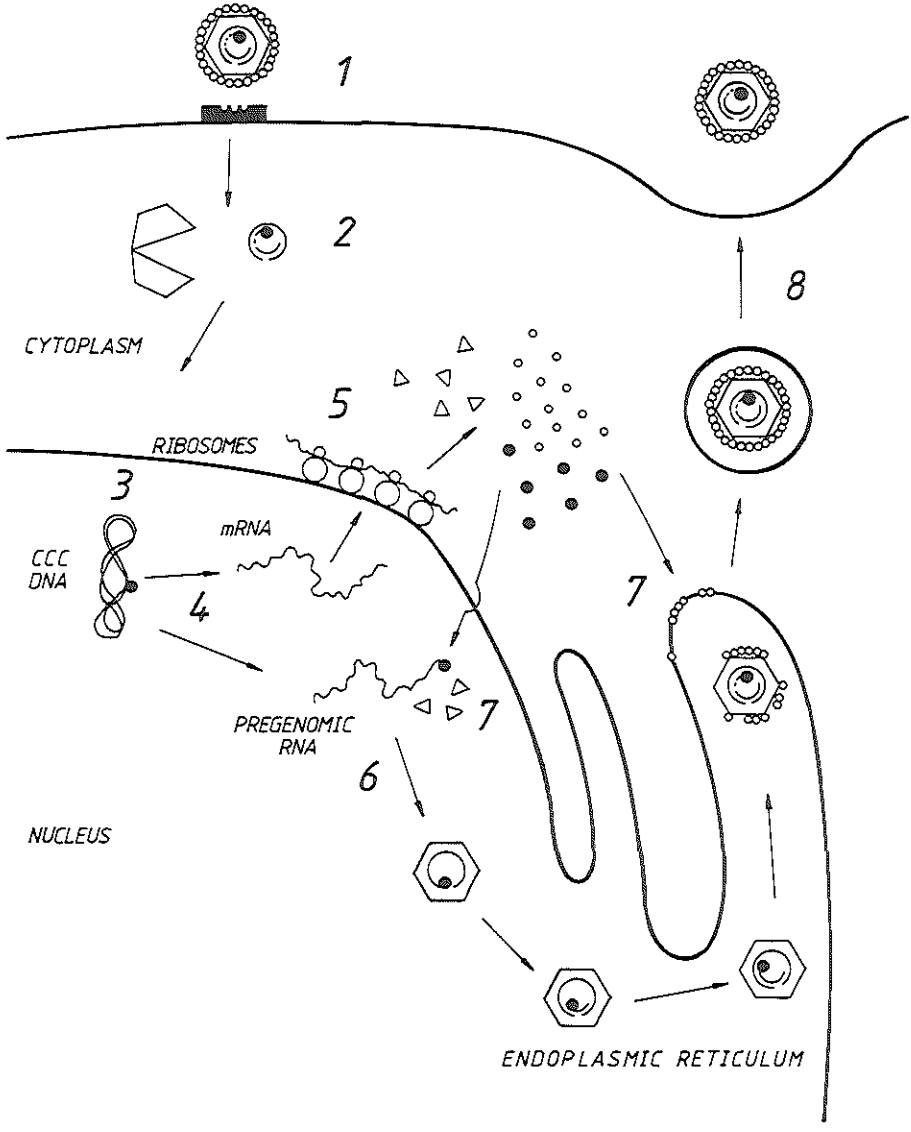


Figure 1. HBV replication cycle in hepatocytes. ○ HBsAg, △ HBcAg, ● DNA polymerase, ⬡ core, ◯ DNA L (-) strand, ◌ DNA S (+) strand, ∿ RNA. The following steps are outlined in the figure: 1 - receptor mediated virus entry, 2 - uncoating, 3 - endogenous DNAp synthetase activity resulting in covalently closed circular DNA (cccDNA), 4 - transcription by cellular RNAp, 5 - viral protein synthesis, 6 - reverse transcription by viral DNAp, 7 - virus assembly, 8 - budding out.

1.2.2 HBV replication cycle

Figure 1 more specifically shows the steps in the HBV replication cycle. The cycle starts with virus entry into hepatocytes. Polymerised human albumin receptors on the hepatocyte membrane and viral surface are reported to play an important role in virus binding to the hepatocyte membrane (21,22). After entry into the hepatocyte, uncoating and transport to the cell nucleus takes place. The incomplete double strand of the viral genome is converted into a completed double strand DNA molecule (covalently closed circular DNA), which supercoiled structure forms the template for RNA production (23). Endogenous DNA dependent DNAp activity is responsible for the completion of the supercoiled DNA structure. Cellular RNA polymerase II then transcribes the L (-) strand into multiple forms of 2.1 kb and 3.5 kb messenger RNA's. The 2.1 kb mRNA encodes the preS2, S and X gene products and the 3.5 kb mRNA encodes all gene products and functions as a pregenome for new viral DNA synthesis in a 1:1 ratio. A new L (-) strand is generated by reverse transcriptase activity (RT) of the viral DNAp with simultaneous degradation of the RNA pregenome. After degradation of the RNA pregenome an oligonucleotide remains that functions as a primer for the S(+) strand synthesis (17,24). At the endoplasmic reticulum the assembly of core particles starts (17) and after completion of the virus particle the progeny of new virus may bud out the hepatocyte and infect neighbouring cells.

1.2.3 treatment strategy for chronic hepatitis B

With the recognition of viral antigens, antibodies, HBV DNA and HBV DNAp it became possible to characterize a state of infection in which the virus is considered no longer as harmful to the host as in case of active replication. This state of virus latency is characterised by a low level of HBV DNA and the absence of HBeAg in serum and HBcAg in the liver. Virus latency is followed by disappearance of clinical symptoms and normalisation of biochemical and histological abnormalities (25,26). The goal of antiviral treatment therefore is the induction of virus latency. There are patients in the Mediterranean and the Far East that remain viraemic after HBe-seroconversion because they carry a virus that has a mutation in the pre-core gene, giving rise to a novel translational stop codon (27,28). This pre-core mutant causes a severe anti-HBe positive hepatitis for which the exact mechanism and geographical distribution remains unclear. Because of the insights in the serology of viral infection, the treatment of a chronic HBV infection can be directed at blocking of viral replication and immune enhancement. The evolution of antiviral treatment over the centuries has evolved from mainly symptomatic and partly rational to mainly rational and partly symptomatic.

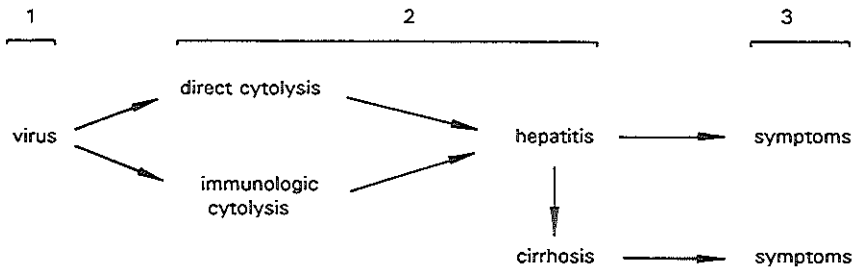


Figure 2 shows three levels of possible intervention in case of a viral infection. Only anamnestic and physical diagnostic data can be used to monitor treatment at level 3. Treatment consisted of supportive and symptomatic measures until the sixth decade of this century. The development of biochemistry allowed recognition of the mechanism of hepatocellular damage with a more rational approach of therapy directed against immunologic damage at level 2. Molecular biology with virus nucleic acid hybridization, radioimmunoassays for viral antigens and antibody detection, now allow rational and specific blocking at level 1.

Treatment at level 1 in figure 1 directed at the viral targets as depicted in figure 2 has not yet been fully explored in present clinical studies. The treatment effects on the separate viral targets can't be assessed by routine clinical tests, but effects on viral antigen production and nucleic acid production are used to assess treatment effect on replication activity. Quantification of these parameters is essential to evaluate the effect of a treatment regime. The goal of treatment is to induce virus latency, usually characterized by absence of serum HBeAg. This dichotomous test result characterizes a patient as a responder only at the moment of HBe-seroconversion, usually after 10 - 20 weeks of antiviral treatment. Since it is of great clinical interest to identify patients as potential responders prior to HBe-seroconversion in order to make decisions about treatment modifications, monitoring during treatment is needed. Decrease of HBV DNA levels in the serum may indicate whether a patient is likely to respond to treatment. Since negative test results for serum HBV DNA with persistence of HBeAg frequently occur during treatment, HBV DNA is not an optimal test to monitor treatment for HBe-seroconversion. Quantification of HBeAg serum levels (29) may be of additional value to monitor patients with chronic hepatitis type B during treatment.

1.2.4 treatment of chronic hepatitis B

Meta-analysis on current treatment regimens for chronic hepatitis B shows several

approaches with effict on HBe-seroconversion (30). Firstly a monotherapy with the nucleoside analogue adenine arabinoside monophosphate (ARA-AMP) or alpha-interferon (α-IFN) and secondly a combination therapy. Several types of combination therapy have been tried such as corticosteroid withdrawal and α-IFN and α-IFN combined with ARA-AMP or acyclovir (ACV).

ARA-AMP as nucleoside analogue interferes with nucleic acid synthesis. Viral DNAp activity is preferentially blocked over mammalian cell polymerases (31). Eight controlled studies with these drugs have been carried out. Administration during 10 to 56 days resulted in an increase of 0 to 42 percent of HBe-seroconversion compared to control patients. Odds ratio on all 8 studies is estimated at 2.37 (95% CI 1.12-5.19). Since the 95% CI excludes an odds ratio of 1, ARA-AMP appears to be an effective drug for the treatment of chronic hepatitis B (30).

Monotherapy with α-IFN is also widely used for chronic hepatitis B. In 1991 the drug has been licensed in the Netherlands for this indication. Viral targets for α-IFN treatment are viral protein synthesis and viral antigen expression on hepatocyte membranes. Diminished protein synthesis and enhanced HLA class I antigen expression leading to cytotoxicity result from α-IFN administration. Sixteen randomized controlled trials with α-IFN have been published. Treatment regimens varied from 3 to 4 months of treatment with doses of 2.5 to 10 MU every day or every other day. Meta analysis shows an increase in HBe-seroconversion rate compared to controls varying from 18 to 37 percent. The odds ratio was 4.2 for the total of the 16 studies (95% CI 2.48-7.37). Since this odds ratio excludes 1, α-IFN is now established as an effective drug in the treatment of hepatitis B (28). Side effects of α-IFN therapy such as influenza-like symptoms, fatigue, general malaise and bone marrow toxicity occur in most patients. Influenza-like symptoms can be adequately suppressed by paracetamol or indomethacin administration which may improve patient compliance to α-IFN therapy (30).

An important approach consists in the concept of combining drugs with different viral targets. A combination of corticosteroid withdrawal followed by either ARA-AMP or α-IFN has been carried out. Prednisolone 40 to 60 mg daily tapered to zero over a period of 6 to 8 weeks was followed by either ARA-AMP or α-IFN in standard regimes. A combination therapy of nucleoside analogues (ARA-AMP and ACV) with α-IFN has also been tried out. The overall beneficial effect on HBe-seroconversion compared to control patients was comparable to monotherapy with either ARA-AMP or α-IFN. The odds ratio was estimated at 4.1 (95% CI 1.67-10.61), indicating that up to now no additional effect of combination therapy compared to monotherapy (30). Whether this is due to smaller numbers in the former group or whether a more effective combination of

antivirals is needed remains to be established.

Therefore, we investigated the effect of ACV and α-IFN combination therapy in a large, controlled study and established the antiviral effect of 2 other drugs in order to evaluate their possible additional value in a treatment regime with α-IFN. Firstly zidovudine was evaluated, since the drug inhibits the enzyme reverse transcriptase and in vitro activity against HBV DNAp (31). Secondly, Phyllanthus amarus was investigated since this herb has been described as an important inducer of HBs-seroconversion (32).

1.3.1 history of hepatitis C virology

In 1975 the existence of a virus was postulated as a cause for post-transfusion hepatitis. Because no serological evidence for hepatitis A, hepatitis B, cytomegalovirus or Epstein-Barr virus could be found, it was called hepatitis non-A,non-B (33). Further evidence for a viral agent causing post-transfusion hepatitis was found in sera of a patient with non-A,non-B hepatitis and in sera from two blood donors. These sera were inoculated in four chimpanzees who developed a raise in aminotransferase levels 2 - 4 weeks after inoculation. Liver biopsy specimens showed evidence of hepatitis, but serological tests for hepatitis A, hepatitis B, cytomegalovirus and Epstein-Barr virus remained negative (34). In 1989 a virus being responsible for most cases of post-transfusion hepatitis was found by isolating a part of its genome (35). It appeared to be a small virus with a single stranded RNA genome and a resemblance to flaviviruses, to which the name hepatitis C virus (HCV) was given. An antibody assay became rapidly avaible for routine serological examination (36). These antiHCV antibodies are helpful in diagnosing HCV infection. Development of antigen tests and RNA hybridisation tests however, are required to elucidate the viral replication cycle.

1.3.2 treatment strategy for chronic hepatitis C

Alpha-IFN treatment has been reported to have beneficial effects in chronic hepatitis C (see section 1.3.3) during administration and in some of the patients after interruption of therapy. This effect can either be due to the protective effect of α-IFN on hepatocytes or to the induction of virus latency or elimination of a cytotoxic virus. The fact that a persistent beneficial effect remains after stopping treatment in a part of the patients, indicates that induction of virus latency may be responsible for the therapeutic effect in these patients. Rational design of therapy for nonresponders to α-IFN is only possible when targets in the replication cycle of the virus can be recognised. Since flaviviruses belong to the family of togaviruses, the replication cycle of the togaviruses (37) may serve to form hypotheses on HCV replication and treatment strategy (fig 3).

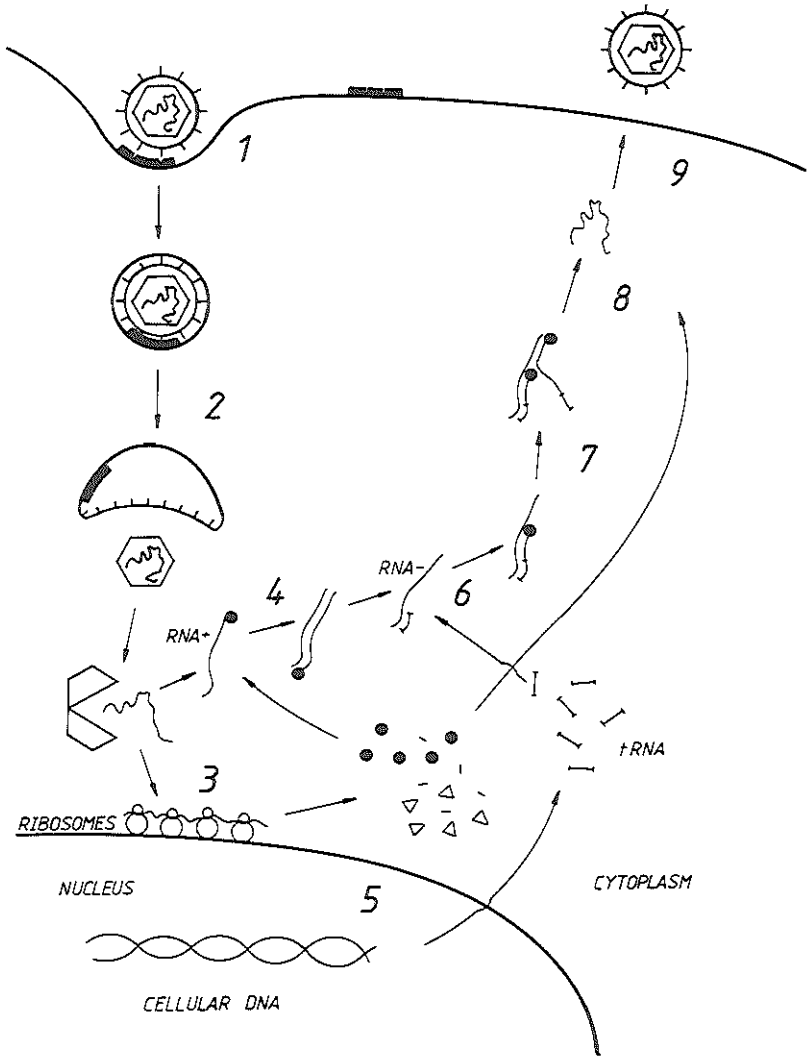


Figure 3. Togavirus replication in vertebrate cells. | | | structural proteins, ● RNA polymerase, messenger RNA and RNA genome, RNA antigenome, cellular transfer RNA. Steps of the replication cycle: 1 - receptor mediated virus entry, 2 - uncoating, 3 - translation of viral genome (RNA^+) in proteins, 4 - viral RNAp activity resulting in antigenomic (RNA^-) RNA, 5 - transcription of cellular transfer RNA (tRNA), 6 - initiation of viral RNA^+ synthesis by tRNA, 7 - viral RNAp activity resulting in genomic RNA, 8 - virus assembly, 9 - budding out.

1.3.3 treatment of chronic hepatitis C

Several drugs have been investigated in the treatment of chronic hepatitis C. So far only α-IFN has proven to be an efficient drug. In 1985 short courses of acyclovir failed to have any beneficial effect on chronic non-A,non-B hepatitis (38). In 1986 a pilot study was reported on ten patients treated with α-IFN 5 MU (n=7) or 1 MU (n=3) daily, subcutaneously, gradually tapered to 1MU thrice weekly. In eight patients alanine aminotransferase (ALT) levels fell within the first month, and normal or nearly normal levels were seen after 4 months of treatment. Interruption of treatment in 2 patients resulted in an immediate rebound of hepatitis activity, so treatment was reinstituted and continued for 1 year in all 8 patients. In 7 biopsies taken after 1 year, marked improvement of liver histology was found (39).

A significant effect of α-IFN on serum ALT levels was observed in three randomized controlled studies (40,41,42). ALT normalisation occurred in approximately 50 % of patients with doses of 2 - 5 MU thrice weekly during 2 - 6 months. Improvement of ALT levels was accompanied by histological improvement in these studies. In over 50 % of responders to therapy positive results were only transient and rebound of aminotransferase levels to pretreatment level occured. Clearly no eradication of virus was achieved in these patients and more specific tests for viral replication activity such as RNA detection or liver immunofluorescence for HCV antigen are needed to assess endpoints and efficacy of antiviral therapy. Beta-interferon has also been reported to suppress ALT levels in chronic non-A,non-B hepatitis, but results are not superior to α-interferon and since the drug has to be administered intravenously, it is not the preferable drug (43,44). Interleukin-2 has been given intravenously in a dose of 100.000 IU/kg three times a week, but severe side-effects in two patients necessitated discontinuation of the scheduled three months course (45).

The cornerstone of therapy for chronic hepatitis C appears to be α-IFN, although the majority of patients appear to be partial or nonresponders to α-IFN. A case is presented of chronic active hepatitis due to HCV infection with a sustained remission after α-IFN therapy despite HIV coinfection with active virus replication. Furthermore, since togaviruses require human transfer RNA fragments and a viral RNA dependent RNA polymerase activity for initiation of replication (fig 3), we investigated the effect of the DNA dependent RNA polymerase inhibitor rifampicin in a partial responder to α-IFN therapy.

1.4.1 history of hepatitis D virology

Since the discovery of the delta agent by Rizzetto in 1977 (46), the defective RNA virus has been found to be a spherical particle of 36 nm coated with HBsAg (47). The genome (HDV RNA) is a circular single stranded RNA molecule of (-) polarity (48). It has the ability to form an unbranched rod structure by folding itself through base pairing like several satellite plant viruses (49,50). HDV RNA contains at least 5 ORF's on its genomic and antigenomic strands (51). ORF 5 on the antigenomic strand is predicted to encode 2 major species of delta antigen (HDAg) (51). HDV genome replication is consistent with a rolling circle model producing antigenomic strands (mRNA's) through self cleavage and ligation in the absence of proteins (52, fig 4).

1.4.2 treatment stragegy for hepatitis D

Evidence has accumulated that little direct interaction between the virus and the host's immune system occurs. The presence of immune reactive cells in the liver as well as presence of HDAg in serum early during infection makes sensitization to HDAg possible. However, since virus particles in serum are enveloped by HBsAg and HDAg is not expressed on hepatocyte membranes, the significance of host immuno reactivity in relation to immunologic clearance of the virus appears low. The role of HBV in the replication cycle of HDV is limited to virus entry or release from host cells (53); therapy should be directed to either interference with virus replication or towards elimination of HBV (development of antiHBs). HDAg is essential for replication of the viral genome (53) and specific targets for therapy directed to HDV infection are therefore an inhibition of HDAg synthesis, an inhibition of cellular RNA polymerase, a blocking of uncoating or assembly of the virus and a clearance of HBsAg (fig 4).

1.4.3 treatment of hepatitis D

There is no established therapy for chronic HDV infection. Immune suppressive therapy with corticosteroids or azathioprine did not ameliorate the course of the infection (54), nor did levamisole (55). However, a 3 to 12 month course of α-interferon has been reported to inhibit HDV replication. HDAg in the liver and serum diminishes or disappears, HDV RNA in serum disappears and AST levels drop. But on stopping treatment, evidence of HDV replication reappeared with elevation of serum AST levels (56-61). For liver transplantation in case of end-stage liver disease due to HDV infection, excellent graft survival has been reported and 2 out of 7 patients cleared HDV as well as HBV infection (62). HBs-seroconversion has been reported during α-IFN therapy in chronic hepatitis B and the combination of α-IFN and ACV was found to

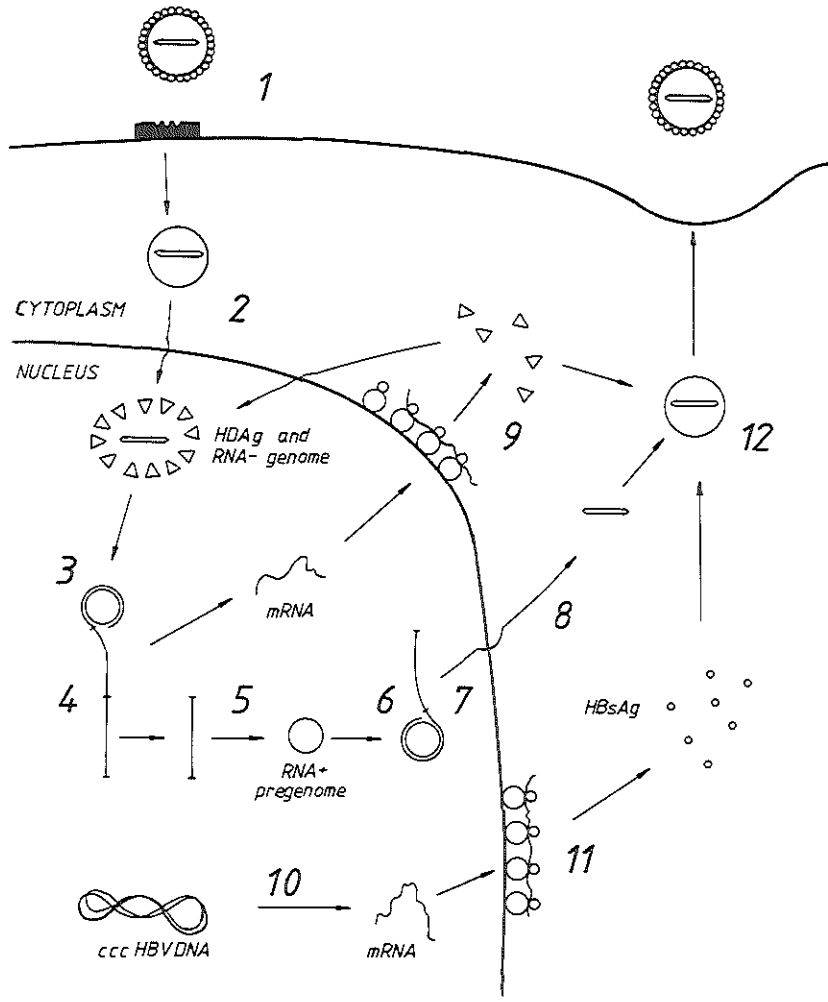


Figure 4. HDV replication in hepatocytes. ○ HBsAg, △ HDAg, ◯ core, ═ genomic RNA (RNA⁻), ◯ antigenomic RNA (RNA⁺), ∿ messenger RNA (mRNA), ∞ covalently closed circular HBV DNA (cccDNA). Steps of the replication cycle: 1 - receptor mediated virus entry, 2 - uncoating, 3 - rolling circle method of RNA⁺ production resulting in generation of multimeres of antigenomic RNA, 4 - selfcleaving of multimeric structure in monomeric RNA⁺, 5 - selfligation of antigenomic RNA, 6 - rolling circle method of RNA⁻ production resulting in multimeric copies, 7 - selfcleaving of RNA⁻, 8 - selfligation of RNA⁻, 9 - viral protein synthesis, 10 - transcription of HBV DNA mRNA, 11 - HBsAg synthesis, 12 - virus assembly.

induce HBV virus latency in 40 % of treated patients (29). Since clearance of HBV results in clearance of HDV infection, we evaluated the effect of combination of α-IFN and ACV treatment in patients with a chronic hepatitis D infection.

