Antiviral Research, 11 (1989) 181–190
Elsevier

AVR 00349

β_2-Microglobulin and antiviral therapy for chronic hepatitis type B

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(Received 8 August 1988; accepted 8 February 1989)

Summary

During a randomized controlled trial of interferon and descyclovir therapy, the β_2-microglobulin and SGOT serum levels in 36 patients with chronic HBe-positive hepatitis B were studied in order to determine whether β_2-microglobulin has prognostic value for HBe seroconversion. Pretreatment levels of β_2-microglobulin were elevated in 39% of patients. Significant differences in mean β_2-microglobulin activity and mean SGOT between treated patients and untreated controls were observed after 4 and 8 weeks of treatment ($P<0.05$). Levels in control patients remained stable. Prior to and during therapy, the mean elevation of β_2-microglobulin and SGOT levels was similar in responders ($N=7$) and non-responders ($N=11$). The outcome of antiviral therapy in our patients was not dependent on β_2-microglobulin levels measured before or during interferon therapy.

Hepatitis B; Antiviral therapy; β_2-Microglobulin; HBV-DNA-polymerase

Introduction

β_2-microglobulin is a low molecular weight (11.8 kDa) protein that is found in low concentrations in serum, saliva and cerebrospinal fluid. Serum levels increase with age, possibly reflecting decreased renal clearance. Elevated β_2-microglobulin levels are found in patients with renal disorders, autoimmune disease, various viral infections (including HIV) and lymphoproliferative diseases. Of the hepatic disorders β_2-microglobulin was found to be elevated in chronic active hepatitis, chronic hepatitis type B, alcoholic liver disease and primary biliary cirrhosis but could not

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be used to discriminate between these diseases (Hallgren, 1979; Beorchia et al., 1981; Rashid et al., 1981; Cooper et al., 1984; Nyberg et al., 1985; Katzmann et al., 1986; Lambin et al., 1986).

In serum β_2-microglobulin occurs as a small protein, while in tissue it is present as part of an HLA class I glycoprotein. Expression of HLA antigens on the cell membrane depends on the presence of β_2-microglobulin; the latter ensures that the three-dimensional configuration of the antigens is retained. Normal parenchymal liver cells do not express either HLA class I antigens or β_2-microglobulin on their membranes. Recently, however, it was found that expression of HLA antigens in the liver of chronic hepatitis B patients coincided with the presence of cytotoxic T cells in periportal infiltrates (Nagafuchi and Scheuer, 1986). It is postulated that hepatocytes infected with hepatitis B-virus are destroyed by these cytotoxic T cells, which respond to the complex of viral antigens and HLA class I antigens on the hepatocyte cell membrane.

Interferon has been shown to enhance the concentration of HLA class I antigens on the surface of infected cells. The therapeutic action of these proteins therefore may be related to an increased presentation of infected hepatocytes to the immune system. Recently a mean increase in serum β_2-microglobulin levels was observed after two weeks of interferon therapy in patients who exhibited a suppression of active virus replication as a result of this therapy (Pignatelli et al., 1986). In a randomized controlled trial we studied the serum levels of β_2-microglobulin as well as markers of viral replication and liver cell necrosis (SGOT) in 36 chronic HBe-positive patients, 18 of whom received α-lymphoblastoid interferon and descyclovir (BW 515). We tried to relate these findings to initial data at the start of the study as well as the therapeutic response, in an attempt to evaluate whether β_2-microglobulin could be of any use in identifying patients who are likely to benefit from antiviral therapy.

Patients and Methods

Patients

Thirty-six patients who participated in a randomized controlled trial of antiviral combination therapy for chronic hepatitis type B were studied. In nearly all cases active virus replication was reflected by HBeAg, HBV-DNA and DNA-polymerase activity in serum (patients without DNA-polymerase activity had stable HBeAg levels for three months prior to the trial). Patients who participated in this study had no history or signs of recent alcohol abuse, drug addiction or non-A, non-B hepatitis.