1.5 aims of the study

Hepatitis B:
- evaluating the effect of acyclovir, zidovudine and Phyllanthus amarus on HBV replication in order to improve current treatment regimes concerning HBe-seroconversion
- defining quantitative HBeAg and HBV DNA analysis as parameters to monitor antiviral treatment
- investigating the influence of indomethacin and paracetamol on the antiviral effect of α-IFN

Hepatitis C:
- evaluating the effect of rifampicin in addition to α-IFN treatment in a partial responder to α-IFN therapy
- documenting that chronic hepatitis C complicated by coinfection with HIV can mimic autoimmune type chronic hepatitis and that antiviral therapy can lead to sustained remission of of hepatitis C.

Hepatitis D:
- evaluating the effect of treatment with α-IFN and ACV on chronic hepatitis D

REFERENCES

1 Schnorrenberger CC and Tsjang T-L. Nei Tsjing, Ling Tsju Tsjing. Stuttgart: Hippocrates Verlag, 1974.

2 Baillière JB. Hippocrates on hepatitis from 'Oevres complètes d'Hippocrates'. Paris, 1851 7:237.

3 Joshi BS, Gawad DH, Pelletier SW, Kartha G and Bhandary K. Isolation and structure (x-ray analysis) of ent-norsecurinine, an alkaloid from Phyllanthus Niruri. J Nat Prod 1986; 49(4): 614-620.

4 Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y and Hikino H. Antihepatotoxic principles of Phyllanthus Niruri herbs. J Ethnopharmacology 1985; 14(1): 41-44.

5 Gupta DR and Ahmed B. Nirurin: a new prenylated flavanone glycoside from Phyllanthus Niruri. J Nat Prod 1984; 47(6): 958-963.

6 Gutzeit K. Die Hepatitis epidemica. Münch Med Wschr, 1950; 29/30: 1161-1166 en 30/31: 1295-1301.

7 Geall MG, Schoenfield LJ and Summerskill WHJ. Classification and treatment of chronic active liver disease. Gastroenterology, 1968; 55: 724-729.

8 Cook GC, Mulligan R and Sherlock S. Controlled trial of corticosteroid therapy in active chronic hepatitis. Q J Med, 1971; 40: 159-185.

9 Soloway RD, Summerskill WHJ, Baggenstoss AH, Geall MG, Gitnick GL, Elveback LR and Schoenfield LJ. Clinical, biochemical and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. Gastroenterology, 1972; 63: 820-833.

10 Murray-Lyon IM, Stern RB and Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. Lancet, 1973; 1: 735-737.

11 Krugman S, Giles JP and Hammond J. Infectious hepatitis: evidence for two distinctive clinical, epidemiological and immunological types of infection. JAMA, 1967; 200 (5): 365-373.

12 Schalm SW, Summerskill WHJ, Gitnick GL and Elveback LR. Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis B s-antigen. Gut, 1976; 17: 781-786.

13 Alter JH and Hoofnagle JH. Non-A,non-B hepatitis: Observations on the first decade. In: Viral hepatitis and liver disease; Vyas GN, Dienstag JL and Hoofnagle JH (eds); Grun and Stratton; New York 1984: 345-354.

14 Rizzetto M, Verme G, Recchia S et al. Chronic HBsAg positive hepatitis with intrahepatic expression of delta antigen: an active and progressive disease unresponsive to immunesuppressive treatment. Ann Intern Med 1983; 98: 437-441.

15 Greenberg HB, Richard B, Pollard RB et al. Effect of human leucocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N Engl J Med, 1976; 295: 517-522.

16 Robinson WS. The genome of hepatitis B virus. Ann Rev Microbiol, 1977; 31: 357-377.

17 Seeger C, Ganem D and Varmus HE. Biochemical and genetic evidence for the hepatitis B virus replication strategy. Science, 1986; 232: 477-483.

18 Le Bouvier GL. The heterogeneity of the australia antigen. J Infect Dis, 1971; 123: 671-675.

19 Uy A, Bruss V, Koechel H, Thomssen R and Gerlich WH. Effect of the precore sequence on functions of the hepatitis B core protein. Meeting on " Molecular biology of hepatitis B viruses", Cold Spring Harbor, may 2-5, 1985.

20 Wang W, London WT, Lega L and Feitelson MA. HBxAg in the liver from carrier patients with chronic hepatitis and cirrhosis. Hepatology 1991; 14: 29-37.

21 Machida A, Kishimoto S, Ohnuma H et al. A hepatitis B surface antigen polypeptide (p31) with the receptor for polymerized human as well as chimpanzee albumins. Gastroenterology 1983; 85: 268-274.

22 Thung SN, Wang D-F, Fasy TM, Hood A and Gerber MA. Hepatitis B surface antigen binds to human serum albumine cross-linked by transglutaminase. Hepatology 1989; (9) 5: 726-730.

23 Gerlich WH and Robinson WS. Hepatitis B virus contains protein attached to the 5' terminus of its complete DNA strand. Cell 1980; 21: 801-809.

24 Miller RH, Marion PL and Robinson WS. Hepatitis B viral DNA-RNA hybrid molecules in particles from infected liver are converted to viral DNA molecules during an endogenous DNA polymerase reaction. Virology 1984; 139: 64-72.
25 Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F and Ruol A. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980:79;195-199.
26 Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A and Realdi G. Clininical, virologic and histologic outcome folllowing seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology 1986:2;167-172.
27 Brunetto M, Stemler M, Bonino F, et al. A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. J Hepatol 1990: 10(2); 258-261.
28 Carman W, Jacyna M, Hadziyannis S et al. Mutation preventing formation of hepatitis e antigen in patients with chronic HBV infection. Lancet 1989: ii; 588-591.
29 de Man RA, Schalm SW, Heijtink RA et al. Interferon plus descyclovir in chronic hepatitis type B: incidence of virus marker elimination and reactivation. In: Viral hepatitis and liver disease; Zuckerman AJ (ed); Alan R. Liss, Inc; New York 1988: 913-916.
30 de Man RA, Berk L, Schalm SW and Stijnen T. Treatment of chronic viral hepatitis. Survey of digestive diseases, 1991; 9: 17-35.
31 Nordenfelt E, Löfgren B, Chattopadhyaya J and Öberg B. Inhibition of Hepatitis B Virus DNA Polymerase by 3'-Azido-3'-Deoxythymidine Triphosphate but not by its Threo Analog. J Med Vir 1987, 22: 231-236.
32 Thyagarajan SP, Subramanian S, Thirunalasundar T, Venkateswaran PS and Blumberg BS. Effect of Phyllanthus Amarus on chronic carriers of hepatitis B virus. Lancet 1988; ii: 764-766.
33 Alter HJ, Purcell RH, Holland PV et al. Clinical and serological analysis of transfusion associated hepatitis. Lancet 1975; ii: 838-841.
34 Tabor E, Gerety RJ, Drucker JA et al. Transmission of non-A,non-B hepatitis from man to chimpanzee. Lancet 1978; i: 463-466.
35 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW and Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A,non-B viral hepatitis genome. Science 1989; 244: 359-361.
36 Kuo G, Choo QL, Alter HJ et al. An assay for circulating antibodies to a major etiologic virus of human non-A,non-B hepatitis. Science 1989; 244: 362-364.
37 Schlesinger RW, ed. The togaviruses. Academic press, New York 1980.
38 Pappas SC, Hoofnagle JH, Young N et al. Treatment of chronic non-A,non-B heaptitis with acyclovir: a pilot study. J Med Vir 1985; 15: 1-9.
39 Hoofnagle JH, Mullen KD,Jones DB, et al. Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986; 315 (25): 1575-1578.
40 Jacyna MR, Brooks MG, Loke RHT, Murray-Lyon IM and Thomas HC. Randomised controlled trial of interferon alfa (lymfoblastoid interferon) in chronic non-A non-B hepatitis. Br Med J 1989; 298: 80-2.
41 Di Bisceglie AM, Kassianides C, Lisker-Melman M et al. Randomized, double-blind, placebo controlled trial of alpha interferon therapy for chronic non-A,non-B hepatitis. Hepatology 1989; 8: 1222.
42 Davis GL, Balart LA, Schiff ER et al. Treatment of chronic hepatitis C with recombinant interferon alfa. N Engl J Med, 1989; 321: 1501-1506.
43 Kiyosawa K. Sodeyama T, Yoda H, et al. Treatment of chronic non-A,non-B hepatitis with beta-interferon. Viral Hepatitis and Liver Disease 1988: 895-897.
44 Arima T, Nagashima H, Shimomura H, et al. Treatment of chronic non-A,non-B hepatitis with human beta-interferon. Viral Hepatitis and Liver Disease; Zuckerman AJ (ed); Alan R Liss, Inc; New York 1988: 898-901.
45 Bach N, Schaffner F and Chan-Lin S. High-dose recombinant interleukin-2 for chronic viral hepatitis. Lancet 1989; 2: 281.
46 Rizzetto M, Canese MG, Aricò S et al. Immunofluorescence detection of a new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and serum of HBsAg carriers. Gut 1977; 18: 997-1003.
47 Rizzetto M, Hoyer BH, Canese MG et al. Delta antigen: association of delta antigen with

hepatitis B surface antigen and ribonucleic acid in the serum of delta infected chimpanzees. Proc Natl Acad Sci USA 1980; 77: 6124-6128.

48 Kos A, Dijkema R, Arnberg AC et al. The hepatitis delta virus possesses a circular RNA. Nature 1986; 323: 558-560.

49 Kuo MY-P, Goldberg J, Coates L, Mason W, Gerin J and Taylor J. Molecular cloning of hepatitis delta virus RNA from an infected woodchuck liver: sequence, structure and applications. J Virol 1988; 62: 1855-1861.

50 Keese P and Symons RH. Physical-chemical properties: molecular structure. In TO Diener (ed), The viroids. Plenum Publishing Corp, New York; 1987: 37-62.

51 Wang KS, Choo QL, Weiner AJ et al. Structure, sequence and expression of the HDV genome. Nature 1986; 323: 508-513.

52 Kuo MY-P, Sharmeen L, Dinter-Gottlieb G and Taylor J. Characterization of self-cleaving RNA sequences on the genome and antigenome of human hepatitis delta virus. J Virol 1988; 62: 4439-4444.

53 Kuo MY-P, Chao M and Taylor J. Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. J Vir 1989; 63; 5:1945-1950.

54 Rizzetto M, Verme G, Recchia S, et al. Chronic HBsAg hepatitis with intrahepatic expression of delta antigen. An active and progressive disease unresponsive to immunosuppressive treatment. Ann Intern Med 1983; 98: 437-441.

55 Arrigoni A, Ponzetto A, Actis G and Bonino F. Levamisole and chronic delta hepatitis. Ann Intern Med 1983; 98: 1024.

56 Rizzetto M, Rosina F, Saracco G, et al. Treatment of chronic delta hepatitis with α-2 recombinant interferon. J Hepatol 1986; 3 (suppl 2): S229-233.

57 Hoofnagle J, Mullen K, Peters M et al. Treatment of chronic delta hepatitis with recombinant human alpha interferon. In Rizetto M (ed):"The delta virus and its infection", 1987, New York: Alan R Liss, pp 291-298.

58 Rosina F, Saracco G, Lattore V et al. Alpha 2 recombinant interferon in the treatment of chronic hepatitis delta virus (HDV) hepatitis. In Rizetto M (ed):"The hepatitis delta virus and its infection", 1987, New York: Alan R Liss, pp 299-303.

59 Thomas HC, Farci P, Shein R et al. Inhibition of hepatitis delta virus (HDV) replication by lymphoblastoid human alpha interferon. In Rizetto M (ed): "The delta virus and its infection", 1987, New York: Alan R Liss, 277-290.

60 Rosina F and Pintus C. Long-term interferon therapy for chronic hepatitis D: A multi-center Italian study (Bologna, Cagliari, Genova, Milano, Napoli, Pavia, Torino). J Hepatol in press.

61 Porres JC, Bas C, Mora I et al. Prolonged treatment of chronic delta hepatitis using recombinant alpha-interferon: A controlled study (abstract). Hepatology 1988; 8: 1408.

62 Rizzetto M, Macagno S, Chiaberge E, et al. Liver transplantation in hepatitis delta virus disease. Lancet 1987; 2: 469-471.

CHAPTER 2

FAILURE OF ACYCLOVIR TO ENHANCE THE ANTIVIRAL EFFECT OF ALPHA LYMPHOBLASTOID INTERFERON ON HBe-SEROCONVERSION IN CHRONIC HEPATITIS B.

A multi-centre randomized controlled trial.

The contents of this chapter have been accepted for publication in The Journal of Hepatology 1991, under the same title and with the following authors: Lucas Berk, Solko W Schalm, Robert A de Man, Rudolf A Heytink, Pierre Berthelot, Christian Brechot, Bernard Boboc, Françoise Degos, Patrick Marcellin, Jean-Pierre Benhamou, Georg Hess, Siegbert Rossol, Karl-Hermann Meyer zum Buschenfelde, Robert AFM Chamuleau, Peter LM Jansen, Henk W Reesink, Beat Meyer, Christoph Beglinger, Georg A Stalder, Jannie W den Ouden-Muller and Maurits de Jong.

SUMMARY.

Serum HBeAg levels and HBe-seroconversion were investigated in patients with chronic HBeAg positive hepatitis who were randomized to receive either alpha lymphoblastoid interferon (5 megaunits subcutaneously daily for 16 weeks) plus acyclovir (2 grams intravenously daily during weeks 1+2 and weeks 9+10) (n=49) or no treatment (n=48). HBeAg levels in serial dilutions of patient serum were assessed quantitatively by radioimmunoassay and compared with the values found for negative control serum. One year after the start of therapy 44 treated patients and 43 control patients were available for follow-up. A complete response (HBe-seroconversion) occurred in 11 treated patients (25%) and 6 controls (14%) (difference: 11%, 95% CI -5 - 28%). A partial response (HBeAg < 50% of initial level) was found significantly more often for treated patients (n=13, 30%) than for controls (n=2, 5%) (difference: 25%, 95% CI 10 - 40%). During acyclovir-interferon combination therapy the decrease in HBeAg level was similar to that achieved during therapy with interferon alone. We conclude that acyclovir does not enhance the effect of interferon on serum HBeAg levels. Since HBeAg levels continue to decline during interferon treatment and rebound thereafter to pretreatment levels, prolongation of therapy may yield a higher response rate.

Keywords: chronic hepatitis B, combination therapy, quantitative HBeAg analysis, interferon

INTRODUCTION.

Chronic hepatitis B virus (HBV) infection is associated with an annual mortality rate of more than a million patients. Decompensated cirrhosis of the liver and hepatocellular carcinoma account for the majority of the losses (1,2). Progression to this end-stage of disease appears to be related to active viral replication (3,4) and is, in Northwestern European patients, almost invariably associated with HBeAg positivity (5). HBe-seroconversion is associated with inactivation of clinical, biochemical and histological signs of disease (3,4). In 10 controlled trials to evaluate the effect of alpha interferon (IFN) therapy on HBe-seroconversion, the response rate for treated patients was about 15% higher than the spontaneous rate found for control patients (6). In a small controlled trial using the combination IFN and descyclovir (DACV), the oral prodrug of acyclovir (ACV), 7 out of 18 treated patients (40%) exhibited HBe-seroconversion versus none of the 18 controls (7). Because this combination therapy appeared more effective than monotherapy with IFN, we designed a large controlled trial to test whether HBe-seroconversion after IFN and ACV combination therapy was at least 40% more frequent than with no treatment.
In order to determine the effect of additional ACV therapy, we measured HBeAg quantitatively and compared decrease of HBeAg during IFN plus ACV with that during IFN monotherapy.

METHODS.

Patients.

Ninety-seven patients entered this study from 7 different hospitals in northwestern Europe: Dijkzigt, Rotterdam; Laennec, Paris; Beaujon, Paris; 1° Medizinische Klinik und Poliklinik, Mainz; Academic Medical Centre, Amsterdam; Kantonspital, Basel; Sint Franciscus Gasthuis , Rotterdam. The study protocol was approved by all human study committees of the participating centres and patients gave written informed consent prior to entry. Inclusion criteria were: indications for therapy (symptoms or progressive liver disease); chronicity, documented by HBsAg positivity for more than one year or histological evidence of chronic disease; stable HBV replication, as indicted by the levels of serum HBeAg and serum HBV DNA or HBV DNA polymerase activity on 2 occassions for six months prior to entry or expression of HBcAg on more than 5% of hepatocytes with stable HBeAg level on 2 occassions. Exclusion criteria were: active hepatotropic viral infections other than HBV (HDV, CMV, EBV, HAV, non-A,non-B); impaired immune

reactivity (transplant and dialysis patients, CD4 positive cell counts less than 400/mm^3; antiviral or immune modulatory treatment in the past 6 months; recent alcohol or drug addiction; decompensated liver disease (ascites, encephalopathy, variceal bleeding); any severe disease affecting the candidate's prognosis.

Randomization.

Patients were enrolled in the study after central assessment of their virological eligibility in Rotterdam. They were centrally randomized in blocks of 6 patients per centre, using a table with random numbers and numbered sealed opaque envelopes (8). The total number of patients to be included in the study was estimated to be between 80 and 100 with an expected HBe-seroconversion rate of at least 50% for the therapy group. After randomization there were forty-nine patients in the therapy group and 48 in the control group. During the last months of enrollment, incomplete blocks of 6 patients were completed without considering the participating centre (9).

Treatment.

Patients in the therapy group received alpha lymphoblastoid interferon (IFN, Wellferon, Beckenham, UK) (5 mega-units daily subcutaneously for 16 weeks) and two 2-week courses of acyclovir (ACV) (2 g daily intravenously) during week 1 and 2 and during week 9 and 10 of IFN administration. ACV administration required hospitalisation. During the first hospital stay, instruction on the IFN injection technique was given so that patients could self-administer the drug. ACV (1 g ACV dissolved in 1000 ml saline) was given twice daily intravenously in 1 hour. Daily urinary output was kept at at least 2000 ml. During the first 3 days of IFN administration, slow release indomethacin (2 x 75 mg daily) or paracetamol (4 x 500 mg daily) was given orally to reduce initial side-effects. Patients in the control group received no treatment for 52 weeks.

Laboratory determinations.

Serum samples were taken weekly during treatment and every four weeks during follow-up in the therapy group and every four weeks in the control group. Quantitative assessment of HBeAg (R.I.A., Abbott, Ill.) was carried out centrally at the Rotterdam hepatitis laboratory (Department of Virology, Erasmus University Rotterdam). Pre-entry serum was tested undiluted and diluted 5, 25, 125 and 625 times. The test results were expressed as the p/n ratio, i.e. the ratio of the counts at the dilution tested and in a negative control serum sample. The highest dilution, with an initial p/n ratio of 10 to 15, was used to observe drops in HBeAg level throughout the study. When indicated,

undiluted serum was also tested to confirm the event of HBe-seroconversion. Serum HBV DNA levels were tested by liquid phase hybridization with a I-125 labeled probe (Genostics, Abbott, Ill.). Aspartate aminotransferase (AST) levels were tested routinely (SMAC, Technicon, NY).

Response.

HBe-seroconversion (complete response) was defined as a negative HBeAg test on two successive occasions during the 52 weeks of follow-up. Patients with a decrease in HBeAg p/n ratio of more than 50% on two successive occasions during follow-up are defined as partial responders. Nonresponders did not exhibit a marked decrease in HBeAg (< 50 %) during follow-up.

Statistics.

Entry data, plotted in histograms to assess the skewness of distribution, showed that medians had to be used for data presentation. Medians of data collected on the basis of intention-to-treat (raw data) were not different from medians of data corrected for patients lost to follow-up (clean data). Clean data are presented in this report. Data were calculated at 2-week intervals during treatment, while blood sampling was performed weekly. As a result, samples missing during treatment could be replaced with the previous blood sample. Two-sample Wilcoxon rank sum analysis was used to compare different groups and the Wilcoxon matched sample sign test to evaluate data within a group during follow-up. Differences between two curves were assessed by log rank testing.

RESULTS.

Balance of randomization.

Randomization resulted in a balanced distribution of sexes, age, AST level, HBV DNA positives, antiHIV positives, cirrhotics and initial serum HBeAg levels between the therapy and control groups (table 1). Chronicity was documented in 83 patients by HBsAg positivity for more than 1 year; in 14 patients with a more recent detection of HBsAg positivity, a liver biopsy provided evidence of chronic disease. One patient died of a myocardial infarction before entry, 2 patients withdrew from the treatment protocol at 4 and 8 weeks, respectively, because of side-effects and 7 patients were lost to follow-up. At 52 weeks 44 patients of the therapy group, who had completed 16 weeks of treatment, and 43 patients from the control group were available for follow-

up. Serum samples for quantitative HBeAg analysis at key-point time intervals (weeks 0, 16, 32 and 52) had been obtained from 79 to 98% of control patients and 82 to 100% of treated patients.

table 1.

PATIENT CHARACTERISTICS AT ENTRY

	therapy group	control group
entered (n)	49	48
lost to follow-up (n)	5	5
female/male	5/44	6/43
age, median/range (y)	34.5 (22-69)	35 (19-62)
antiHIV, + ve (n)	3/43	5/43
antiHCV, + ve (n)	1/44	0/43
cirrhosis (n)	2/37	3/34
AST, median/range (U/l)**	49 (11-515)	48 (22-412)
HBV DNA, + ve (n)	49	48
HBeAg, serum dilution*		
undiluted (n)	3	4
5 x	5	2
25 x	10	13
125 x	25	24
625 x	1	0

* serum dilution to obtain a p/n ratio of 10 -15 (see methods, laboratory determinations)

** normal value < 30 U/l

Qualitative HBeAg analysis.

HBeAg seroconversion occurred in 11 out of 44 (25%, 95% CI 12 - 38%) treated patients and 6 out of 43 (14%, 95% CI 7 - 21%) control patients (fig 1, no significant difference between treatment and control groups). In addition to a complete response, a

partial response was observed in 13 out of 44 (30%, 95% CI 17 - 45%) treated patients versus 2 out of 43 (5%, 95% CI 1 - 16%) control patients. The difference in partial response between the treated and control groups was 25% (95% CI 10 - 40%); this is statistically significant since zero is not included in the confidence interval.

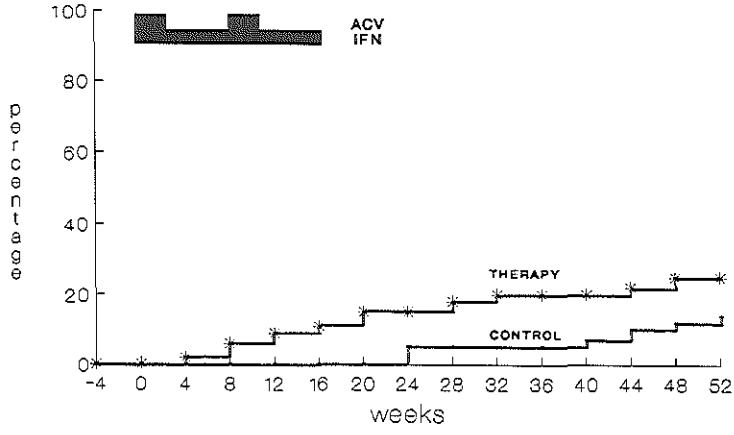


fig 1. Cumulative percentage of patients with HBe-seroconversion in the therapy group versus spontaneous HBe-seroconversion in the control group.

Quantitative HBeAg analysis.

For treated patients quantitative HBeAg assessment showed a marked decrease 12 (38%) and 16 weeks (49%) after initiation of treatment compared to the pretreatment level ($p = 0.0001$, fig 2[a]). A significant difference in the decrease in HBeAg between the treated group and the control group occurred at 12 weeks and persisted at 16 weeks ($p = 0.003$ and $p = 0.0003$); however at 32 and 52 weeks no significant difference was observed. Within the treatment group three types of responses were observed (fig 2[b]). Complete responders were HBeAg negative in the fixed serum dilution at a median of 12 weeks of treatment; partial responders showed a marked decrease in HBeAg level but were on average not negative at 16 weeks. Nonresponders showed little variation in HBeAg level. When treatment was interrupted, a rebound to

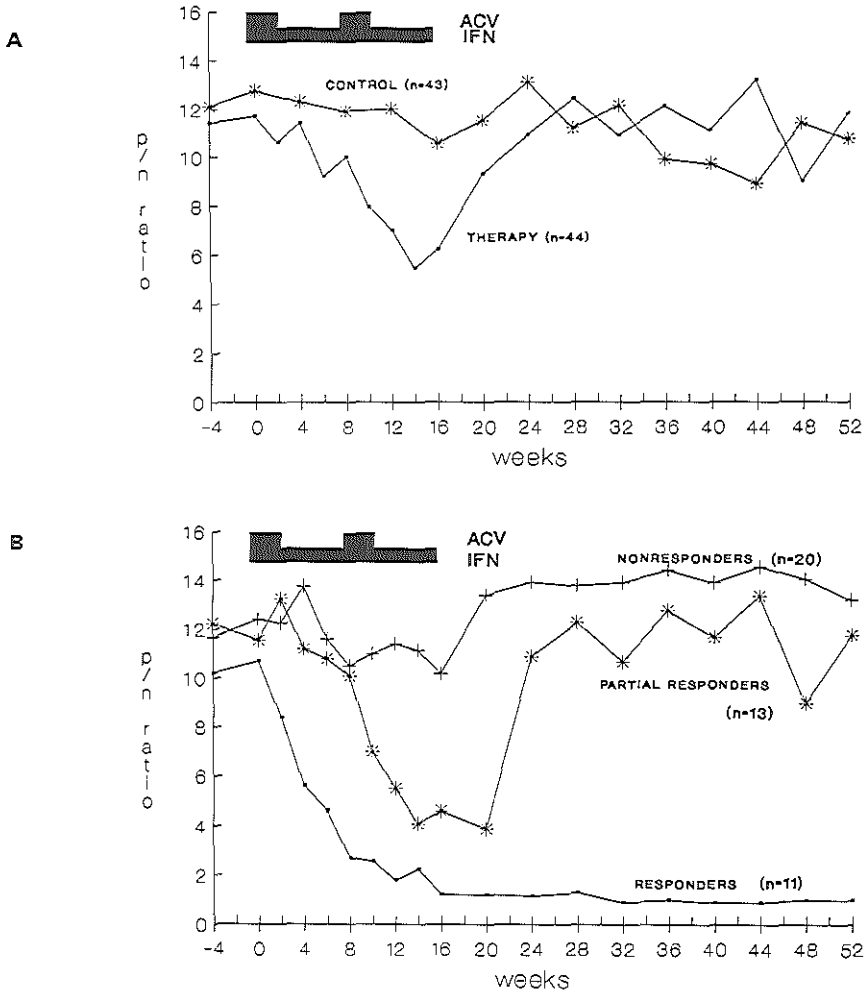


fig 2. Decrease in median HBeAg level in the therapy group resulted in a statistically significant reduction of HBeAg level at 12 - 16 weeks of treatment; the difference in HBeAg level with the control group became statistically significant at 12 weeks (fig 2[a], see results, quantitative HBeAg analysis). Median HBeAg p/n ratio for responders, partial responders and nonresponders in the therapy group, reveals a decrease for the first 2 groups with a persistent low HBeAg level in responders and a rebound to pretreatment levels in partial responders (fig 2[b]).

pretreatment levels occurred in the partial responders. No cases of reactivation were observed among responders throughout the follow-up period.

Combination therapy versus monotherapy.

The decrease in HBeAg level per 2 weeks of therapy was evaluated separately for monotherapy (IFN) and combination therapy (IFN + ACV). For every patient from the therapy group, the 16-week treatment period was divided in two 2-week periods during which combination therapy was given and six 2-week periods during which monotherapy was given. For every 2-week period the decrease in HBeAg level was calculated and expressed as percentage of the HBeAg level at the beginning of that 2-week period. Median percentual decrease per 2-week treatment period is presented in tabel 2. No additional decrease in HBeAg level could be related to ACV therapy.

Biochemical response.

Pretreatment AST level in responders appeared higher than in partial responders and nonresponders, but no statistically significant differences were observed. The median levels with 95 % CI respectively were: 65 (50-238), 53 (37-79) and 48 (36-66) U/l. Serum AST level normalized in 10 out of 11 responders at week 52 (median 25 U/l, 95% CI 16-39 U/l, $p=0.0016$). In partial responders and nonresponders no significant decrease occurred, at week 52 serum levels respectively were 49 U/l (95% CI 19-90) and 38 U/l (95% CI 27-62)

Side effects.

Symptoms, either related to chronic hepatitis B or treatment with IFN and ACV, were reported by 43 out of 44 therapy patients and by 27 out of 43 control patients. Fatigue was reported by 32 therapy patients and 8 control patients. Myalgia, hairloss, anorexia, arthralgia and headache respectively occcurred in 12, 12, 8, 6 and 6 therapy patients, but only incidentally in the control patients. Fever shortly after initiation of IFN treatment was reported by 12 patients. Neurotoxic symptoms due to IFN occurred in 4 patients of whom 1 had a seizure at 10 weeks after onset of IFN treatment. In 29 patients leucopenia was found during therapy with white blood counts under 3.0×10^9 cells/l. Dose reduction due to side effects was required in 17 patients. Administration of ACV caused frequent episodes of phlebitis at the site of canulation. Symptoms of fatigue and diminished performance were not comparable with the period of IFN monotherapy because of hospitalization during ACV administration. Diminished renal function due to ACV was not observed. Nor were any neurotoxic effects observed due

to ACV administration.

table 2.

THE MEDIAN PERCENTAGE DECREASE IN HBeAg FOR EACH 2-WEEK PERIOD OF TREATMENT TO DETERMINE THE ADDITIONAL EFFECT OF ACV

period (week)	therapy	HBeAg decrease (%)
0 - 2	IFN + ACV	-1.2
2 - 4	IFN	8.5
4 - 6	IFN	4.8
6 - 8	IFN	8.1
8 - 10	IFN + ACV	8.3
10 - 12	IFN	8.3
12 - 14	IFN	8.7
14 - 16	IFN	6.8

The decrease in HBeAg level at the end of each 2-week period during treatment is expressed as a percentage of the HBeAg level at the beginning of that 2-week period. Decrease HBeAg = $((HBeAg_{week\ n} - HBeAg_{week\ n+2})/ HBeAg_{week\ n}) \times 100$. Median levels are presented in the tabel. No additional decrease was observed during combination treatment (week 0 - 2 and week 8 - 10).

DISCUSSION.

An additional effect of ACV on HBe-seroconversion during IFN treatment was indicated in a pilot study (10) and a small controlled study (7). The present large controlled study failed to cause an additional beneficial effect of ACV on HBe-seroconversion in chronic hepatitis B. At the start of the study it was stated that combination therapy would be considered worthwhile for our patient population if a HBe-seroconversion rate of more than 50% was obtained. This result was not observed. The marked difference with

respect to the previously mentioned pilot study may be due to patient selection in an uncontrolled setting. Meta-analysis of 16 randomized controlled trials with alpha interferon showed only a modest benefit for alpha interferon (odds ratio 4.2, 95 % CI 2.48 - 7.37) compared to no treatment (11). Modification of usual 12 - 16 week IFN treatment regimens is therefore necessary. Following advise of our statistician, we assessed the therapeutic effect of IFN on a single endpoint, the HBeAg level, while other studies also used HBV DNA, HBV DNA polymerase activity and HBsAg as endpoints. When several markers are used, positive results are more easily obtained. HBe-seroconversion was choosen as single endpoint because of the great clinical implications of this event for patients with a chronic hepatitis B infection (3,4). Therefore, changes in HBeAg level were more indicative for response to treatment than changes in HBV DNA level. HBV replication activity monitored by serum HBV DNA hybridization is used by many investigators as endpoint. A disadvantage of the HBV DNA assay is that the detection limit varies considerably. Furthermore, the HBV DNA level will decline below the detection limit before a situation of virus latency will be obtained. Therefore HBV DNA testing, although of great importance for monitoring effects of antiviral agents, may not be the test-of-choice for assessment of an endpoint of therapy. Quantitative HBeAg analysis as performed in this study, appeared a very usefull test to determine response to treatment, as indicative for HBe-seroconversion. Because of the short halflife of the serum level of HBeAg, this test appeared also usefull to evaluate the decline per two week period during either IFN or IFN plus ACV treatment.

The concept of a partial response to treatment is often used to indicate patients who become negative for HBV DNA while remaining positive for HBeAg. Quantitative serum HBeAg analysis leads to a different concept of partial response. A marked reduction of HBeAg level is clearly related to IFN therapy, as shown in this study, while a steady decrease in HBeAg level during treatment suggests that prolongation of treatment might yield more responders.

We conclude that 16 weeks of IFN (5 MU daily subcutaneously) plus two 2-week courses of ACV (2 g daily intravenously) result in HBeAg seroconversion in 25 percent of patients. No additional benefit of adding ACV to IFN treatment was observed.

REFERENCES.

1. Weissburg JI, Andres LJ, Smith CI et al. Survival in chronic hepatitis B; an analysis of 379 patients. Ann Int Med, 1984; 101: 613-616.
2. World Health Organization. Prevention of primary liver cell cancer. Report on a meeting of a WHO scientific group (Zuckerman AJ, chairman). Lancet, 1983; i: 463-465.
3. Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F and Ruol A. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980:79;195-199.
4. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A and Realdi G. Clininical, virologic and histologic outcome folllowing seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology 1986:2;167-172.
5. Fagan EA and Williams R. Serological responses to HBV infection. Gut 1986: 27; 858-867.
6. Schalm SW, De Man RA, Thomas HC, Jacyna M, Hadziyannis SJ and Manessis E. Chronic hepatitis B: therapeutic controversies and randomised controlled trials. Gastroenterology International 1989: 2(1); 16-24.
7. De Man RA, Schalm SW, Heijtink RA, Chamuleau RAFM, Reesink HW, Den Ouden J, Grijm R, De Jong M, Van der Heijden JTM and Ten Kate FJW. Interferon plus descyclovir in chronic hepatitis type B: incidence of virus marker elimination and reactivation. In: Viral hepatitis and liver disease; Zuckerman AJ (ed); Alan R. Liss, Inc; New York 1988:913-916.
8. Peto R, Pike MC, Armitage P, Breslow E, Cox DR, Howard V, Mantel N, McPherson K, Peto J and Smith PG. Design and analysis of randomized clinical trials requiring prolonged observation of ech patient. I introduction and design. Br J Cancer 1976: 34; 585-612.
9. Altman DG and Doré CJ. Randomisation and baseline comparisons in clinical trials. Lancet 1990: 335; 149-153.
10. Schalm SW, Van Buuren HR, Heijtink RA and De Man RA. Acyclovir enhances the antiviral effect of interferon in chronic hepatitis type B. The Lancet 1985:2;358-360.
11. De Man RA, Berk L, Schalm SW and Stijnen T. Treatment of chronic viral hepatitis. Survey of Digestive Diseases 1991; 9: 17-35.

CHAPTER 3

COMPLEMENTARY QUANTITATIVE HBeAg MONITORING DURING ANTIVIRAL THERAPY FOR CHRONIC HEPATITIS B

The contents of this chapter have been submitted for publication to Hepatology under the same title and with the following authors: Luuk Berk, Harry LA Janssen, Robert A de Man, Solko W Schalm and Rudolf A Heijtink.

SUMMARY

Markers of viral replication, such as HBeAg and HBV DNA, are used to monitor treatment of chronic hepatitis B. To quantify hepatitis B virus replication, serum HBV DNA levels are usually assessed; however, it is uncertain how accurately a negative serum HBV DNA predicts response to therapy. Since in most cases the serum HBeAg level reflects HBcAg production in the liver, a quantitative assay for HBeAg might also be suitable for monitoring therapeutic effects. We determined the HBeAg levels quantitatively and compared them with the results of HBV DNA analysis found for 44 patients with chronic HBeAg-positive hepatitis who received alpha lymphoblastoid interferon for 16 weeks.

HBe-seroconversion (HBeAg-response) occurred in 11 out of 44 patients with a sustained response during follow-up; 13 patients showed a decrease of > 50 % of the initial HBeAg level (partial HBeAg-response); 20 patients (HBeAg-nonresponders) did not exhibit a significant decrease in HBeAg level. HBV DNA became negative in 17 out of 44 patients, but 6 patients again had positive HBV DNA levels before the end of the study. Serum HBV DNA levels decreased significantly during therapy in HBeAg-responders ($p=0.03$) and partial HBeAg-responders ($p<0.001$) as well as HBeAg-nonresponders ($p=0.001$).

We conclude that HBe-seroconversion is of more prognostic significance than HBV DNA negativity. A quantitative HBeAg assay can be used for both monitoring and endpoint determination of antiviral therapy and is - in most cases - sufficient as single parameter until HBe-seroconversion. Additional HBV DNA monitoring may identify potential responders as a subgroup of patients with high initial replication activity.

INTRODUCTION

Definition of the stage of a chronic hepatitis B virus (HBV) infection requires the assessment of several markers of disease. Clinical, biochemical, virological and histological measurements have to be made since they determine the prognosis for and management of a patient. However, for monitoring antiviral therapy, virological measurements are the most important since induction of virus latency is the aim of treatment. Serum HBeAg positivity is associated with ongoing viral replication and HBe-seroconversion is generally considered to be an important event that indicates a state of virus latency in patients with wild type HBV infection (1,2,3). A standard quantitative test for HBeAg is not available. Therefore, the serum HBV DNA level is used to quantify hepatitis B virus (HBV) replication activity. We compared the values of a standard HBV DNA test with an in house quantitative serum HBeAg assay during antiviral combination treatment with alpha lymphoblastoid interferon (IFN).

MATERIALS AND METHODS

Patients.

The effect of IFN and acyclovir (ACV) combination therapy was investigated in a controlled trial (4). We compared the HBeAg and HBV DNA analyses of the 44 patients who were randomized to the treatment group. Inclusion and exclusion criteria are described elswhere (4). Patients received 5 mega-units of alpha lymphoblastoid interferon (IFN, Wellferon, Beckenham, UK) daily subcutaneously for 16 weeks and two 2-week courses of acyclovir (ACV) (2 g daily intravenously), as described previously (4). No effect of ACV was demonstrated, so patients are considered to have received only IFN as effective therapy.

Laboratory determinations.

Serum samples were taken weekly during treatment and every four weeks during follow-up. Quantitative assessment of HBeAg (R.I.A., Abbott, Ill.) was carried out centrally at the Rotterdam hepatitis laboratory (Department of Virology, Erasmus University Rotterdam). Pre-entry serum was tested undiluted and diluted 5, 25, 125 and 625 times. The test results were expressed as the p/n ratio, i.e. the ratio of the counts at the dilution tested to the mean of 5 negative control serum samples. The cut-off was the mean of 5 negative control samples plus three standard deviations. The highest dilution, with an initial p/n ratio of 10 to 15, was used to monitor the HBeAg level

throughout the study. When indicated, undiluted serum was also tested to confirm HBe-seroconversion. Serum HBV DNA levels were determined by means of a liquid phase hybridization assay with an I-125 labeled probe (Genostics, Abbott, Ill.).

Response terminology.

An HBeAg response was defined as a negative HBeAg test result for undiluted serum samples on two successive occasions during the 52 weeks of follow-up. A partial HBeAg response was defined as a decrease in the HBeAg p/n ratio of more than 50% that persisted on two successive occasions; this differs from the usual definition of HBV DNA levels below 1 pg/ml with persisting HBeAg. HBeAg-nonresponders did not exhibit a marked decrease in HBeAg (< 50%).

Statistics.

Entry data, plotted in histograms to assess the skewness of distribution, showed that medians had to be used for data presentation. Medians of data collected on the basis of intention-to-treat (raw data) were not different from medians of data corrected for patients lost to follow-up (clean data). Clean data are presented in this report. The Wilcoxon matched sample sign test was used for comparison of data before, during and after treatment. The two-sample Wilcoxon rank sum analysis was applied to compare different groups.

RESULTS

Qualitative analysis.

HBeAg seroconversion occurred in 11 out of 44 (25%, 95% CI 12-38) patients who remained HBeAg-negative throughout the follow-up period of 52 weeks. HBV DNA negativity occurred in 17 out of 44 patients after entry to the study (39%, 95% CI 24-53, table 1) ; 2 patients became HBV DNA-negative in the 4-week period between entry and the start of treatment. For 8 out of 10 responders serum HBV DNA was negative prior to HBe-seroconversion; in 1 patient serum HBV DNA disappeared at the time of HBe-seroconversion; in another patient HBV DNA persisted at low levels for 25 weeks after HBe-seroconversion. In 1 HBeAg-responder HBV DNA could not be tested before HBe-seroconversion. Of the 17 patients who became serum HBV DNA-negative, only 11 were still negative at 52 weeks. One patient was negative for HBeAg at 52 weeks and positive for HBV DNA; reactivation occurred shortly afterwards.

table 1: Qualitative changes in serum HBeAg and HBV DNA status

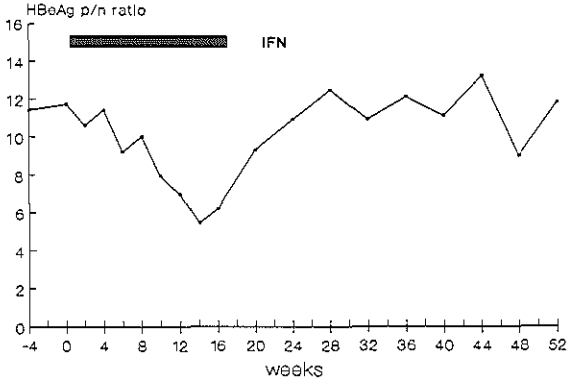
	HBeAg negative (n)	HBV DNA negative (n)	
	total (n=44)	total (n=44)	responders (n=11)
week -4	0/44	0/44	0/11
week 0	0/44	2/42	2/11
week 16	4/44	13/44	8/11
week 32	8/44	11/42	9/11
week 52	11/44	11/44	10/11

Quantitative analysis.

Quantitative HBeAg assessment showed a marked decrease 12 (38%) and 16 weeks (49%) after initiation of treatment compared to the pretreatment level ($p < 0.001$, fig 1). Three subgroups of patients were recognized on the basis of the HBeAg levels (fig 2); For HBeAg-responders (n=11) the fixed serum dilution was HBeAg-negative after a median of 12 weeks of treatment; partial HBeAg-responders (n=13, 30%, 95% CI 16-43) showed a marked decrease in HBeAg level but were not negative at 16 weeks; HBeAg-nonresponders (n=20) showed little variation in HBeAg level. When treatment was interrupted, a rebound to pretreatment levels occurred in the partial responders. No reactivation was observed among the responders within the 52 weeks of follow-up.