In addition those with decompensated liver disease (ascites, variceal bleeding, encephalopathy, hepatoma), a malignancy (other than basocellular type skin cancer) within the past 5 years or impairment of renal function were excluded from this study. All patients were evaluated on day 0 with a standardized history, physical examination, laboratory investigations, esophagogram and liver biopsy. Ran-

domization was done in blocks of six. In the treatment group, there were 18 males with a median age of 36 years (range 23–64 years). The control group consisted of 14 males and 4 females with a median age of 33 years (19–49 years). Ten controls and seven treated patients were homosexuals. All patients were tested for the presence of HIV antibodies by a commercially available ELISA (Wellcozyme); positive results were confirmed by Western blotting. The HIV antibody-positive patients who participated in this trial (3 treated, 5 control) belonged to class I or II of the Walter Reed classification system which implies normal immunology (Redfield et al., 1986). In addition all patients were tested for the presence of concomitant viral infections using IgM CMV, IgM EBV (VCA) and anti HDV (delta) antibodies at the beginning and the end of the study; none were found.

The study was approved by the Medical Ethics Committee of the participating hospitals and written informed consent was obtained.

Study design and follow-up

Eighteen patients received 5 megaunits of α-lymphoblastoid interferon (Wellferon) once daily by subcutaneous injection in combination with 1 g of the oral prodrug of acyclovir, descyclovir (BW 515) twice daily, for sixteen weeks. Treated patients were instructed to maintain fluid intake above 2.0 l/day. Descyclovir was started three days after interferon therapy had commenced. Control patients did not receive any treatment. Treated patients were seen after three days and then weekly during treatment. Control patients and those who had completed the sixteen-week course of therapy were seen monthly. Blood was sampled at each visit for assessment of the hematological, biochemical and viral parameters; part of the serum was prepared and stored immediately at −20°C for determination of β_2-microglobulin. Treatment response was defined as absence of HBe antigen on two successive occasions with absence of serum HBV-DNA.

In study A, we compared β_2-microglobulin with entry features, liver biochemistry, liver histology and virology.

In study B we determined β_2-microglobulin, viral markers, SGOT, creatinine and creatinine clearance every 4 weeks and compared the results obtained for 18 treated and 18 untreated control patients.

In addition, we compared these markers after 3, 7, 14, 21, 28 days of interferon therapy in 7 treatment responders and 8 non-responders. (For 3 non-responders insufficient serum was available for testing.)

β_2-Microglobulin assay

Serum β_2-microglobulin concentrations were measured in duplicate with the commercially available β_2-microglobulin radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). The assay was carried out according to the Manufacturer's instructions. The mean and upper limits of normal (+ 2 SD) for serum β_2-microglobulin levels for normal subjects under the age of 60 are 1.7 and 2.4 mg/l and for subjects over the age of 60 2.0 and 3.0 mg/l, respectively. The initial stand-

ard curve was repeated after 50% and 100% of the samples had been analyzed to test for trend. No trend was observed. Pooled serum samples were used to determine the intra-assay coefficient of variation. This coefficient equaled 6% when the β_2-microglobulin concentration was 2.8 mg/l.

Laboratory evaluation of the liver disease

HBeAg was measured by radioimmunoassay (Abbott, IL, USA). DNA-polymerase activity was measured by standard methodology as described by Fang et al. (1981). The results are expressed as a ratio of the activity in patient serum to that in normal control serum. The serum amino-transferases, alkaline phosphatase, bilirubin, serum albumin and serum creatinine were measured in our clinical chemistry laboratory using an automated system (SMA-12). Immunological activity was indicated by the immunoglobulin G and smooth muscle autoantibody concentrations as well as the C1q binding activity. Liver biopsy samples were obtained from eighteen treated patients and sixteen controls. One sample was processed for routine histological determinations while the other was snap-frozen in liquid nitrogen for immunofluorescence studies. The liver biopsy samples were scored blindly on the basis of the criteria established by an international group. Both the density and the penetration into liver tissue of the portal inflammatory infiltrate were scored on a scale of 0 to 3 (Bianchi et al., 1977).