Serum HBV DNA decreased from a median level of 103 pg/ml at week 0 to 13 and 6.5 pg/ml at weeks 8 and 16, respectively ($p < 0.001$). After termination of treatment a rebound to 82.5 pg/ml occurred at week 52, a level which was no longer significantly different from the pretreatment value (fig 2[a]). Subgroup analysis of HBeAg-responders, partial HBeAg-responders and HBeAg-nonresponders showed a significant decrease in HBV DNA in all groups after 16 weeks of treatment. The median HBV DNA level for HBeAg-responders decreased from 18.5 pg/ml at week 0 to under the detection limit at week 16 ($p = 0.03$), for partial HBeAg-responders the level decreased from 126.5 pg/ml at week 0 to 4 pg/ml at week 16 ($p < 0.001$) and for HBeAg-nonresponders from 130 pg/ml at week 0 to 73 pg/ml at week 16 ($p = 0.001$) (fig 2[b]). Within the group of HBeAg-nonresponders, only 5 patients did not show a decrease in HBV DNA level during therapy. The pretreatment HBV DNA level found for HBeAg-responders was

A



B

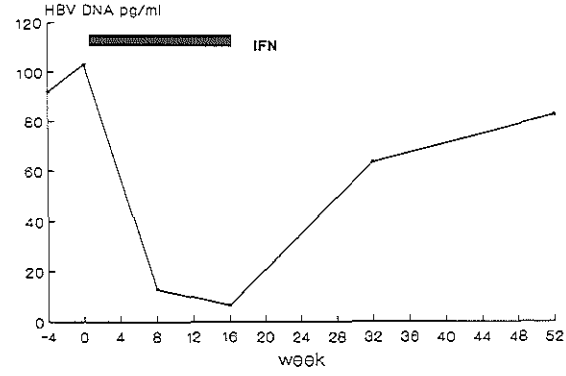
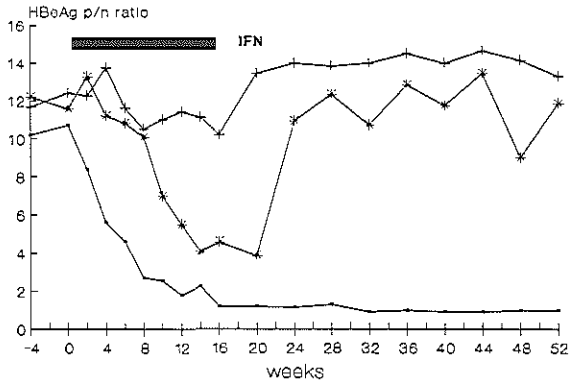


fig 1. A decrease in the median HBeAg level resulted in a statistically significant reduction of the HBeAg level at 12 and 16 weeks of treatment. A statistically significant decrease in median the HBV DNA level occurred at 8 and 16 weeks of treatment (see results, quantitative HBeAg and HBV DNA analysis).

significantly lower than that for partial HBeAg-responders and HBeAg-nonresponders ($p = 0.05$).

Paired observations of serum HBV DNA 4 weeks prior to the start of treatment and at the start of treatment for 36 patients showed a spontaneous decrease in HBV DNA level of 50 % or more in 10 patients (28%, 95% CI 14-45). Such a decrease was observed during treatment, i.e. between HBV DNA levels at the start of treatment and after 16 weeks of treatment, in 30 out of 39 patients (77%, 95% CI 61-89). Because a

A



B

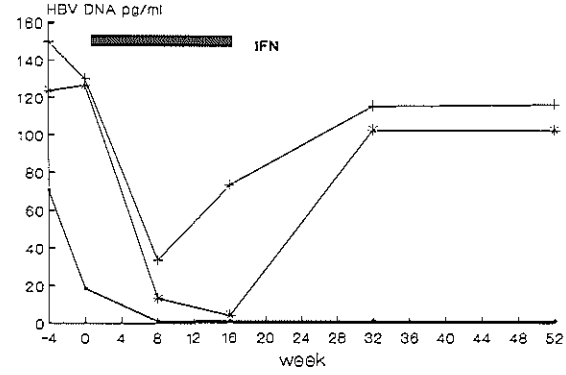


fig 2. Median HBeAg p/n ratio for HBeAg-responders (———), partial HBeAg-responders (*———*) and HBeAg-nonresponders (+———+) reveals a decrease for the first 2 groups with a persistent low HBeAg level for HBeAg-responders and a rebound to pretreatment levels for partial HBeAg- responders. The median level of HBV DNA in HBeAg-responders, partial HBeAg-responders and HBeAg-nonresponders is shown. A rebound to pretreatment levels is observed for partial HBeAg-responders and HBeAg-nonresponders. There is a statistically significant difference in pretreatment levels between HBeAg-responders and partial and nonresponders (see text, results)

decrease in HBV DNA level of 50% or more occurred significantly more often during treatment than spontaneously (28%), this event was related to therapy in approximately 70% of patients. We therefore evaluated this response to therapy in the subgroups of HBeAg-responders, partial responders and nonresponders (table 2). Two patients from

the HBeAg-nonresponder group did not show a marked decrease in serum HBV DNA level. Since all other patients showed a decrease in either serum HBeAg level or serum HBV DNA level, only 11 % were true nonresponders in this study.

table 2: Cumulative number of patients with a 50% HBV DNA decrease

	HBeAg-resp n=11	HBeAg-partial resp n=13	HBeAg-nonresp n=20
week 0	4/9	4/10	4/17
week 16	10/10	11/12	15/18
week 32	10/10	11/12	16/18
week 52	10/10	11/12	16/18

DISCUSSION

During IFN treatment 3 major decisions have to be made: 1 - stop therapy because of success, 2 - continue therapy because of partial response, 3 - stop therapy because of failure. Decision 1 can be based on information about HBeAg status. In the present study HBeAg negativity on 2 successive occassions appeared to be a stable endpoint of therapy. Decision 2 can be based on the presence or absence of a declining HBeAg level. The decrease in HBeAg and subsequent rebound to pretreatment levels after cessation of therapy in all partial HBeAg-responders together indicate that spontaneous HBe-seroconversion is unlikely to occur and that only prolongation of therapy may result in HBe-seroconversion (5). If no decrease in serum HBeAg level can be found, additional HBV DNA measurement is required. When the HBV DNA level has decreased, further therapy is indicated. Decision 3 requires absence of a response of the HBeAg or HBV DNA level after a period of 16 weeks of IFN treatment. Of course, it may be necessary to stop therapy because of unacceptable side effects.

The HBeAg assay described in this study requires serial dilutions of patient serum and the quantity of HBeAg cannot be expressed in weight units/volume unit. Because of the clinical relevance of the monitoring of HBeAg levels, as described above, development of a standard quantitative HBeAg assay is highly desirable.

We conclude that a quantitative HBeAg assay can be used for both monitoring and endpoint determination of antiviral therapy. The value of HBV DNA testing is that it identifies a response to treatment in patients with high initial levels of replication activity who do not exhibit a marked decrease in serum HBeAg level.

REFERENCES

1. Weissburg JI, Andres LJ, Smith CI et al. Survival in chronic hepatitis B; an analysis of 379 patients. Ann Intern Med 1984; 101:613-6.
2. Realdi G, Alberti A, Rugge M, Bortolotti F, Rigoli AM, Tremolada F and Ruol A. Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980; 79:195-9.
3. Fattovich G, Rugge M, Brollo L, Pontisso P, Noventa F, Guido M, Alberti A and Realdi G. Clininical, virologic and histologic outcome folllowing seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. Hepatology 1986; 2:167-72.
4. Berk L, Schalm SW, de Man RA et al. Failure of acyclovir to enhance the antiviral effect of alpha lymphoblastoid interferon on HBe-seroconversion in chronic hepatitis B. J Hep 1991, in press.
5. Janssen HLA, Berk L, de Man RA, Heijtink RA and Schalm SW. The antiviral effect of prolonged intermittent lymphoblastoid alpha interferon treatment in chronic hepatitis B. Gut 1991; in press.

CHAPTER 4

MODULATION OF ALPHA-INTERFERON EFFECTS BY INDOMETHACIN AND PARACETAMOL IN CHRONIC HEPATITIS B.

The contents of this chapter have been submitted for publication to the Journal of Hepatology under the same title with the following authors: Luuk Berk, Robert A de Man, Jan Lindemans, Rudolf A Heijtink and Solko W Schalm.

SUMMARY

Indomethacin and paracetamol can prevent influenza-like symptoms. We investigated whether antiviral effects of alpha-interferon in patients with chronic hepatitis B (suppression of active viral replication as well as enhanced expression of HLA class I antigens) occur during indomethacin and paracetamol administration.

Patients with chronic HBeAg positive hepatitis received for 2 weeks either: leukocyte alpha-interferon intramuscularly (group I, n=8), lymphoblastoid alpha-interferon subcutaneously (group II, n=10), acyclovir intravenously (group III, n=11) or a combination of lymphoblastoid alpha interferon and acyclovir (group IV, n=5). In addition group I received paracetamol (500 mg 4 times daily) and groups II and IV indomethacin retard (75 mg 2 times daily) during the first 3 days of antiviral therapy.

Indomethacin and paracetamol successfully prevented influenza-like symptoms. The drop in DNA polymerase activity after initiation of treatment was comparable in groups I, II and IV. Beta 2 microglobulin concentration, a measure of HLA class I antigen turnover, rose to a similar degree in groups I, II and IV; no rise was found for group III. A statistically significant decrease in DNA polymerase activity and increase in beta 2 microglobulin level was noticed in groups I and II.

Administration of alpha-interferon results in a decrease in markers of viral replication and enhanced expression of HLA antigens, despite indomethacin and paracetamol administration. Since indomethacin and paracetamol prevented the influenza-like symptoms of alpha-interferon treatment, these drugs can be given to avoid these symptoms.

Keywords: chronic hepatitis B, α-interferon, side effects, indomethacin, paracetamol.

INTRODUCTION

Therapy with alpha interferon (α-IFN) evokes influenza-like symptoms at initiation of treatment. The first week of treatment is characterized by fever, sometimes as high as 40 °C, with headache, myalgia, arthralgia and general malaise. Reduction of the dose of αIFN will decrease these side effects, but they tend to diminish of their own accord within two or three weeks of the start of therapy. However, the severity of these symptoms may cause interruption of professional activities and even hospitalization during the initial phase of therapy. Therefore the administration of analgesics to prevent influenza-like symptoms would seem to be justifiable. It is, however, not known whether analgesics interfere with the antiviral effect of α-IFN. Since the antiviral state of the cells, as induced by IFN, is reported to be related to continuous activity of cyclo-oxygenase to catalyze the first step of prostaglandin biosynthesis (1), inhibition by analgesics may have a negative influence on the effects of α-IFN treatment. On the other hand prostaglandins of the E series have been reported to inhibit induction of natural killer cell activity and killer cell activity by IFN (2). Arachidonic acid metabolites clearly have a dual effect on the antiviral activity induced by α-IFN. We therefore investigated the effects of indomethacin and paracetamol administration during α-IFN treatment for patients with a chronic active hepatitis B infection.

METHODS AND MATERIALS

Patients and treatment.

All patients had chronic HBeAg positive hepatitis and no history or signs of recent alcohol abuse, drug addiction or posttransfusion hepatitis. Leukocyte alpha interferon (α-IFN-le) was supplied by Cantell (3), lymphoblastoid alpha interferon (α-IFN-ly) and acyclovir (ACV) by Wellcome, Beckenham, United Kingdom. Four different therapies were given for 2 weeks: group I (n=8) received α-IFN-le (12 MU daily) intramuscularly, group II (n=10) received α-IFN-ly (5 MU daily) subcutaneously, group III (n=11) received ACV (30 mg/kg daily) intravenously and group IV (n=5) received a combination of the treatments of groups II and III. During ACV administration a urinary output of 2000 ml daily was maintained to preserve normal renal function. In addition group I received paracetamol (4 x 500 mg daily) and groups II and IV indomethacin retard (2 x 75 mg daily) during the first 3 days of therapy.

Serologic tests.

All serum samples taken before (week 0), during (week 1) and after treatment (week 2) were assayed for beta 2 microglobulin (B2M) by RIA (Pharmacia Diagnostics AB, Uppsala, Sweden), aspartate aminotransferase (AST) and creatinine using an automated system (SMA 12), HBeAg by RIA (Abbott laboratories, Ill., USA) and hepatitis B virus DNA polymerase activity (DNAp) by standard methodology: 800 μl of serum were centrifuged for 4 hours at 30,000 rpm and 4 °C in a SW41 rotor. The virus pellet was resuspended in 40 μl PBS and added to 105 μl of the following reaction mixture : 160 mM Tris-HCl, pH 7.5, 40 mM MgCl2, 120 mM NH4Cl, 50 μM dATP, 50 μM dGTP, 50 μM dCTP, 0.3 μM 3HdTTP (50 Ci/mmol), 1.0 % Nonidet P40, 0.3 % 2-Mercaptoethanol, 33 % bidest, 9 % PBS. Incubation for 3 h at 37 °C was followed by spotting 100 μl on a Whatmann 3M chromatography paper which was air-dried for 30 min. DNA was fixed on the filter by overnight incubation in trichloroacetic acid (TCA) (5 % w/v), which also eluted the excess radioactive label, and then dried at 80 °C for 1 h. Subsequently the incorporation of 3HdTTP was assayed by measuring the counts per minute (cpm) of the filters in insta-gel (United Technologies Packard) in a PW4540 liquid scintilation analyzer. In each test run 2 positive control samples and 5 negative control samples were included and assayed as described above. The test cut-off was the mean of the negative samples plus 3 standard deviations.

Statistics.

Data were compared by the Wilcoxon signed-rank test using an Olivetti M240 personal computer and STATA (Computing Resource Center, Los Angeles, USA).

RESULTS

Both indomethacin and paracetamol prevented or diminished influenza-like symptoms when administered during the first 3 days of α-IFN treatment. Body temperature remained normal to subfebrile in most patients (fig 1). Some patients experienced mild influenza-like symptoms, such as headache, fatigue, myalgia and general malaise, but continuation of normal daily routine remained possible. Hospitalization was only required for intravenous administration of ACV.

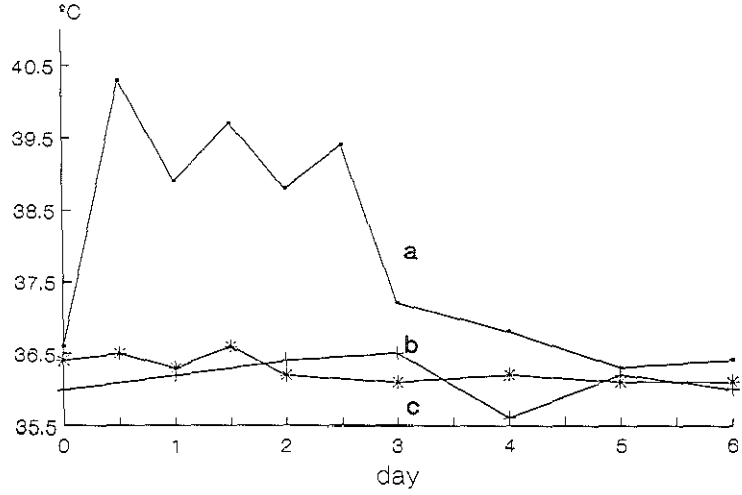


Figure 1. Body temperature during the first week of α-IFN treatment of a patient who did not receive paracetamol or indomethacin (a), median body temperature of 3 patients receiving paracetamol (500 mg 4 times daily) (b) and in 3 patients receiving indomethacin (75 mg 2 times daily) (c) during the first 3 days of α-IFN therapy.

Virus replication.

Inhibition of viral replication, as measured by DNAp activity, occurred in all groups after 1 and 2 weeks. The median percentage of activity after 1 and 2 weeks compared to the pretreatment level was 44 and 47 % for group I ($p < 0.05$), 32 and 20 % for group II ($p < 0.05$), 45 and 59 % for group III and 17 and 15 % for group IV (fig 2), respectively. No decrease in the serum HBeAg titer was observed except for a non-significant drop in group IV at 2 weeks.

Beta 2 microglobulin levels.

Serum B2M levels were tested to assess HLA class I antigen turnover during α-IFN therapy. Only 3 patients showed slightly elevated pretreatment levels of B2M; 1 patient in group II (3.7 mg/l), 1 patient in group III (3.6 mg/l) and 1 patient in group IV (4.6 mg/l, normal level < 3.0 mg/l). B2M levels in groups I, II and IV exhibited a rise after

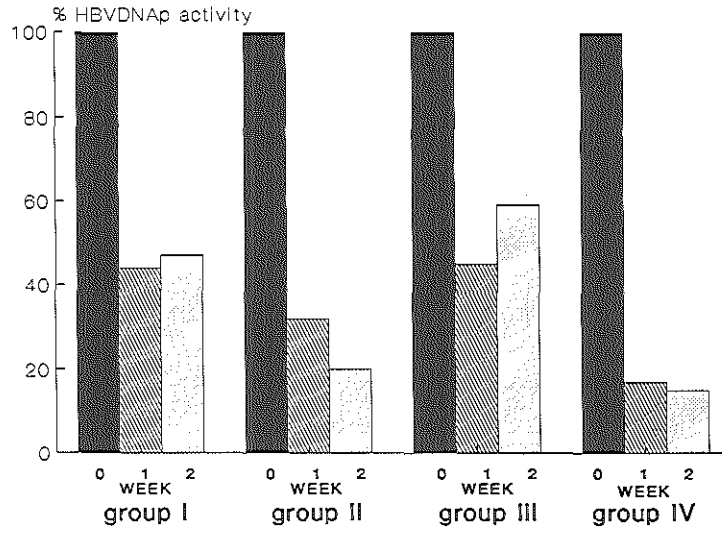


Figure 2. Percentage of HBV DNAp activity before (week 0), during (week 1) and after treatment (week 2) in groups I, II, III and IV. Statistically significant decreases were found at week 1 and 2 for groups I and II. A significant decrease was not reached in group IV because of the small number of patients.

the start of treatment. No increase was found for group III (fig 3). One and 2 weeks after initiation of treatment, the median percentual increments in serum level were: 65 and 74 % for group I ($p < 0.05$), 61 and 59 % for group II ($p < 0.05$), 20 and 17 % for group III and 58 and 54 % for group IV, respectively.

Hepatitis activity.

Serum AST levels were assessed to determine the change in hepatocellular lysis as a possible cause of the increment in B2M. Compared to baseline levels, no significant increase was found for any of the treatment groups. The median percentages of the AST level at weeks 1 and 2 compared to week 0 were: 98 and 90 % in group I, 110 and 113 % in group II, 105 and 122 % in group III and 106 and 98 % in group IV, respectively.

Serum creatinine levels remained stable in all groups.

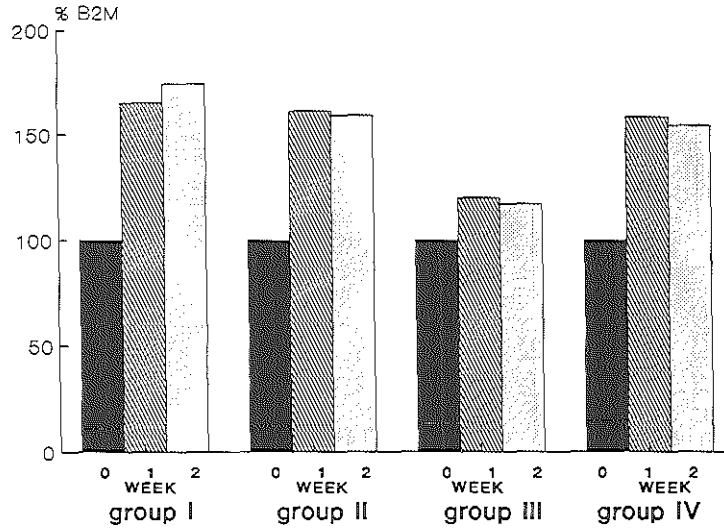


Figure 3. Percentual increments in B2M level before (week 0), during (week 1) and after treatment (week 2) in groups I, II, III and IV. Statistically significant increments were found for groups I and II.

DISCUSSION

The antiviral effect of α-IFN consists of depression of viral particle synthesis and cellular membrane changes with enhanced expression of HLA class I antigens (4,5). Depression of viral particle synthesis is reflected by the serum HBV DNAp activity, which is associated with virus particles in serum and responds to antiviral treatment within days (6). We therefore used serum HBV DNAp activity to demonstrate the antiviral effect of α-IFN. As an indirect parameter of HLA class I antigen expression, B2M serum levels were tested. Increase in HLA class I antigen turnover is associated with an increase in serum B2M concentration. If renal function and cytolysis remain stable, an increase in serum B2M concentration must be due to an increase in HLA class I antigen expression on cellular membranes. The stable levels of serum AST excluded an increase in hepatocellular necrosis and stable serum creatinine levels indicated that the renal clearance of B2M did not change. A statistically significant decrease in serum DNAp activity and increase in serum B2M level were found for patients of group I, indicating

that despite paracetamol administration inhibition of viral particle synthesis and increased HLA class I antigen expression occurred during α-IFN therapy. The same results were found for group II which received indomethacin during the first 3 days of α-IFN administration. Patients in group III showed no increase in B2M level, as was expected because ACV does not increase HLA class I antigen expression. The decrease in DNAp activity and increase in B2M level during combination α-IFN and ACV therapy was at least as evident in group IV as in groups I and II, despite indomethacin administration during the first 3 days of treatment. We conclude that indomethacin and paracetamol can prevent the influenza-like symptoms of α-IFN treatment, when administered during the first 3 days of treatment. Furthermore, since a significant effect of α-IFN on viral replication activity and HLA class I antigen turnover could still be demonstrated, these drugs can be used to prevent the influenza-like side effects of α-IFN treatment.

REFERENCES

1 Pottathil R, Chandrabose KA, Cuatrecasas P and Lang DJ. Establishment of the interferon-mediated antiviral state: Role of fatty acid cyclooxygenase. Proc Natl Acad Sci USA 1980; 77 (9):5437-40.

2 Flodgren P and Sjögren HO. Influence in vitro on NK and K cell activities by cimetidine and indomethacin with and without simultaneous exposure to interferon. Cancer Immunol Immunother 1985; 19:28-34.

3 Cantell K and Hirvonen S. Large-scale production of human leucocyte interferon containing 10 units per ml. J Gen Virol 1978; 39:54.

4 Lockart RZ. Criteria for acceptance of a viral inhibitor as an interferon and a general description of the biological properties of known interferons. In: Finter NB, editor. Interferons and Interferon Inducers. Amsterdam: North-Holland, 1973: 11-27.

5 Pignatelli M, Waters J, Brown D et al. HLA class I antigens on hepatocyte membrane: increased expression during acute hepatitis and interferon therapy of chronic hepatitis B. Hepatology 1986; 6:349-53.

6 Schalm SW, van Buuren HR, Heijtink RA and de Man RA. Acyclovir enhances the antiviral effect of interferon in chronic hepatitis B. The Lancet 1985; ii:358-60.

CHAPTER 5

ZIDOVUDINE INHIBITS HEPATITIS B VIRUS REPLICATION

The contents of this chapter have been submitted for publication to Antiviral Research under the same title with the following authors: Luuk Berk, Solko W Schalm and Rudolf A Heijtink.

SUMMARY

Hepatitis B virus DNA polymerase is a viral enzyme that can use viral DNA as well as viral RNA as a template for DNA synthesis. Since both activities are essential for the production of new virus particles, blockage of this enzyme should reduce viral replication activity.

In the present study the in vitro effect of zidovudine triphosphate on hepatitis B virus DNA polymerase activity and the in vivo effect of zidovudine on viral replication activity in chronic HBsAg-positive patients are investigated. Zidovudine triphosphate inhibited in vitro DNA polymerase activity by fifty percent at a concentration of 0.3 μM. Serum DNA polymerase activity was significantly reduced in seven patients who received zidovudine (200 mg orally four times daily) for one week. A dose response effect was suggested by the results found for six patients who received 100 mg, 200 mg and 300 mg orally four times daily for one week with 2 drug-free weeks between each course.

We conclude that zidovudine may be of value for nonresponders to alpha-interferon therapy or patients with high initial levels of viral replication prior to the start of interferon treatment.

INTRODUCTION

The hepatitis B virus (HBV) has an endogenous DNA polymerase (DNAp) activity that can be detected in the serum of patients with active virus replication (1). This DNAp induces synthesis of viral DNA by DNA-dependent DNAp activity and RNA-dependent DNAp activity (2). Both activities are essential for HBV replication. Inhibition of these steps of the replication cycle therefore should reduce the formation of new virus particles.

Alpha interferon (α-IFN) can be considered the cornerstone of antiviral therapy for chronic hepatitis B. Treatment for 12 -16 weeks with 5 MU daily or 10 MU every other day results in the induction of virus latency (HBe-seroconversion) in approximately 25 % of patients (3). Response to α-IFN treatment is related to the initial level of viral replication, with poor results for patients with high levels of replication activity (4). To increase the response rate for α-IFN, it therefore appears rational to add to the treatment regimen an antiviral agent with a different target in the multiplication cycle than those influenced by α-IFN. Administration of nucleoside analogues in combination with α-IFN to patients who do not respond to monotherapy or have high initial replication levels prior to the start of treatment may thus increase the response to treatment. Several nucleoside analogues have already been included in the treatment of chronic hepatitis B: acyclovir with poor success (5) and adenine-arabinoside (-monophosphate) with moderate success but severe toxicity (6).

Zidovudine (3'-azido-3'-deoxythymidine, AZT) is widely used to inhibit in vivo human immunodeficiency virus (HIV) replication since it has been demonstrated that AZT leads to a significant improvement in the survival of AIDS patients (7). Zidovudine triphosphate (AZT TP) has also been reported to inhibit HBV DNAp activity in vitro (8). In order to confirm these findings, we investigated the in vitro effect of AZT TP on HBV DNAp activity and the in vivo effects of zidovudine on hepatitis B virus replication activity.

METHODS AND MATERIALS

Virus isolation.

Hepatitis B virus (HBV) was isolated from HBV DNA positive serum by ultracentrifugation in a SW41 rotor for 3 h at 30,000 rpm and 4 °C. The pellet was resuspended in phosphate buffered saline (PBS), layered on a discontinuous gradient of 10 to 20 % sucrose in sodium, tris HCl and EDTA (NTE) buffer, pH 7.5, and centrifuged overnight in

a SW27 rotor at 25,000 rpm and 4 °C. The pellet was resuspended in PBS and immediately assayed for DNAp activity.

HBV marker assays.

DNAp activity measurements were performed in duplo as described by Howard using a modification of the elution of unincorporated tritiated thymidine-methyl-5'-triphosphate (3HdTTP) as described by Fang et al (9,10). Forty µl of virus suspension were added to 105 µl of the following reaction mixture : 160 mM Tris-HCl, pH 7.5, 40 mM MgCl2, 120 mM NH4Cl, 50 µM dATP, 50 µM dGTP, 50 µM dCTP, 0.3 µM 3HdTTP (50 Ci/mmol, Amersham, UK), 1.0 % Nonidet P40, 0.3 % 2-Mercaptoethanol, 33 % bidest, 9 % PBS. Incubation for 3 h at 37 °C was followed by spotting 100 µl on Whatmann 3M chromatography paper which was air-dried for 30 min. DNA was fixed on the filter by overnight incubation in trichloroacetic acid (TCA) (5 % w/v) which also eluted the excess 3HdTTP, and then dried at 80 °C for 1 h. Incorporation of 3HdTTP was assayed by placing the filters in 10 ml scintillation counting fluid (Insta-gel, Packard, Ill.) in a PW4540 liquid scintillation analyzer.

To assay HBV DNAp activity in patient serum 800 µl samples were diluted to 11 ml in PBS and centrifuged for 4 hours at 30,000 rpm and 4 °C in a SW41 rotor. The virus pellet was resuspended in 40 µl PBS and added to 105 µl assay mix, as described above. Five negative control samples and 2 positive control samples were included in each test run. Samples were considered positive when the cpm exceeded the mean cpm of the negative control samples plus 3 standard deviations (approximately 150 cpm). HBV DNA was measured by liquid phase hybridization (Genostics, Abbott, Ill., U.S.A.); results exceeding 5 pg/ml were considered positive. Serum HBsAg and HBeAg were tested by RIA (Abbott, Ill., U.S.A.)

HBV DNAp inhibitors.

A sodium salt of 3'azido 2'3'dideoxythymidine triphosphate (AZT TP) was donated by Prof. E. de Clercq, Rega Institute, Leuven. A stock solution of 200 µM AZT TP in bidest was kept at -20 °C. The concentration of AZT TP in bidest was assessed by high pressure liquid chromatography (dr. J. Balzarini, Rega Institute, Leuven). A sodium salt of phosphonophormate (foscarnet, PFA) was provided by Dr. M. Kuipers, Astra, the Hague. A stock solution was made of 10 mM in bidest and stored at 4 °C.

Patients.

All patients (n=7) had had HBsAg positive hepatitis for more than 1 year, with stable

levels of HBeAg, DNAp activity and HBV DNA in serum as well as HBcAg in the liver. None of the patients had antiHDV antibodies in serum or HDAg in the liver. Two had cirrhosis and 3 were antiHIV positive with normal OKT4 positive cell counts.

Treatment.

Zidovudine was given in 100 mg capsules, orally. Seven patients (Group I) received 4 x 200 mg zidovudine daily for 1 week. Six patients (group II) then received increasing doses of the drug: 4 x 100 mg, 4 x 200 mg and 4 x 300 mg zidovudine daily for 1 week with 2 drug-free weeks between each course.

Statistics.

Pre- and posttreatment results were compared by the Wilcoxon sign test.

RESULTS

In vitro results.

Characteristics of the virus suspension, obtained from the serum of a patient with chronic hepatitis B, are given in table 1. Inhibition of 3HdTTP incorporation by foscarnet (PFA) was used to identify HBV DNA-specific DNAp activity and inhibition by actinomycin D (act-D) to identify the proportion of activity due to DNA-dependent DNAp activity and RNA-dependent DNAp activity (11). A final concentration of 10 μM PFA in the assay mixture resulted in an inhibition of HBV DNAp-specific activity of 91 %. Addition of act-D in a final concentration of 100 μM resulted in an inhibition of (DNA dependent-) DNAp activity of 83 %. Inhibition by AZT TP is presented in figure 1. A 50 % inhibition can be estimated for an AZT TP concentration of 0.3 μM in the test. Results were reproducible for sera obtained from different patients and in different sets of tests.

In vivo results.

DNAp activity decreased in all patients of group I after 4 days of treatment and were still low on day 7 (fig 2). Median DNAp activity diminished from 3036 cpm on day 0 to 819 cpm on day 4 (median percentual decrease compared to baselines values: 50%, 95% CI 14-73%) and 999 cpm on day 7 (29%, 95% CI 12-72%, $p=0.008$). In group II a dose-response effect was observed; median DNAp activity on days 4 and 7 was 85% (95% CI 54-188%) and 76% (95% CI 57-115%), respectively, of initial activity during 4 x 100 mg, 57% (95% CI 19-107%) and 65% (95% CI 31-108) during 4 x 200 mg and 40% (95% CI 25-67%) and 54% (95% CI 6.6-136) during 4 x 300 mg zidovudine

Table 1: Characteristics of HBV suspension for in vitro assay

	DNAp activity (cpm)
40 µl sample	1070
negative control sample	46
40 µl sample + PFA 10 µM	102
40 µl sample + act D 100 µg/ml	173
background radiation	22

The serum, obtained from a male donor who had a chronic HBeAg, HBV DNA positive hepatitis acquired during his work as an ambulance nurse, was concentrated 20-fold after sedimentation and resuspension in PBS as described under Methods and Materials. No clinical or serological indications of other viral infections were found. The patient was antiHDV, antiHIV, IgM antiHAV, IgM anti EBV and IgM antiCMV negative. The negative control sample contained 40 µl of pelleted serum from a healthy human donor. PFA = foscarnet, act D = actinomycin D.

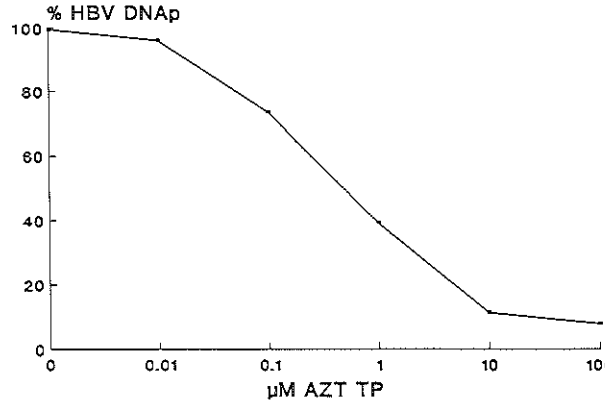


Figure 1. Percentage DNAp activity of a human HBV serum preparation during different concentrations of AZT TP.

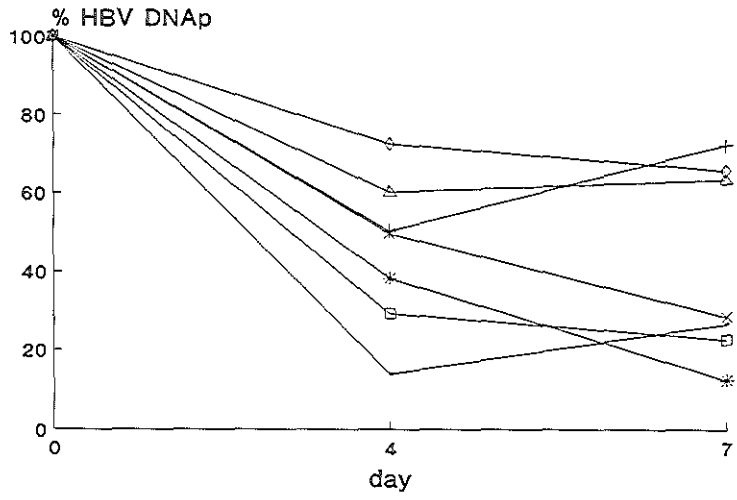


Figure 2. Percentage residual HBV DNAp activity in serum during treatment with zidovudine (200 mg orally 4 times daily).

daily (fig 3). No statistical significance was observed because 1 out of 6 patients did not respond to therapy. HBV DNA also tended to decline but no statistical significance could be found because 1 patient did not respond. During 4 x 300 mg zidovudine the median HBV DNA level decreased from 227 pg/ml on day 0 to 196 pg/ml on day 4 (median percentage compared to baseline values: 105%, 95% CI 62-109%) and 161 pg/ml on day 7 (77%, 95% CI 41-124%).

DISCUSSION

The results of our in vitro study confirm those reported by Nordenfelt et al. Since the concentration of AZT TP that yields 50 % inhibition is comparable to the concentration of the label used in this assay, the drug has an affinity for viral DNAp similar to that of the natural substrate thymidine TP. In vitro AZT TP appears to be a moderately effective inhibitor of DNA dependent HBV DNAp activity.

The results show that a statistically significant suppression of HBV DNAp activity is achieved in chronically infected patients by the administration of zidovudine 4 times daily. The repeated 1-week courses with increasing doses ranging from 400 to 1200

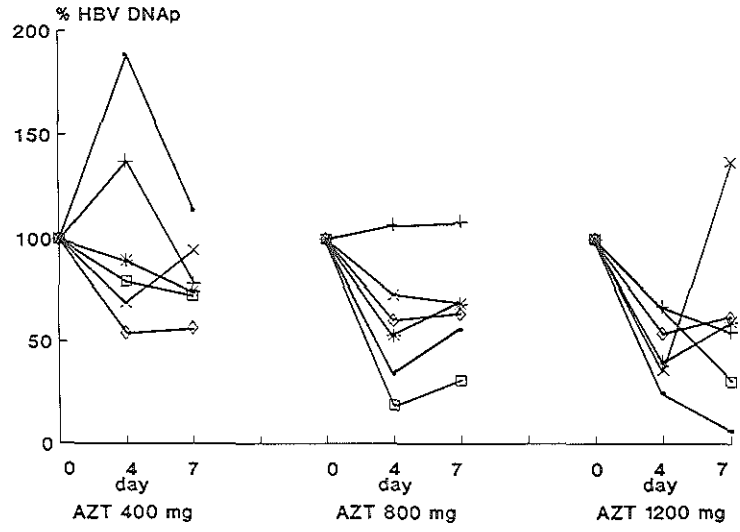


Figure 3. HBV DNAp activity expressed as a percentage of residual activity during increasing doses of zidovudine, suggesting a dose-response effect.

mg daily, provides an estimation of the dose versus response effect.

These findings are not in accordance with data of other investigators who found no effect of zidovudine on HBV replication in patients with concurrent HBV and HIV infections (12,13,14). Such patients usually have high levels of HBV replication activity. A lack of effect of zidovudine on HBV replication in symptomatic HIV-infected patients may be explained by the use of the insensitive semiquantitative dot-blot method for HBV DNA measurement (15).

In vitro experiments with HBV-producing cell lines and AZT showed inhibition by noncytotoxic doses of the drug, indicating that sufficient phosphorylation of AZT occurs in these models (16,17,18). We found the most pronounced inhibition of HBV DNAp on day 4 during each course with no further decline and sometimes even an increase in some patients. An escape mechanism of the virus might explain the lack of further effect on replication activity. Our results for immunologically competent patients should be confirmed by a randomized placebo-controlled study to evaluate the effect of zidovudine as a single agent on HBV replication activity. If this study confirms the antiviral effect of zidovudine in chronic hepatitis B, a randomized placebo-controlled

study to investigate the effect of zidovudine in combination with α-IFN in patients who do not respond to monotherapy with α-IFN is indicated.

The present results suggest that zidovudine may be effective in lowering viral replication activity in immunocompetent chronic HBV-infected patients. Since AZT can be given orally, further study is indicated to assess the clinical efficacy and risks of this drug.

REFERENCES

1. Robinson WS and Greenman RL. DNA Polymerase in the Core of the Human Hepatitis B Virus Candidate. J Virol 1974: 13; 1231-6.
2. Fowler MJF, Monjardino J, Tsiquaye KN, Zuckerman AJ and Thomas HC. The Mechanism of Replication of Hepatitis B Virus: Evidence of Asymmetric Replication of Two DNA Strands. J Med Vir 1984: 13; 83-91.
3. Schalm SW, De Man RA, Thomas HC, Jacyna M, Hadziyannis SJ and Manessis E. Chronic hepatitis B: therapeutic controversies and randomised controlled trials. Gastroenterology International 1989: 2(1); 16-24.
4. De Man RA, Schalm SW, Heijtink RA et al. Interferon plus descyclovir in chronic hepatitis type B: incidence of virus marker elimination and reactivation. In: Viral hepatitis and liver disease; A. J. Zuckerman (ed); Alan R. Liss, Inc; New York 1988: 913-916.
5. Alexander GJM, Fagan EA, Hegarty JE, Yeo J, Eddleston ALWF and Williams R. Controlled clinical trial of acyclovir in chronic hepatitis B infection. J Med Vir 1987: 21; 81-7.
6. Lok ASF, Wilson LA and Thomas HC. Neurotoxicity associated with adenine arabinoside monophosphate in the treatment of chronic hepatitis B virus infection. J Antimicrob Chemother 1987: 14; 93-9.
7. Fischl MA, Richman DD, Grieco MH et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind placebo-controlled trial. N Engl J Med 1987: 317; 185-91.
8. Nordenfelt E, Löfgren B, Chattopadhyaya J and Öberg B. Inhibition of Hepatitis B Virus DNA Polymerase by 3'-Azido-3'-Deoxythymidine Triphosphate but not by its Threo Analog. J Med Vir 1987: 22; 231-6.
9. Howard CR. The detection of DNA polymerase activity in the diagnosis of HBV infection. J Med Vir 1978: 3; 81-6.
10. Fang CT, Nath N, Pielech M and Dodd RY. A modified technique for the detection of hepatitis B virus-specific DNA polymerase. J Vir Methods 1981: 2; 349-56.
11. Tao P-Z, Löfgren B, Lake-Bakaar D, Johansson NG, Datema R and Öberg B. Inhibition of human hepatitis B virus DNA polymerase and duck hepatitis B virus DNA polymerase by triphosphates of thymidine analogs and corresponding nucleosides. J Med Vir 1988: 26; 353-62.
12. Gilson RJC, Hawkins AE, Kelly GK, Gill SK and Weller IVD. No effect of zidovudine on hepatitis B virus replication in homosexual men with symptomatic HIV-1 infection. AIDS 1991: 5; 217-20.
13. Farraye FA, Mamish DM and Zeldish JB. Preliminary evidence that azido thymidine does not affect hepatitis B virus replication in aquired immunodeficiency syndrome (AIDS) patients. J Med Virol 1989: 29; 266-7.
14. Marcellin P, Pialoux G, Girard P-M et al. Absence of effect of zidovudine on replication of hepatitis B virus in patients with chronic HIV and HBV infection. N Engl J Med 1989: 321; 1758.
15. Lin HJ, Wu P-C and Lai C-L. An oligonucleotide probe for the detection of hepatitis B virus DNA in serum. J Virol Methods 1987: 15; 139-49.
16. Lampertico P, Malter JS and Gerber MA. Development and application of an in vitro model for screening anti-hepatitis B virus therapeutics. Hepatology 1991: 13; 422-6.
17. Ueda K, Tsurimoto T, Nagakata T, Tschisaka O and Matsubara K. An in vitro system for screening anti-hepatitis B virus drugs. Vir 1989: 169; 213-6.
18. Galle PR and Theilmann L. Inhibition of hepatitis B virus polymerase-activity by various agents. Drug Research 1990: 40; 1380-2.

CHAPTER 6

BENEFICIAL EFFECTS OF PHYLLANTHUS AMARUS FOR CHRONIC HEPATITIS B, NOT CONFIRMED

Part of this chapter has been published in the Journal of Hepatology 1991, 12(3); 405-406, under the same title with the following authors: Luuk Berk, Robert A de Man, Solko W Schalm, Rudi P Labadie and Rudolf A Heijtink.

SUMMARY

The positive results of Thyagarajan et al with P. amarus to eradicate HBsAg in chronic HBV infected patients, stimulated us to investigate the effects of this plant on HBsAg seroconversion in our own patient population. We designed a cross-over study for 10 patients who were HBsAg positive for more than 1 year. All patients were antiHD and antiHIV negative. They were randomly assigned to receive double blind either P. amarus from Suriname 3 x 200 mg daily for 28 days followed by placebo (spinach) 3 x 200 mg daily for 28 days (group 1) or placebo followed by P. amarus (group 2). Clinical evaluation was done four-weekly to register side effects. Blood sampling was done weekly to monitor changes in HBsAg (RIA), HBeAg (RIA) or AST status. Follow-up was performed until 12 weeks after onset of therapy.

No marked clinical changes were observed during treatment nor during follow-up. No changes in HBsAg, HBeAg or HBV DNA level nor in AST level were observed during treatment with P. amarus or placebo. No difference between HBV DNA positive patients (n=6) or HBV DNA negative patients (n=4) could be observed.

We conclude that P. amarus from Suriname did not effect the clinical, biochemical or HBV virological status of our patient population. In view of our negative results and those of Leelarasamee et al with P.amarus from Thailand, we think that the results of Thyagarajan et al need to be confirmed with P. amarus from India in a European centre to conclude that P. amarus might be an effective drug in the treatment of chronic hepatitis type B in European patients.

INTRODUCTION

Phyllanthus amarus is a tropical plant that is used as a herbal medicine in southern India for over 2000 years against icteric and various other diseases. Several antihepatotoxic and potential antiviral substances have been isolated from P. amarus (1,2,3). In 1982 in vitro inhibition of HBsAg-antiHBs binding was reported by aqueous extracts of P. amarus (4). Further evaluation of this herb in the woodchuck (Marmota Monax) model infected with woodchuck hepatitis virus showed in vitro inhibition of WHsAg-antiWHs binding and WHV DNA polymerase activity. In vivo studies showed decrease of serum WHsAg titers and inhibition of WHV DNA polymerase activity (5). In 1988 a placebo controlled study reported a high incidence of HBsAg eradication in chronic HBV infected patients treated with a dried plant preparation (6). These positive results stimulated us to investigate the effects of this plant on HBsAg seroconversion in our own patient population.

METHODS

Patients.

Ten patients who had been HBsAg positive for more than 1 year were entered in the study. All patients were antiHD and antiHIV negative. Patient characteristics are given in the table.

Treatment and design.

Patients were randomly assigned to one of two groups to receive double blind either P. amarus 3 x 200 mg daily for 28 days followed by placebo 3 x 200 mg daily for 28 days (group 1) or placebo followed by P. amarus (group 2). Clinical evaluation was done four-weekly to register side effects. Blood sampling was done weekly and follow-up was performed until 12 weeks after onset of therapy.

Medication.

P. amarus plants were gathered in Suriname and kept fresh frozen. After freeze-drying and grinding preparations were sterilized by gamma-radiation, and dispersed in 100 mg capsules. For placebo (spinach) the same procedure was performed.

Laboratory assessments.

Serum HBsAg and HBeAg were assessed by RIA (Abbott, Ill.). Serum HBV DNA was measured by liquid phase hybridisation (Abbott, Ill.) and serum aspartate aminotransferase (AST) by using an automated system (SMA 12).

Statistics.

Cross-over analysis was performed for HBsAg p/n ratio to assess treatment effect, period effect or a combined treatment x period effect (7).

RESULTS

No marked clinical changes were observed during treatment nor during follow-up. Tabel 1 shows the results of P. amarus on HBsAg and HBeAg level and on HBV DNA level in individual patients. No changes in HBsAg, HBeAg or HBV DNA level nor in AST level were observed during treatment with P. amarus. No difference between HBV DNA positive patients (n=6) or HBV DNA negative patients (n=4) could be observed. Table 2 presents the cross-over analysis of HBsAg levels during P. amarus and placebo treatment.

DISCUSSION

The cornerstone of therapy for chronic hepatitis B is alpha interferon (IFN) that induces virus latency in a significantly greater proportion of patients than spontaneously occurs in control patients (8). However, a considerable amount of patients does not benefit from a standard IFN treatment of 12 to 16 weeks. Addition of an antiviral agent with a different target in the viral replication cycle than the enhanced cytotoxic immunity induced by IFN would therefore be likely to improve the treatment response. The in vitro and in vivo results of P. amarus from India on HBsAg-antiHBs binding and HBs-seroconversion (4,6) suggested that P. amarus could be of value to improve IFN treatment results.

We did not find a beneficial effect of P. amarus from Suriname on the virological status of our patients, nor on the clinical or biochemical status. The quality of the herbs used in our study was guaranteed by preparation according to routine procedures in a pharmacological laboratory (Prof R.P. Labadie, department of Pharmacology, University of Utrecht). Leelarasamee et al found no effect of P. amarus from Thailand either (2). P. amarus is a common herb found in several tropical regions world-wide. In view of the

table 1.

EFFECT OF P. AMARUS ON HBsAg AND HBeAg TITERS AND SERUM AST LEVEL

			before therapy			end of therapy		
pat	sex	age	HBsAg (p/n)*	HBeAg (p/n)*	HBV DNA (pg/ml)**	HBsAg p/n)*	HBeAg (p/n)*	HBVDNA (pg/ml)**
1	m	28	60.6	8.2	98	63.2	4.3	73
2	m	36	37.7	9.5	32	50.1	7.1	12
3	m	36	199.4	10.5	76	31.7	9.4	48
4	f	26	79.7	10.8	<1.6	86.9	10.4	4.4
5	m	40	41.8	8.6	6.2	59.7	9.7	3
6	f	52	71.1	7.9	13	63.4	6.5	7.7
7	m	24	40.1	4.0	<1.6	41.8	2.5	<1.6
8	m	30	76.4	8.1	27	84.8	12.8	25
9	m	35	55.8	0.9	<1.6	64.5	0.7	<1.6
10	m	59	55.4	0.8	<1.6	44.5	0.8	<1.6

* HBsAg and HBeAg were tested in a fixed dilution of serum throughout the study and expressed as a ratio of cpm in patient serum (p) and in negative control serum (n).

** AST normal < 30 U/l

results of Thyagarajan et al (4,6) and the support of these data by in vitro and in vivo results in the woodchuck model, there may be a difference in fenotype of these plants depending on the region of origin. We think that the data of Thyagarajan et al need to be confirmed with P. amarus from India in a European centre to conclude that P. amarus might be an effective drug in the treatment of chronic hepatitis type B in European patients.

table 2.

CROSS-OVER ANALYSIS OF HBsAg

period	1	2		
group 1 treatment	PA	plac	difference	sum
	mean + SD	mean + SD	mean + SD	mean + SD
HBsAg p/n ratio	58.8 + 27.7	56.6 + 18.3	2.2 + 11.1	115.4 + 45.6
group 2 treatment	plac	PA		
HBsAg p/n ratio	63.7 + 16	61.5 + 19	2.1 + 7.1	125.2 + 34.4
p	0.26	0.31	d1 = d2: 0.01 d1 = -d2: 0.52	0.29

PA = Phyllanthus amarus; plac = placebo; difference = (HBsAg p/n ratio) period 1 - (HBsAg p/n ratio) period 2, d1 = difference group 1, d2 = difference group 2; summ = (HBsAg p/n ratio) period 1 + (HBsAg) period 2; p = p value obtained by t-test for unequal means. Equality of d1 and d2 indicates that no treatment effect was found. The sum of d1 and d2 excluding 0 indicates that no period effect was found. Equality of the sum of period 1 and period 2 for group 1 and 2 indicates that no treatment x period effect was found.

REFERENCES

1 Joshi BS, Gawad DH, Pelletier SW, Kartha G and Bhandary K. Isolation and structure (x-ray analysis) of ent-norsecurinine, an alkaloid from Phyllanthus niruri. J Nat Prod 1986; 49(4): 614-620.

2 Syamasundar KV, Singh B, Thakur RS, Husain A, Kiso Y and Hikino H. Antihepatotoxic principles of Phyllanthus niruri herbs. J Ethnopharmacology 1985; 14(1): 41-44.

3 Gupta DR and Ahmed B. Nirurin: a new prenylated flavanone glycoside from Phyllanthus niruri. J Nat Prod 1984; 47(6): 958-963.

4 Thyagarajan SP, Thiruneelakantan K, Subramanian S and Sundaravelu T. In vitro inactivation of HBsAg by Eclipta alba Hassk and Phyllanthus niruri Linn. Indian J Med Res 1982; 76 Suppl: 124-130.

5 Venkateswaran PS, Millman I and Blumberg BS. Effects of an extract from Phyllanthus niruri on hepatitis B and woodchuck hepatitis viruses: in vitro and in vivo studies. Proc Natl Acad Sci USA 1987; 84 (1): 274-278.

6 Thyagarajan SP, Subramanian S, Thirunalasundari T, Venkateswaran PS and Blumberg BS. Effect of Phyllanthus amarus on chronic carriers of hepatitis B virus. Lancet 1988; ii: 764-766.

7 Armitage P and Berry G. The simple crossover design. In: Statistical methods in medical research. 2nd ed Oxford: Blackwell Scientific Publications, 1987; 222-226.

8 de Man RA, Berk L, Schalm SW and Stijnen T. Treatment of chronic viral hepatitis. Survey of digestive diseases, 1991; 9: 17-35.

9 Leelarasamee A, Trakulsomboon S, Maunwongyathi P, Somanabandhu A, Pidetcha P, Matrakool B, Lebnak T, Ridthimat W and Chandanayingyong D. Failure of Phyllanthus amarus to eradicate hepatitis B surface antigen from symptomless carriers. Lancet 1990; i: 1600-1601.

CHAPTER 7

ALPHA LYMPHOBLASTOID INTERFERON AND ACYCLOVIR FOR CHRONIC HEPATITIS DELTA

The contents of this chapter have been published in "The Hepatitis Delta Virus", J.L. Gerin, M. Rizzetto and R.H. Purcell (ed), New York, Wiley-Liss, Inc., 1991; 411-420, under the same title and with the following authors: Luuk Berk, Rob A. de Man, Chantal Housset, Pierre Berthelot and Solko W. Schalm

SUMMARY

Ten patients with chronic hepatitis type D were treated during 4 months with alpha lymphoblastoid interferon in combination with two 2-week courses of acyclovir. Median percentage of HDAg-positive hepatocytes decreased from 11 to 1, $p = 0.0225$. Patients with no liver HDAg expression after treatment ($n=5$) showed improved AST levels (normal in 4) and diminished liver cell inflammation. One patient, who cleared HDAg has complete biochemical remission of his liver disease with 2 years of follow-up. Five patients with persistent, albeit low, HDAg expression in the liver, had continued liver cell destruction (AST elevated and/or abnormal biopsy). No evidence for an enhancing effect of acyclovir for interferon therapy was observed.

INTRODUCTION

Hepatitis Delta virus (HDV) complicating a hepatitis B virus (HBV) infection, although uncommon in North-Western Europe, is a serious disease. Cirrhosis is often found in patients between 30 and 40 years of age (1). Results of treatment with corticosteroids or interferon (IFN) have been inconclusive. Suppression of serum HDV RNA by IFN is frequently observed but sustained inactivation of the liver disease is seldom obtained (2,3,4). During the last few years, several aspects of the replication cycle of HDV and the role of delta antigen (HDAg) for genome replication have been elucidated (5,6). No viral enzyme activity has been recognized as a potential target for antiviral therapy. Furthermore, it now appears that HDV is not in itself cytopathic but livercell necrosis usually occurs in condition of replication of both HDV and HBV. A rational goal of therapy is to interfere with HDAg synthesis and to suppress HBV replication. Alpha-IFN is considered an antiviral agent acting through interference with viral messenger RNA and the most efficacious drug available for suppressing HBV replication. In 1985, when this study was designed, combination therapy of alpha lymphblastoid IFN and acyclovir (ACV) was thought to be the most promising therapy for chronic HBV infection (7). In this study we evaluated this combination therapy in patients with chronic hepatitis D.

METHODS

Patients.

Ten patients from 2 hospitals (Hopital Laennec, Paris and University Hospital Dijkzigt, Rotterdam), with chronic HDV infection were entered in this study between december 1985 and june 1988. All patients were male and antiHIV negative. Patient characteristics are listed in table 1.

Therapy.

Alpha lymphoblastoid IFN (Wellferon, Wellcome, Beckenham, U.K.) 5 mega-unit daily was administered by subcutaneous injection during 4 months. Two 2-week courses of ACV 2 g daily intravenously were given during week 1 and 2 and week 8 and 9 of IFN administration. Slow release indomethacin 2 x 75 mg daily, or paracetamol 4 x 500 mg orally was given during the first 3 days of IFN administration to reduce side-effects.

Approval for the study was obtained from the human study commitees of both participating hospitals.

table 1: patient characteristics at entry

patient (nr)	age (y)	trans-mission	known antiHD	duration pos (mo)	AST (xULN)	histology	hepatocytes (%) pos for HDAg	HBcAg	HBeAg	HBV DNA > 5 pg/ml	antiHCV*
1	48	stay in endemic area		4	2.4	mild CAH cirrhosis	18	0	-	-	+
2	24	i.v. drug-abuse		21	2.4	mild CAH cirrhosis	5	0	-	-	+ +
3	50	stay in endemic area		24	1.4	mod CAH cirrhosis	1	0	+	-	-
4	31	i.v. drug abuse		96	2.4	sev CAH cirrhosis	0	0	-	-	-
5	26	i.v. drug abuse		5	2.4	mod CAH mild fibr	18	0	-	-	-
6	48	stay in endemic area		36	1.5	mod CAH cirrhosis	4	0	-	-	+
7	27	homosexual contacts		12	1.4	sev CAH mild fibr	20	0	+	-	-
8	29	i.v. drug abuse		11	0.9	mod CAH mild fibr	40	0	-	-	+ +
9	36	unknown		18	4.6	mod CAH mild fibr	0	0	-	-	-
10	27	i.v. drug abuse		10	1.4	mod CAH sev fibr	2	0	-	-	+ +

* +: absorbance > 0.473 (=detection limit), + +: absorbance of positive control
CAH = chronic active hepatitis, fibr = fibrosis, mod = moderate, sev = severe

Laboratory assessments.

Pre-treatment sera were tested for HBsAg (R.I.A., Abbott), HBeAg (R.I.A., Abbott), HBV DNA (Genostics, Abbott) and antiHCV antibodies (E.I.A., Ortho). AntiHDV antibodies were tested by E.I.A. (Abbott). During treatment serum AST levels were assessed every 2 weeks in addition to assays for leucocytes, platelets and creatinin. Liver biopsies were taken before and within 10 weeks after treatment, and tested for HDAg by immunofluorescence and/or immunoperoxidase. Indirect immunofluorescence

was performed on paraffine slides. After deparaffination and pronase pretreatment, slides were incubated with human serum containing a high titer of IgG antiHDV antibodies, giving a specific staining for HDAg in a dilution of 1/30 in PBS and no staining for HBcAg and HBsAg. Paired pre- and post treatment biopsies were tested by this method. Fresh frozen tissue was tested by direct immunofluorescence and/or immunoperoxidase, using human serum derived antiHDV antibodies labeled with FITC or peroxidase. This serum gave a specific staining in a dilution of 1/80 in PBS for HDAg, while negative results were obtained for HBcAg and HBsAg.

RESULTS

0 - 16 weeks.

Eight out of 10 patients were positive for liver HDAg before treatment. A marked decrease in liver HDAg expression was observed in post treatment biopsies. The median percentage of positive hepatocytes diminished from 11 to 1, $p = 0.0225$ (Wilcoxon, signed-rank test). Three patients became negative for liver HDAg during treatment in addition to the 2 patients that were negative before treatment and remained negative in the post treatment biopsy (fig 1).

The 4 patients with high HDV replication activity (HDAg in 15 - 40 % of hepatocytes) did not respond differently to treatment than the 6 patients with low HDV replication activity (HDAg in 0 - 5 % of hepatocytes). In the former group 2 patients became liver HDAg negative with normalization of serum AST and reduction in periportal infiltrate. In the latter group 1 patient became liver HDAg negative, 3 showed normalization of AST and 3 had a reduction in periportal infiltrate.

Nine patients had elevated serum AST levels prior to treatment. Median AST level decreased from 2.0 to 1.0 x the upper limit of normal (ULN). Although this decrease was not statistically significant, AST normalized in 5 patients. Of 7 patients with a decrease of AST level during treatment, 5 showed a reduction of periportal infiltrate and only 2 remained low positive for liver HDAg expression.

Furthermore, all patients with a reduction in periportal infiltrate in the post treatment biopsy ($n=5$) had a decrease in AST level and no liver HDAg expression after treatment.

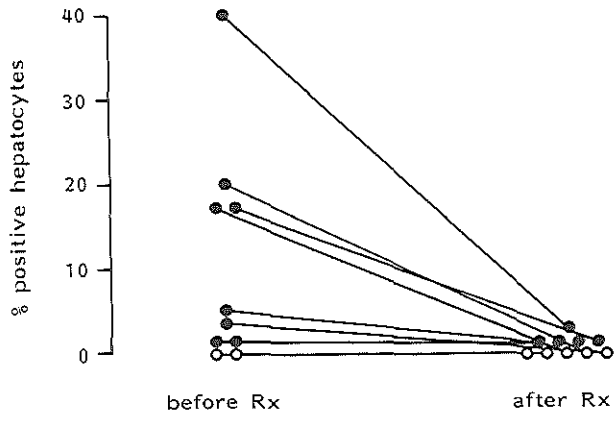


Figure 1. Liver HDAg expression before and after combination therapy with IFN/ACV. Open circles denote absence of HDAg.

These 5 liver HDAg negative patients showed a decrease of periportal infiltrate and a decrease of serum AST level (4 normalised during therapy). In the other 5 patients, in whom liver HDAg remained low positive in the post treatment biopsy, severity of the periportal infiltrate remained stable or showed progression (fig 2).

Most apparent is the relation between the absence of liver HDAg expression after treatment and the biochemical and histological improvement.

HBeAg was positive in 2 out of 10 patients, 1 seroconverted to anti HBe after treatment. None of the patients had HBcAg expression in the pre- or post treatment biopsy.

16 - 52 weeks.

A sustained normalisation of serum AST at 52 weeks follow-up was observed in patients n° 6 and n° 10. All other patients showed elevated AST levels as sign of liver cell inflammation, thus 3 patients relapsed after discontinuation of treatment. Patient n° 6 was HBcAg, HBeAg and HBV DNA negative prior to treatment and cleared HDAg in the post treatment biopsy. An AST peak occured immediately after treatment that normalised thereafter and remained normal with 2 years of follow-up (fig 3). Patient n°

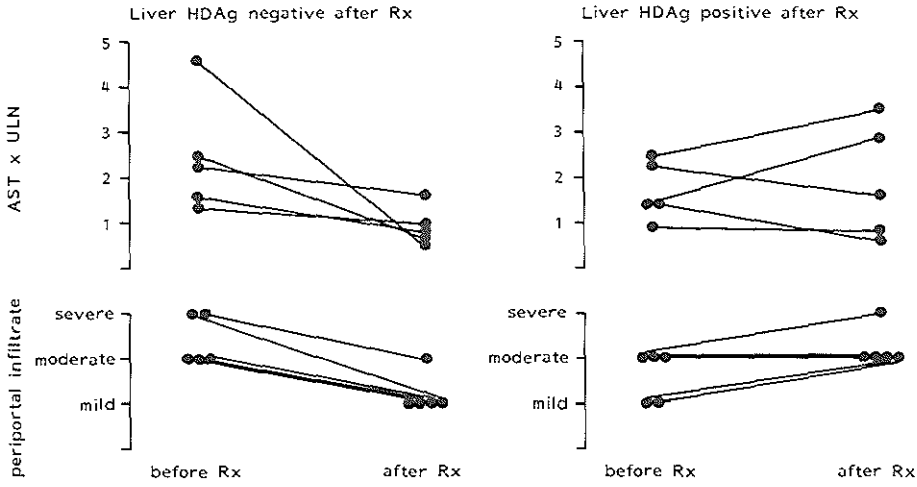


Figure 2. Markers of liver cell inflammation (AST and periportal infiltrate) before and after IFN/ACV therapy. Note the decrease in inflammation in patients with absence of HDAg in the liver biopsy after treatment.

5 showed no sign of active HBV replication and was antiHCV negative before treatment. He cleared the HDAg from the liver, AST normalized during treatment and a marked reduction in periportal infiltrate was observed in the post treatment biopsy. Despite the absence of HDAg and HBcAg in the liver, the liver disease reactivated with elevation of AST 3 months after stopping IFN administration (fig 4).

DISCUSSION

Sustained response to treatment in case of chronic hepatitis D defined as permanent absence of viral replication, normal serum transaminases and no signs of progressive liver disease was observed in 1 patient. This low frequency is similar to observations of others (2,3,4). Although the role of antiviral treatment cannot be proven in this single patient, a clear effect of therapy on liver HDAg expression was observed in 3 out of 8 patients (38%). Complete suppression of liver HDAg was associated with diminished

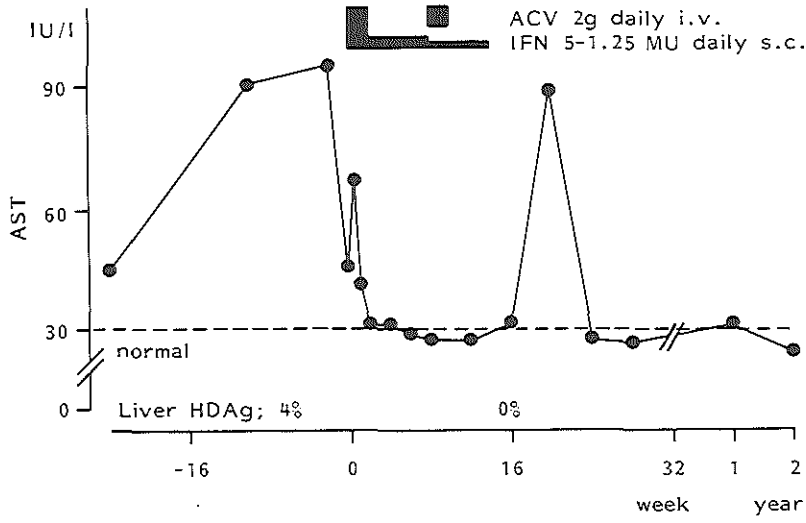


Figure 3. AST levels of patient 6 before, during and after IFN/ACV therapy. A sustained remission followed antiviral therapy. The single elevated AST value after treatment remained unexplained.

liver cell inflammation as assessed by serum AST and liver biopsy. These observations strongly suggest that antiviral treatment including IFN can be beneficial for patients with chronic hepatitis D. Results of combination therapy with IFN and ACV are not clearly different from those reported for IFN alone.

The mechanism of action of IFN is still unclear. Since none of the patients had liver HBcAg expression, no evident HBV replication was present. The post treatment improvement was therefore more likely to be related to suppression of HDV than to suppression of HBV. This hypothesis is supported by the reactivation of the liver disease in antiHCV negative patients that were also negative for HBcAg in the post treatment biopsy. Why IFN, while sometimes completely suppressing HDAg expression, did not eliminate HDV is not clear. Like HBV, suppression of viral core antigen is fairly uniform but immune mediated cytolysis of virally infected hepatocytes apparently does not occur in responders to treatment. AST-peaks after 8 - 12 weeks of treatment, as sometimes observed in HBV infection (8), were not noticed. In cell culture experiments treatment with ribavirin abolished HDV RNA replication but HDV RNA apparently

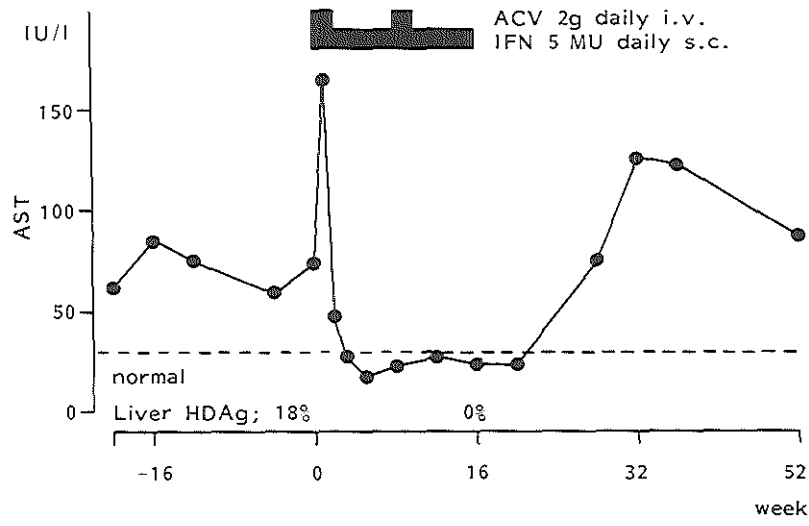


Figure 4. Response to IFN/ACV therapy in a patient without HBV DNA and antiHCV. Note the relapse of liver disease 12 weeks after discontinuation of antiviral therapy.

remained present since it recurred after withdrawal of 2-weeks drug treatment (9). Longterm suppression of HDV RNA may be required to allow elimination of HDV RNA by natural mechanisms.

Chronic hepatitis D was observed in a heterogenous group of patients with various stages of the disease (mild fibrosis to cirrhosis), various degrees of HDV replication and - in intravenous drugabusers - associated hepatitis C virus infection. Heterogeneity for HBV in these patients was limited since all were negative for liver HBcAg. Also HIV was negative in all patients.

Based on observations in this study, future evaluation of antiviral therapy in chronic hepatitis D probably should be limited to patients negative for HCV and HIV with stratification for HBV replication activity. The goal of induction therapy should be a repeated negative test for liver HDAg and HBcAg. Serum analysis for HDV RNA and HBV DNA as well as HDAg and HBeAg might be helpfull for defining the optimal time for performing the liverbiopsy. Maintainance therapy should be attempted, since reactivation occurs even in patients negative for liver HDAg and HBcAg. Future developments with PCR for HDV and HBV may help in defining endpoints for therapy.

REFERENCES

1 Rizzetto M, Ponzetto A, Bonino F and Smedile A. Hepatitis delta infection: clinical and epidemiological aspects. In Zuckermans AJ (ed): "Viral Hepatitis and Liver Disease", New York: Alan R Liss, 1988; 389-394.

2 Thomas HC, Farci P, Shein R, Karayiannis P, Smedile A, Caruso L and Gerin J. Inhibition of hepatitis delta virus (HDV) replication by lymphoblastoid human alpha interferon. In Rizetto M (ed): "The delta virus and its infection", New York: Alan R Liss, 1987; 277-290.

3 Hoofnagle J, Mullen K, Peters M, Avigan M, Park Y, Waggoner J, Gerin J, Hoyer B and Smedile A. Treatment of chronic delta hepatitis with recombinant human alpha interferon. In Rizetto M (ed):"The delta virus and its infection", New York: Alan R Liss, 1987; 291-298.

4 Rosina F, Saracco G, Lattore V, Quartarone V, Rizzetto M, Verme G, Trinchero P, Sansalvadore F and Smedile A. Alpha 2 recombinant interferon in the treatment of chronic hepatitis delta virus (HDV) hepatitis. In Rizetto M (ed):"The hepatitis delta virus and its infection", New York: Alan R Liss, 1987; 299-303.

5 Chen PJ, Kalpana G, Goldberg J, Mason W, Werner B, Gerin J and Taylor J. Structure and replication of the genome of the hepatitis δ virus. Proc Natl Acad Sci USA 1986, 83; 8774-8778.

6 Kuo MY-P, Chao M and Taylor J. Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. J Vir 1989, 63; 5:1945-1950.

7 Schalm SW, Heijtink RA, van Buuren HR and de Man RA. Acyclovir enhances the antiviral effect of interferon in chronic hepatitis B. Lancet 1985; ii:358-360.

8 Thomas HC. Hepatitis B viral infection. Am J Med 1988; 85 (Suppl 2A): 135-140.

9 Choi S -S, Rasshofer R and Roggendorf M. Inhibition of hepatitis delta virus RNA replication in primary woodchuck hepatocytes. Antiviral Res 1989, 12; 4: 213-222.

CHAPTER 8

SEVERE CHRONIC ACTIVE HEPATITIS (AUTOIMMUNE TYPE) MIMICKED BY COINFECTION OF HEPATITIS C AND HUMAN IMMUNODEFICIENCY VIRUS

The contents of this chapter have been excepted for publication in Gut under the same title and with the following authors: Luuk Berk, Solko W Schalm and Rudolf A Heijtink.

SUMMARY

Severe chronic active hepatitis, defined as the presence of a five-fold elevation of serum aminotransferases in conjunction with a two-fold elevation of gammaglobulin for at least 10 weeks, is considered a progressive immunological liver disease requiring corticosteroid medication, particularly when serum autoantibodies and a marked lymphoplasmacellular periportal infiltrate in the liver biopsy are found.

A case is described in which a 38-year-old male fullfilled the criteria for severe chronic active hepatitis. However his sex, his homosexuality and the presence of antibodies against human immunodeficiency virus led to the suspicion of a coinfection with hepatitis C virus rather than autoimmune disease. The rapid and complete response to alpha-interferon therapy and a recently available positive antibody test for hepatitis C virus indicate that a hepatitis C virus-related chronic active hepatitis, as illustrated by this case, can present as the severe autoimmune type of chronic active hepatitis. Moreover, as in hepatitis B, the response to therapy in hepatitis C virus-related severe chronic active hepatitis differs from that of autoimmune severe chronic active hepatitis.

Keywords: alpha-lymphoblastoid-interferon, autoimmune type chronic active hepatitis

INTRODUCTION

In 1968 severe chronic active hepatitis (CAH) was defined as an entity characterized by at least a 10-fold elevation of the serum aminotransferases levels for 10 weeks or a two-fold elevation of gammaglobulin together with a five-fold increase in serum aminotransferase levels (1). The disease, thought to be of autoimmune origin, usually progressed rapidly with a mortality rate of 50% in 2 years. Corticosteroid therapy was shown to increase life expectancy significantly (2,3,4). Subsequently the features of severe CAH were encountered in liver diseases of other etiology, such as primary biliary cirrhosis, primary sclerosing cholangitis, chronic hepatitis B infection (5,6,7), and metabolic disorders requiring therapeutic modalities other than corticosteroids. The diagnosis of autoimmune severe CAH is based on the presence of CAH plus autoantibodies in serum and portal and periportal lymphoplasmacellular infiltrates as well as piecemeal necrosis in the liver biopsy.
The following case suggests that chronic hepatitis C virus infection can mimic autoimmune severe CAH and that treatment with alpha-interferon (8) may induce clinical and biochemical remission in such patients.

CASE-REPORT

History

A 38-year-old man, born in the Netherlands Antilles, was admitted for treatment of syphilis with intravenous penicillin. He had had a hepatitis B virus infection in the past as well as repeated gonococcal and Treponema pallidum infections; 1 year prior to admission he was found to be antiHIV- positive. Apart from a tonsillectomy at age 17 and a perianal abscess and lobar pneumonia at age 37, there were no hospital admissions or surgical procedures in the patient's history. During admission elevated serum transaminase levels were found. Fatigue was his only symptom. There were no signs of alcohol abuse or intravenous drug abuse; he had never had a blood transfusion or undergone earpiercing or tattooing; there was no hepatitis in the family history; he took no medication. He was, however, a promiscuous homosexual. Physical examination revealed lymph node enlargement; liver and spleen were normal; apart from palmar erythema, there were no signs of chronic liver disease. Over a period of 26 weeks there

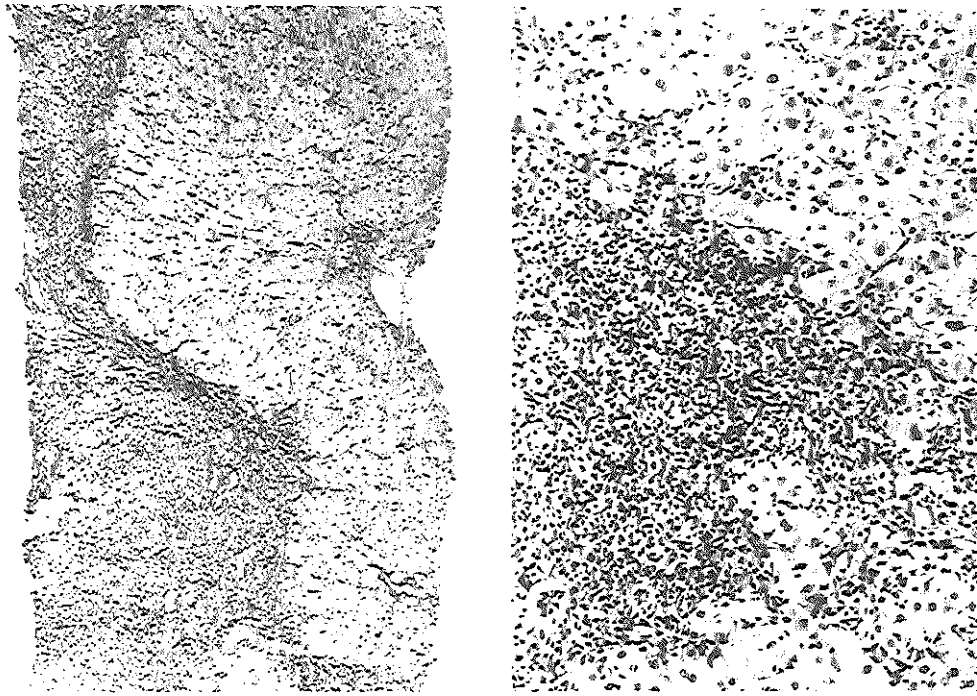
was a progression of hepatitis activity. Bilirubin concentration rose to 42 μmol/l (normal less than 17), serum AST to 405 IU/l (normal less than 30), ALT to 300 IU/L (normal less than 30); serum albumin decreased from 34 to 28 g/l. Blood clotting tests remained normal: normotest 83%, fibrinogen 2.0 g/l, antithrombin III 86%. Alpha-fetoprotein was below 3 μg/l. IgG was 72.5 g/l, IgM 4.8 g/l, IgA 2.4 g/l; anti-nuclear antibodies were positive (titer 1:160);anti-smoothmuscle antibodies and anti-mitochondrial were negative; anti-liver-kidney microsomal antibodies were not tested. Virus serological tests revealed the following: HBsAg negative, antiHBs positive, antiHBc positive, antiHD (delta) negative, antiCMV-IgM negative, antiEB-VCA IgM negative and antiHIV positive. CD4 cell count was 0.6 x 10^9/l (600/mm^3) and in vitro lymphocyte stimulation tests were normal. Ultrasound examination of the abdomen revealed a slightly enlarged liver and spleen. A liver biopsy showed probable cirrhosis (fig 1A + B) with CAH. The enlarged portal tracts and fibrous septa contained an extensive infiltrate, consisting of lymphocytes, plasma cells and some neutrophilic granulocytes. Piecemeal necrosis with severe degeneration of liver parenchyma and many Councilman bodies was seen (fig 1B).

Diagnosis and treatment

According to the Mayo Clinic criteria (1,9) this patient had a severe CAH with immunological features. Immunosuppressive therapy appeared to be indicated. Because of the HIV infection and a suspected non-A,non-B hepatitis, corticosteroid therapy was postponed and a course of 5 MU alpha lymphoblastoid interferon (Wellcome Laboratories, Beckenham, UK) subcutaneously daily was given (8).

RESULTS

Serum transaminases normalized in 2 weeks and serum IgG levels fell to less than 50% of the initial value (fig 2). When the dose of interferon was tapered to 2 MU every other day, the serum transaminase levels remained normal. Interferon was well-tolerated, and the patient spontaneously reported the gradual disappearance of fatigue during treatment. The dose of IFN was increased to 2 MU daily at month 11 in an attempt to suppress HIV replication, since HIV-associated p24 antigen had become detectable in the serum. HIV p24 antigen became negative at month 13 only to



A B

Figure 1. Liver biopsy showing disturbed architecture and fibrosis compatible with probable cirrhosis (x75) (A) and dense periportal lymphoplasmacellular infiltrate and piecemeal necrosis (x185) (B).

reappear 2 months later. The patient rejected treatment with zidovudine and the dose of interferon was increased to 3 MU daily at month 20. At 22 months the patient withdrew from therapy. He felt physically well, and apart from an elevated serum IgG level, his liver disease was in biochemical remission. Bilirubin was 0.4 x ULN, serum aminotransferases 0.5 x ULN, albumin 39 g/l and IgG 37.6 g/l. Anti-nuclear antibodies became negative. Clotting factors were 100%. No reactivation of his liver disease has been observed in 6 months of follow-up. Serological testing for hepatitis C virus, available since month 28, showed the presence of antiHCV antibodies at the time of admission to our department, during treatment and at follow-up.

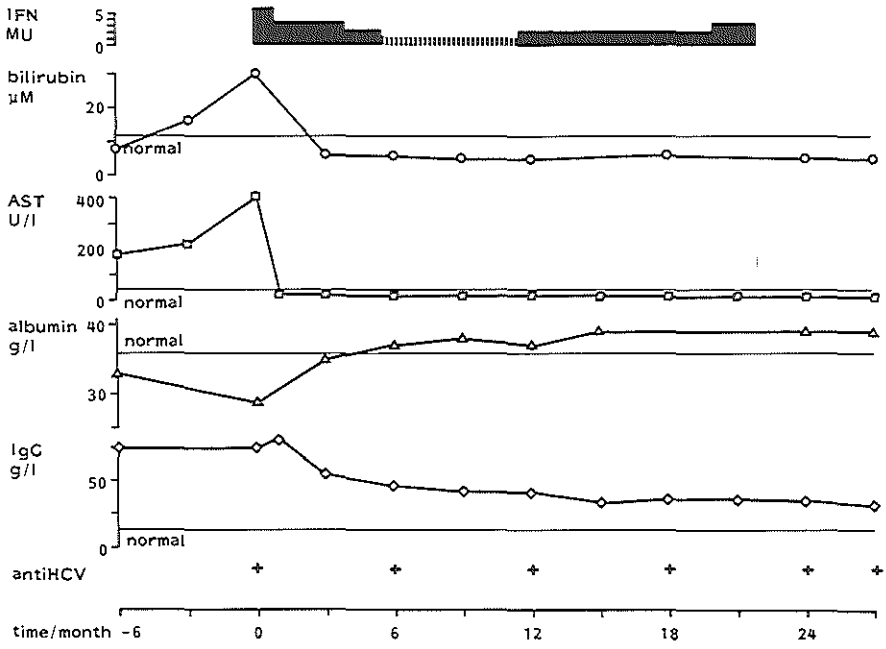


Figure 2. Serologic findings before, during and after alpha-interferon therapy in a 38-year-old male patient with severe CAH due to HCV and HIV coinfection. The dose of alpha-interferon was increased at month 11 and month 20 because of active HIV infection (see text). Note the rapid normalization of AST after start of therapy and the absence of relapse in the 6 months since discontinuation of alpha-interferon therapy.

DISCUSSION

The patient described in this report suffered from a severe CAH that presented serologically and histologically as CAH of autoimmune origin.

The diagnosis severe autoimmune CAH, however, depends on non-specific criteria such as a ten-fold increase in serum transaminase level, a two-fold increase in IgG level, the presence of auto-antibodies and dense lymphoplasma cellular infiltrate in the liver. Since the patient was a promiscuous homosexual male, he was considered more likely

to have a viral disease than autoimmune type CAH. Chronic non-A,non-B hepatitis was the most likely diagnosis and probably explained the elevated serum aminotransferase levels. The high IgG levels might be related to the HIV infection (10). The response to 2 weeks of interferon treatment supported our suspicion of an HCV infection, later virtually confirmed by the detection of antiHCV antibodies. The confirmatory value of the finding of persistent anti-HCV reactivity in this patient with high serum IgG level however, should be questioned in view of the recent finding of the nonspecific reactivity of the antiHCV test in CAH.

Prednisone treatment, probably life-saving in the event of severe autoimmune CAH, was no longer considered once the beneficial effect of IFN treatment was observed. Interestingly, persistent clinical and biochemical remission of the severe CAH occurred in this antiHIV positive patient after discontinuation of IFN treatment. Such a result is remarkable, since remission for 6 or more months of discontinuation of IFN therapy for chronic non-A,non-B hepatitis occurs in the minority of patients (12) and supposedly reflects adequate immune control.

This patient illustrates that diagnosis of severe CAH as the autoimmune type also requires the exclusion of HCV infection as well as of HBV and HDV infection, toxic hepatitis, metabolic disorders, primary biliary cirrhosis and primary sclerosing cholangitis. In addition, this case supports the observation that chronic hepatitis C can be rapidly progressive in case of HIV coinfection. Still, such patients, as recently described by Martin et al (13), may be as responsive to IFN therapy as the usually milder form of hepatitis C virus infection.

ACKNOWLEDGEMENTS

We thank F.J.W. ten Kate, MD for the photographs of the liver biopsy slides.

Wellcome Research Laboratories, Beckenham, England supplied the alpha-lymphoblastoid interferon.

REFERENCES

1. Geall MG, Schoenfield LJ and Summerskill WHJ. Classification and treatment of chronic active liver disease. Gastroenterology, 1968; 55: 724-729.
2. Cook GC, Mulligan R and Sherlock S. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. Q J Med 1971; 40: 159-185.
3. Soloway RD, Summerskill WHJ, Baggenstoss, Geall MG, Gitnick GL, Elveback LR and Schoenfield LJ. Clinical, biochemical and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. Gastroenterology 1972; 63: 820-833.
4. Murray-Lyon IM, Stern RB and Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. Lancet 1973; 1: 735-737.
5. Geubel AP, Baggenstoss AH and Summerskill WJH. Responses to treatment can differentiate chronic active hepatitis with cholangitic features from the primary biliary cirrhosis syndrome. Gastroenterology 1976; 71: 444-449.
6. LaRusso NF, Wiesner RH, Ludwig J and MacCarty RL. Primary sclerosing cholangitis. N Engl J Med 1984; 310: 899-903.
7. Schalm SW, Summerskill WHJ, Gitnick GL and Elveback LR. Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis B s-antigen. Gut 1976; 17: 781-786.
8. Hoofnagle JH, Mullen KD, Jones DB, et al. Treatment of chronic non-A,non-B hepatitis with human recombinant alpha interferon. New Engl J Med 1986; 315: 1575-1578.
9. Czaja AJ and Dickson ER. Chronic active hepatitis, the Mayo Clinic experience. New York: Marcel Dekker Inc., 1986.
10. Schnittman SM, Lane HC, Higgins SE, Folks T and Fauci AS. Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. Science 1986; 233: 1084-1086.
11. McDonald JA, Caruso L, Karayiannis P, Scully LJ, Harris JRW, Forster GE and Thomas HC. Diminished responsiveness of male homosexual chronic hepatitis B carriers with HTLV-III antibodies to recombinant α-interferon. Hepatology 1987; 7(4): 719-723.
12. Davis GL, Balart L, Schiff E, Lindsay K, Bodenhelmet H, Perrillo R, Carey W, Jacobson I, et al. Multicenter randomized controlled trial of alpha interferon treatment for chronic non-A,non-B hepatitis. Gastroenterology 1989; 96: A 501.
13. Martin P, Di Bisceglie AM, Kassianides C, Lisker-Melman M and Hoofnagle JH. Rapidly progressive non-A,non-B hepatitis in patients with human immunodeficiency virus infection. Gastroenterology 1989; 97: 1559-1561.

CHAPTER 9

RIFAMPICIN FOR CHRONIC HEPATITIS C WITH INCOMPLETE RESPONSE TO ALPHA-INTERFERON

SUMMARY

Many patients with chronic hepatitis C on standard doses of alpha interferon (IFN) treatment experience only an incomplete normalization of serum aminotransferase levels. This situation may still lead to development of liver failure, aspecially in patients who do not tolerate higher doses of IFN. With the current knowledge of hepatitis C virus concerning its RNA genome and requirement of RNA dependent RNA polymerase activity for replication, combination therapy of IFN and an inhibitor of RNA synthesis could be beneficial.

We treated a male hemophiliac with hepatitis C, liver cirrhosis and portal hypertension, and an incomplete response to maximally tolerated doses of IFN, by administring additional rifampicin. Immediately after the start of rifampicin a normalization of serum aminotransferase levels occurred and biochemical remission persisted for more than six months after stopping rifampicin. In the ensuing 2 years no progression of the liver disease was observed.

This case suggests that rifampicin may have antiviral activity against hepatitis C. Definite proof of this effect of rifampicin can only come from measurement of serum HCV RNA levels. If an inhibition can be demonstrated, the additional value of rifampicin in treating hepatitis C infection in incomplete responders to IFN should be evaluated in a controlled trial.

INTRODUCTION

In the absence of a reliable test for measurement of HCV replication, the therapeutic aim is complete normalization of serum aminotransferases; as only after such a normalization improvement in liver histology has been documented. The cornerstone for treatment of chronic hepatitis C is alpha interferon (IFN). Still many patients experience an incomplete response to monotherapy with only partial reduction in serum transaminase levels (1,2,3). Available data on the virus replication strategy are insufficiently precise to design a combination schedule of drugs to suppress viral replication at several levels. However, the HCV is a member of the flavivirus family that require RNA dependent RNA polymerase activity for replication, in addition to cellular tRNA's for priming antigenomic RNA synthesis (4). Inhibition of this replication cycle logically occurs when a RNA polymerase inhibitor is administered. We therefore tested the effect of rifampicin (5) in a patient with chronic hepatitis C and an incomplete response to monotherapy with maximally tolerated doses of IFN.

CASE HISTORY

A 52 years old male hemophiliac developed hepatitis after administration of blood-products 6 years prior to admission to our department for progressive splenomegaly. He was working as an electromechanic engineer and appeared in good health. The only symptom was fatigue; he showed no signs of alcohol or drug abuse; he took no medication. Physical examination showed an enlarged spleen. Normal laboratory results were obtained for serum albumin and bilirubine; quick-test was 45 % (normal 65 - 135 %); platelet count was 92 * 10^9/l (normal 130 - 350 *10^9/l); ALT and AST were markedly elevated with respectively 209 and 166 U/l (normal 30 U/l) α-foetoprotein 29 μg/l. Serological tests for hepatitis B, hepatitis D, cytomegalovirus, Epstein-Barr virus and human immuno deficiency virus or autoimmune disease were all negative. Ultrasonography showed an enlarged spleen of maximally 17 cm and dilated portal veins. Esophageal X-ray showed moderately large varices. Liver histology showed an active cirrhosis with bile-duct proliferation and focal steatosis, compatible with hepatitis C. AntiHCV antibodies were present in the serum. In this patient with a child A liver cirrhosis due to hepatitis C, IFN treatment was initiated with a daily dose of 5 MU subcutaneously and tapered to 2.5 MU daily because of fatigue and malaise.

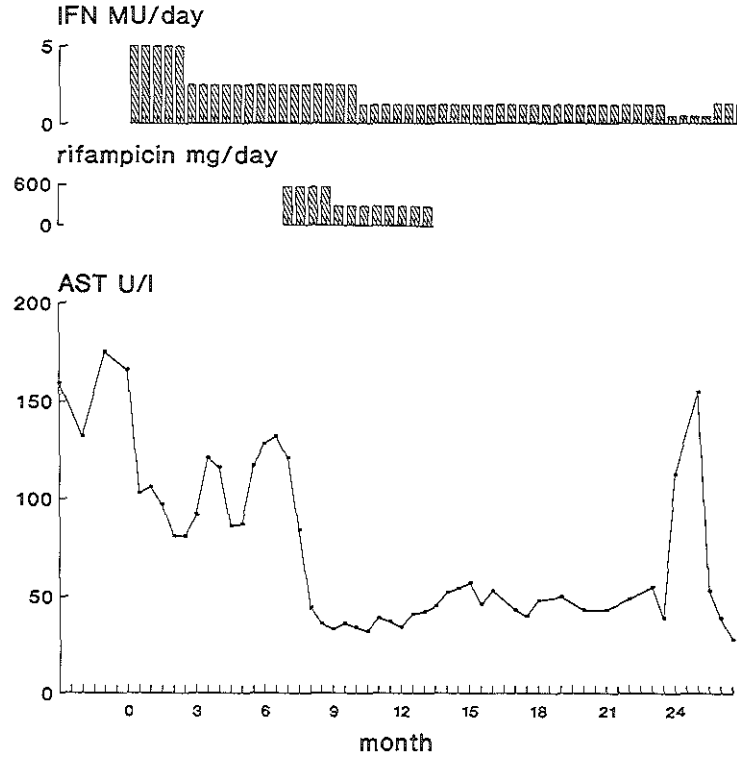
RESULTS

Serum aspartate aminotransferase (AST) levels fell from 6-7 times the upper limit of normal to 3-4 times the upper limit of normal but decreased no further (fig). After 6 months of IFN treatment rifampicin 4 x 150 mg was daily added orally to the IFN and the AST level rapidly fell to nearly normal. The dose of IFN was further reduced to 2.5 MU every other day and after a period of 2.5 months with stable transaminase levels rifampicin administration was stopped. Normal serum transaminases persisted for 14 months but after reduction of the IFN dose to 1 MU every other day, a flare-up of hepatocytoloysis occurred. With increment of the IFN dose to 2.5 MU 3 times a week normalization of aminotransferase levels occurred again and this dose was maintained for further treatment.

DISCUSSION

This case documents normalization of serum AST by IFN treatment with additional rifampicin administration. Normalization of AST levels in the setting of a progressive liver cirrhosis is considered to be important in view of our hypothesis that complete suppression of inflammatory activity may stop progression of the liver disease, even in case of cirrhosis (6). Increase of IFN doses may induce normalization of serum AST in patients with incomplete response to standard IFN therapy. However, increased doses of IFN are poorly tolerated for prolonged periods of administration, especialy in patients with liver cirrhosis. Rifampicin is a RNA polymerase inhibitor that could interfere with viral RNA synthesis either by inhibition of virus induced RNA dependent RNA polymerase or by inhibition of cellular tRNA synthesis required for priming new RNA genomes. The normalization of AST after rifampicin could have been due to an antiviral effect or increased clearance of aminotransferases by a non-specific enzyme induction. The persistence of the effect on serum AST after discontinuation of therapy suggests an antiviral effect rather than an enzyme induction.

Recently ribavirin has also been shown to lower ALT level in chronic hepatitis C (7). Clearly the effect of these drugs on HCV RNA need to be determined before a choice can be made for more rational combination therapy. Eventually the efficacy of combination therapy versus IFN monotherapy needs to be tested in a controlled trial.



The figure shows the serum AST level with an incomplete response to IFN therapy. Nearly normal serum levels of AST are obtained when rifampicin is added to the treatment regime.

REFERENCES

1 M.R. Jacyna, M.G. Brooks, R.H.T. Loke, I.M. Murray-Lyon and H.C. Thomas. Randomized controlled trial of interferon alfa (lymfoblastoid interferon) in chronic non-A non-B hepatitis. Br Med J 1989; 298: 80-2.

2 Di Bisceglie AM, Kassianides C, Lisker-Melman M et al. Randomized, double-blind, placebo controlled trial of alpha interferon therapy for chronic non-A,non-B hepatitis. Hepatology, 1989; 8: 1222.

3 Davis GL, Balart LA, Schiff ER et al. Treatment of chronic hepatitis C with recombinant interferon alfa. N Engl J Med, 1989; 321: 1501-1506.

4 Schlesinger RW, ed. The togaviruses. Academic press, New York 1980.

5 S. Riva and L.G. Silvestri, Rifampicins: A General View. Annu Rev Microbiol 1972; 26: 199-224.

6 de Man RA, de Jongh FE, Janssen HLA, Schalm SW, Hop WCJ and van Blankenstein M. Survival and prognostic indicators in HBeAg-positive liver cirrhosis. J Hep 1991, abstract.

7. Reichard O and Andersson J. Ribavirin: A possible alternative for the treatment of chronic non-A,non-B hepatitis. Scand J Infect Dis 1990; 22: 509.

CHAPTER 10

DISCUSSION

Alpha-IFN is the cornerstone for treatment of chronic hepatitis B, C and D. Inhibition of virus replication in chronic hepatitis B occurs in most patients during α-IFN treatment. Induction of virus latency after 16 weeks of therapy occurs in 25 % of patients (chapter 2). For chronic hepatitis C approximately 50 % of patients experience a decrease in hepatocellular cytolysis as a result of α-IFN treatment. Induction of HDV latency in chronically infected patients during treatment, as measured by disappearance of liver HDAg expression, occurs in 35 % (chapter 7, 1) and is not always associated with a decrease in hepatocytolysis.

The physician treating patients with chronic viral hepatitis will be confronted with the following problems: - nonresponse; - incomplete response (= incomplete suppression of active virus replication, which nearly always leads to reactivation of replication activity after stopping interferon treatment); - and response followed by relapse to active virus replication in patients showing a response during therapy. Relapse reflects insufficient virus-specific immunity. The magnitude of these problems are summarized in the table.

table

MAGNITUDE OF RESPONSE FAILURES IN CHRONIC VIRAL HEPATITIS

	response	relapse	nonresponse	incomplete response
HBV wild type	25 %	10 %	15 %	60 %
HCV	50 %	50 %	20 %	30 %
HDV	35 %	90 %	65 %	?
(HBV pre-core mutant	70 %	90 %	30 %	?)

This table summarizes the magnitude of response failures in the various forms of chronic viral hepatitis.

In patients infected with the wild type HBV approximately half of the patients do not respond to therapy by a decline of serum HBeAg levels. Measurement of HBV DNA levels shows a significant decline in HBeAg-nonresponders. Therefore, only 10 to 15 % of patients are true nonresponders (chapter 3). Relapse of HBeAg-responders to active viral replication occurs as an unusual event and incomplete response remains as the

major problem to be dealt with.

For chronic hepatitis C it is not yet established which virus parameters indicate a state of virus latency. For the time being response to therapy can only be identified by decreasing HCV RNA levels and/or decreasing aminotransferase levels. Definition of immunologic control depends on the relevance of HCV RNA negativity in serum and liver. The major clinical problems are incomplete response and relapse of serum transaminase elevation after interruption of treatment.

In chronic hepatitis D, complete response cannot be differentiated from incomplete response with HDAg measurement in the liver, since patients negative for HDAg expression in hepatocytes showed both sustained response as well as reactivation to aminotransferase elevation. Relapse in chronic HDV infected patients is a frequent fenomenon (2). Immunologic control is a rare event in chronic HDV infection, probably because the only virus specific protein (HDAg) is expressed in the hepatocyte nucleus (3). Therefore, development of antiHBs antibodies seems the only possibility to obtain immunologic control over HDV infection (4); this appears to be a rare spontaneous event in chronically infected patients. The most frequent problems for the treatment of chronic hepatitis delta are nonresponse and relapse.

Currently the inadequacies of therapy for chronic viral hepatitis are twofold: inadequate suppression of virus replication by α-IFN and inadequate induction of virus specific immunity. According to most investigators, virus specific immunity is difficult to induce in patients with chronic hepatitis D, C and the pre-core mutant (2,5,6). It usually follows however complete virus suppression in chronic wild type HBV infection and is characterized by HBe-seroconversion.

This thesis focusses on various attempts to enhance virus suppression of α-IFN by combination with other antiviral agents.

For HBV we investigated the synergistic effect of acyclovir (ACV) as a competitive inhibitor of HBV DNA polymerase or as a chain terminator of HBV DNA, but no increase in HBe-seroconversion rate was found. Zidovudine with a comparable mechanism as ACV, gave a modest inhibition of HBV DNA polymerase, suggesting this drug might give some additional virus suppression in a regime with α-IFN. However, considering the side effects of zidovudine, severe toxicity may occur during simultaneous administration of α-IFN. If zidovudine shows a significant effect on virus replication in a placebo controlled study, combination therapy with zidovudine and α-IFN may be evaluated. To avoid side effects of effective, but toxic drugs, such as zidovudine, an interesting

development is the targeting of drugs by conjugation to lactosaminated serum albumin (L-HSA). L-HSA has the capacity of binding to hepatocyte receptors and can release an antiviral drug into the target organ in a pharmacologically active form (7). Besides the concept of combination therapy for nonresponders and incomplete responders, improvement of treatment results can also be attempted by prolongation of α-IFN administration (chapter 2). Therapy for the pre-core mutant was not evaluated since this variety is rare in The Netherlands (8).

Limited experience with incomplete response of a patient with chronic HCV infection to α-IFN suggested an additional effect of rifampicin. If an effect of this RNA polymerase inhibitor can be demonstrated on HCV RNA levels, further evaluation in a controlled setting appears indicated. Ribavirin as a broad spectrum inhibitor of RNA virus replication may be another candidate to evaluate in a controlled setting (9).

Combination therapy for chronic HDV infection was started because addition of ACV was believed to increase the chance of HBV elimination. As for chronic HBV, no additional effect of ACV was found in chronic HDV infection. The concept of treatment for chronic hepatitis D is to eradicate HBV infection, since HDV depends on HBsAg for cell-entry. Therefore monoclonal antibodies to HBsAg may block spread of HDV to neighbouring hepatocytes, which in time could eliminate the infection.

The concept of combination therapy of α-IFN with nucleoside analogues for chronic viral hepatitis, as discussed in the various chapters, is applicable to nonresponders and incomplete responders to α-IFN monotherapy. However, the problem of relapse that especially occurs in chronic hepatitis C and D, requires enhancement of virus specific immunity. Recent studies with interleukin 1-ß, interleukin 2 and tumor necrosis factor indicate that virus specific immunity may be enhanced by these immunomodulators (10,11). Future studies are needed to assess whether prolongation of α-IFN monotherapy, combination therapy of α-IFN with nucleoside analogues and immunomodulatory therapy with other biological response modifiers than α-IFN will result in safe and effective therapies for chronic viral hepatitis.

In the history of treatment of hepatitis, empirics have led to intriguing proposals for therapy in ancient times. We should bear in mind that even in modern medicine empirical learning processes dominate the treatment of chronic viral hepatitis. Sophisticated laboratory methods allowed insight in the replication cycle of the viruses with the development of rational treatment regimes, but "trial and error" learn the clinician whether the therapy works.

REFERENCES

1. Hadziyannis S. Use of α-interferon in the treatment of chronic delta hepatitis. J Hepatol 1990: 13;S21-S26.
2. Rosina F and Pintus C. Long-term interferon therapy for chronic hepatitis D: A multicenter Italian study (Bologna, Cagliari, Genova, Milano, Napoli, Pavia, Torino). J Hepatol, in press.
3. Kuo MY-P, Chao M and Taylor J. Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. J Vir 1989: 63 (5); 1945-1950.
4. Hansson BG, Norkrans G, Weibull M et al. Epidemiology of delta infection in Scandinavia. Prog Clin Biol Res 1983: 143; 155-159.
5. Davis GL, Balart LA, Schiff ER et al. Treatment of chronic hepatitis C with recombinant interferon alpha. N Engl J Med 1989: 321; 1501-1506.
6. Brunetto M, Oliveri F, Rocca G et al. Natural course and response to interferon of chronic hepatitis B accompanied by antibody to hepatitis B e antigen. Hepatology 1989: 10; 198-202.
7. Fiume L, Torrani-Cerenzia ML, Bonino F et al. Inhibition of hepatitis B virus replication by vidarabine monophosphate conjugated with lactosaminated serum albumin. Lancet 1988: ii; 13-15.
8. Brown JL, Carman WF and Thomas HC. The hepatitis B virus. Baillière's Clin Gastroenterol 1990: 4 (3); 721-747.
9. Reichard O and Andersson J. Ribavirin: A possible alternative for the treatment of chronic non-A,non-B hepatitis. Scand J Infect Dis 1990: 22; 509.
10. Bartlett JA, Blankenship K, Waskin H et al. Zidovudine and interleukin-2 in WR2 HIV infected patients: evidence for stimulated immunologic reactivity against HIV (abstr.). Sixth International Conference on AIDS, San Fransisco 1990: 3; SB 421.
11. Daniels HM, Meager A, Eddleston ALWF et al. Spontaneous production of tumor necrosis factor alpha and interleukin-1beta during interferon-alpha treatment of chronic HBV infection. Lancet 1990: 335; 875-877.

CHAPTER 11

SUMMARY/SAMENVATTING

DANKWOORD

CURRICULUM VITAE

SUMMARY

Chapter 1 presents the history of hepatitis treatment. Although only indirect evidence exists, viral hepatitis may exist for several thousands of years. Therapy has been symptomatic until recently the development of molecular biology allowed to design a rational therapy aimed at eradication of the viruses causing hepatitis. Current ideas about replication of HBV, HCV and HDV are discussed with possible therapeutic targets in the replication cycle. Finally the aims of the studies are outlined.

The thesis focusses mainly on improvement of the antiviral effects of α-IFN by combination with potentially synergistic drugs. The first study (chapter 2) describes the effect of 4 months of treatment with α-IFN in combination with 2 2-week courses of ACV on HBe-seroconversion in chronic hepatitis B. In a randomized controlled study this treatment resulted in a slightly higher HBe-seroconversion compared to untreated control patients. Quantitative HBeAg measurement showed a significant decrease of HBeAg titer and separate analysis of HBeAg-responders, partial HBeAg-responders and HBeAg-nonresponders in the treatment group suggested that prolongation of therapy may yield more HBe-seroconverters. No additional effect of ACV was found.

The second study was performed to evaluate the additional effect of HBV DNA measurement during antiviral therapy for chronic hepatitis B (chapter 3). Quantitative HBeAg and HBV DNA measurements in the therapy group of the previously reported study showed that quantitative HBeAg measurement can be used to monitor antiviral therapy and determine endpoint of treatment. HBV DNA levels were of additional value to identify response in patients with high levels of viral replication activity.

Patients on α-IFN treatment may suffer serious influenza-like side effects that can be countered largely by non-steroidal-anti-inflammatory drugs. Therefore, the influence of paracetamol and indomethacin on α-IFN effects was investigated (chapter 4). Satisfactory control of side effects was obtained without a demonstrable negative effect on inhibition of viral replication (HBV DNA polymerase activity) nor on the elevation HLA class I turnover (beta 2 microglobulin level).

Since the additional effect of ACV on HBe-seroconversion could not be demonstrated, the effect of zidovudine on HBV replication was investigated in an uncontrolled setting. A moderate decrease in DNA polymerase activity was found with a dose response effect that allows further evaluation. Because of the toxicity of zidovudine a report in the Lancet about HBs-seroconversion induced by the herb Phyllanthus Amarus,

appeared interesting to be investigated in our patient population. A small double blind cross-over study was designed that showed no effect of the herb on serum levels of HBsAg, HBeAg or HBV DNA (chapter 6).

The effect of ACV and α-IFN combination treatment was also investigated in chronic hepatitis type D (chapter 7). This therapy that was aimed at eradication of the HBV coinfection that is obligatory for HDV infection, showed a marked decrease in liver HDAg expression and serum aminotransferase levels during treatment. However, reactivation of liver disease occurred after interruption of therapy in all but one patient. No additional effect of ACV was found.

Chapter 8 reports on the treatment of a patient with a severe chronic active hepatitis, occurring as an auto-immune type hepatitis. A combined HIV and HCV infection appeared to be responsible for this disease. Although prednisone treatment has been reported to be life-saving in case of a severe chronic active auto-immune type hepatitis, this case clearly belonged to a sub-group of patients in which prednisone therapy is contra-indicated. Alpha-IFN therapy resulted in normalization of serum AST level and restoration of the liver-function with sustained success after interruption of therapy two years later.

Another case of chronic hepatitis C is described in chapter 9. Combination therapy with rifampicin and α-IFN was started because of a poor reaction on α-IFN monotherapy. An additional effect of rifampicin was suggested with normalization of serum AST level. Since rifampicin as a RNA polymerase inhibitor may be effective in the treatment of hepatitis C, this drug should be tested for its effect on serum HCV RNA levels.

In the discussion (chapter 10) the problems concerning antiviral therapy for chronic viral hepatitis are outlined and the implicatons for future treatment strategies are discussed.

SAMENVATTING

Hoofdstuk 1 beschrijft de geschiedenis van de behandeling van virale hepatitis. Er bestaan aanwijzingen dat virale hepatitis reeds vele duizenden jaren bestaat. Behandeling van geelzucht was symptomatisch, tot in de twintigste eeuw de ontwikkeling van moleculair biologische technieken de herkenning van virale verwekkers mogelijk maakte. De huidige therapie is gericht op het klaren van deze verwekkers en het verkrijgen van immunologische controle over de infectie. Huidige inzichten in de vermenigvuldiging van HBV, HCV en HDV worden besproken, samen met de mogelijke therapie doelwitten in de vermenigvuldigingscyclus van deze virussen. Tenslotte worden de doelstellingen van de studies geformuleerd.

Alfa-interferon (α-IFN) wordt beschouwd als basis bestanddeel van elke behandeling van chronische virale hepatitis. Deze dissertatie behandelt overwegend de mogelijke verbetering van de standaard therapie met α-IFN.

De eerste studie (hoofdstuk 2) beschrijft de behandeling van combinatie therapie van α-IFN met acyclovir (ACV) voor chronische HBeAg positieve hepatitis B. Tijdens een gerandomizeerd, gecontroleerd onderzoek trad een HBe-seroconversie op in vijfentwintig procent van de therapie patiënten en in veertien procent van de onbehandelde controle patiënten. Kwantitatieve analyse van serum HBeAg spiegels toonde een significante daling in de therapie groep in vergelijking met de controle groep. Verder bleek uit subgroep analyse van responders, partiële responders en nonresponders uit de therapie groep dat verlenging van de therapie duur tot een hoger percentage HBe-seroconversie zou kunnen leiden. Een additioneel effect van ACV kon niet worden aangetoond.

In hoofdstuk 3 wordt de waarde van een kwantitatieve HBeAg bepaling vergeleken met de waarde van een kwantitatieve HBV DNA standaard test. Deze vergelijking toont aan, dat in het merendeel van de patienten een kwantitatieve HBeAg bepaling voldoende is om therapie resultaten te vervolgen en het eindpunt van de behandeling vast te stellen. Additionele HBV DNA meting is nodig om in patiënten met hoge activiteit aan virale vermenigvulding, bij uitblijven van een daling van het serum HBeAg, toch een respons op de therapie te kunnen vaststellen.

Omdat patiënten hevige griepachtige bijwerkingen kunnen vertonen bij het instellen van de behandeling met α-IFN, werd het effect van paracetamol en indomethacine op bijwerkingen en antivirale effecten van α-IFN bestudeerd (hoofdstuk 4). Indomethacine

en paracetamol werden met succes toegediend om griepachtige verschijnselen van α-IFN te voorkomen. Er kon desondanks een significante remming van α-IFN op virus vermenigvuldiging (serum HBV DNA polymerase activiteit) en op HLA klasse I turnover (beta 2 microglobuline concentratie) worden vastgesteld. Geconcludeerd werd dat indomethacine en paracetamol goed de initiële bijwerkingen van α-IFN konden voorkomen zonder aantoonbaar negatief effect op de antivirale werking van α-IFN.

Vanwege het teleurstellende effect van ACV toevoeging aan de α-IFN therapie werd de werking van zidovudine op HBV replicatie onderzocht (hoofdstuk 5). Naast een matige remming van serum HBV DNA polymerase activiteit, duidde toediening van verschillende doseringen AZT op een mogelijk dose-response effect. Deze studie lijkt verder onderzoek naar zidovudine als HBV DNA replicatie remmer te rechtvaardigen in een gecontroleerde opzet.

Vanwege de toxiciteit van zidovudine was het aantrekkelijk om een niet toxisch plantaardig geneesmiddel (Phyllanthus amarus) te onderzoeken, waarvan een hoge mate van HBs-seroconversie beschreven was. Er werd een kleine gecontroleerde studie gedaan met een cross-over opzet (hoofdstuk 6). Helaas werd geen effect op serum spiegels van HBsAg, HBeAg of HBV DNA waargenomen, hetgeen ook door andere onderzoekers werd bevestigd.

Een combinatie therapie van α-IFN en ACV werd ook voor chronische hepatitis D onderzocht met als doel de HBV infectie te elimineren (hoofdstuk 7). Delta antigeen expressie in de lever en serum AST spiegels toonden een daling gedurende de behandeling, maar reactivatie van de hepatitis werd gezien na onderbreken van de therapie. Er werd geen extra effect van ACV waargenomen.

Hoofdstuk 8 beschrijft de behandeling van een patiënt met een ernstige chronisch actieve hepatitis die zich presenteerde als een auto-immuun hepatitis. Een HIV en HCV coïnfectie, die goed reageerde op behandeling met α-IFN in plaats van met prednison zoals gebruikelijk is bij ernstige auto-immuun hepatitis, bleek aan dit ziektebeeld ten grondslag te liggen.

Behandeling van een patiënt met chronische hepatitis C met de combinatie α-IFN en rifampicine wordt beschreven in hoofdstuk 9. Toevoeging van rifampicine als RNA polymerase inhibitor aan de bestaande α-IFN therapie werd gedaan vanwege onvoldoende respons de op α-IFN monotherapie. De serum transaminasen normaliseerden na start van de rifampicine zodat een additief effect verondersteld werd wat verder onderzocht zal moeten worden met behulp van serum HCV RNA bepalingen.

Het laatste hoofdstuk bespreekt de problemen rond de behandeling van chronische virale hepatitis. Suggesties voor toekomstige behandelingen worden besproken.

DANKWOORD

Dit proefschrift is tot stand gekomen onder supervisie van Prof. dr. S.W. Schalm. Solko, je creativiteit in combinatie met je streven naar perfectie zijn eigenschappen die de drijfveer vormden voor dit onderzoek. Je talent om uit een verwarrend probleem de essentie te halen heeft dikwijls mijn bewondering gewekt.

De leden van de promotiecommissie, Prof. dr. N. Masurel, Prof. dr. J. Huisman en Drs. M. van Blankenstein, dank ik voor hun bereidheid het manuscript te willen beoordelen en voor hun waardevolle aanvullingen.

De uren die ik op het hepatitis laboratorium van de Erasmus Universiteit doorbracht hebben tot inzicht in de problemen rond virus onderzoek geleid en tot beter begrip van de replicatie strategie van de hepatitis virussen. Dr. R.A. Heijtink, beste Ruud, bedankt voor de gesprekken die we over deze onderwerpen hebben gehad. De medewerkers van het lab wil ik bedanken voor het tolereren van mijn aanwezigheid daar en voor de vele testen die voor de klinische studies zijn verricht.

Aan de hepatologische secretariaatsmedewerkers denk ik vol weemoed terug vanwege de uren die we achter de tekstverwerker sleten met databestanden en tabellen. "Bij nood, bel Boot!" is herhaaldelijk mijn redding geweest bij kennismaking met een nieuw computerprogramma. De medewerkers van de Interne II polikliniek wil ik met name bedanken voor het opvangen van patienten die op het einde van mijn altijd uitlopende spreekuur gepland stonden en voor de plezierige samenwerking.

Dr. R.A. de Man, beste Rob, mijn leven op het werk is heel wat gemakkelijker gemaakt met jou als voorganger, bedankt voor je raad en daad.

Drs. H.R. van Buuren, beste Henk, behalve voor medische adviezen, je halve kamer en de nodige gezelligheid, ben ik je ook erkentelijk voor alle sigaren die we gerookt hebben.

Dhr. P. van der Heijden en mw. G. Bieger dank ik voor de engelse correcties.

Dr. A. Schelling, Adrie, bedankt voor het creëren van de mogelijkheid het manuscript te drukken en te financieren.

De firma's Glaxo b.V., Essex Nederland B.V., Wellcome Pharmaceuticals B.V. en Abbott B.V. hebben een financiele bijdrage geleverd aan de kosten voor de promotie, waarvoor mijn hartelijke dank.

Last but not least moet aan jou, Gerty, worden toegeschreven dat we nog vrienden hebben overgehouden en als ik voortaan aan ons huwelijk evenveel tijd besteed als de afgelopen jaren aan mijn proefschrift, gaan we met z'n drieën gouden tijden tegemoet.

CURRICULUM VITAE

Lucas Berk werd op 12 januari 1960 in Delft geboren. In 1979 werd in Purmerend aan de Rijks Scholen Gemeenschap het Atheneum-B diploma behaald. Van 1979 tot 1982 studeerde hij aan het Rijks Universitair Centrum te Antwerpen, waar hij in 1982 het kandidaatsexamen geneeskunde behaalde. Zijn studie werd in Rotterdam aan de Erasmus Universiteit vervolgd en het artsexamen werd in 1987 afgelegd. Tijdens zijn middelbare school opleiding en studie nam hij deel aan meerdere nationale en internationale vlakwater kanokampioenschappen. Van 1983 tot 1984 werkte hij als student assistent op het laboratorium voor Experimentele Cardiologie van de Erasmus Universiteit Rotterdam (hoofd: Prof. dr. P.D. Verdouw). Van 1984 tot 1985 werden deze werkzaamheden als voltijd baan voortgezet. Aansluitend aan het artsexamen werkte hij van 1987 tot 1990 op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Rotterdam (hoofd: Prof. J.H.P. Wilson) onder leiding van Prof. dr. S.W. Schalm en op de afdeling Virologie van de Erasmus Universiteit (hoofd: Prof.dr. N. Masurel) onder leiding van dr. R.A. Heijtink aan de experimenten voor dit proefschrift. In 1990 werd de opleiding tot internist in het Sint Franciscus Gasthuis te Rotterdam gestart (opleider: dr. M. de Jong, later dr. H.S.L.M. Tjen). Hij is gehuwd met Gerty Custers en zij hebben een dochter Lisan.