Statistical analyses

β_2-microglobulin levels at entry to this study were analyzed in relation to age, HIV antibody status, immunological measurements, liver function and liver biopsy score using a multiple matrix plot and calculation of linear correlation. Significance was tested with a Spearman rank correlation test. Changes in β_2-microglobulin levels in treated patients and controls were analyzed by the method of variance using age and HIV status as covariates. Differences in SGOT, DNA-polymerase and HBeAg levels were tested using a Mann Whitney U test for non-paired samples. All statistical analyses were carried out with STATA. (Computing Resource Center, Los Angeles, U.S.A.) on an Olivetti M24 personal computer.

Results

Antiviral treatment

Baseline characteristics are shown in Table 1. No significant differences were observed between control patients, treatment responders and treatment non-responders with regard to age, sex, HIV status, histology, transaminase activity or IgG levels.

Seven treated patients responded (seroconversion from the HBe-positive to the HBe-negative state with absence of HBV-DNA) to therapy. Antibodies against

TABLE 1

Characteristics of patients participating in our studies

	Control	Therapy	Responders	Non-responders
Number of patients (*N*)	18	18	7	11
Age (years, median, range)	33 (19–49)	36 (23–64)	35 (23–64)	42 (26–63)
Male/female (*N*)	14/4	18/0	7/0	11/0
Homosexual (*N*, %)	10 (28)	7 (39)	3 (43)	4 (36)
anti-HIV positive (*N*, %)	5 (28)	3 (17)	2 (28)	1 (9)
IgG (g/l, median, range)	16.3 (9.6–34.7)	14.1 (6.9–14.2)	20.4 (6.9–26.2)	12.6 (9.3–27.2)
SGOT (IU/l, median, range)	43 (12–161)	43 (18–133)	81 (18–133)	40 (22–127)
β_2-microglobulin (mg/l, median, range)	2.4 (1.6–4.9)	1.8 (0.3–4.0)	1.89 (1.5–2.4)	1.6 (0.3–4.0)
Cirrhosis (*N*, %)	5 (28)	5 (28)	1 (14)	4 (36)

HBe developed in five of these patients. No serum HBV-DNA could be detected in any of the seven responders after one year of follow-up. Liver histology after one year of follow up showed clearance of HBcAg from the liver. In control patients HBeAg seroconversion was not observed. The results of antiviral treatment have been described in more detail elsewhere (De Man et al., 1986, 1987, 1988).

Serum β_2-microglobulin levels in chronic hepatitis type B

In study A, serum β_2-microglobulin levels were elevated in 14 of the 36 patients (39%).

The initial levels of β_2-microglobulin could not be related to hepatitis activity, as indicated by the SGOT levels (Table 1), nor was there a relation with alkaline phosphatase or bilirubin levels, liver histology or the penetration or density of the portal inflammatory infiltrate (Table 2). There was no correlation between the levels of β_2-microglobulin and either immunoglobulin-G concentrations or the presence of smooth muscle antibodies. Of the eight patients with HIV antibodies, four showed elevated β_2-microglobulin levels (50%). Renal function, assessed by serum creatinine levels and creatinine clearance, was normal in all patients.

Serum β_2-microglobulin during treatment

In study B the β_2-microglobulin serum levels at the start of therapy found for patients who responded to treatment (responders), patients who did not respond to treatment (non-responders) and untreated control patients were comparable. During treatment mean β_2-microglobulin levels rose in responders and non-responders, while the levels remained stable in untreated controls (Fig. 1). Signifi-

TABLE 2

β_2-microglobuin levels in relation to portal infiltrate and histological classification of liver biopsy

Histological classification	*N*	Infiltrate density				Infiltrate penetration				β_2-Microglobulin (mg/l)	
		0	1	2	3	0	1	2	3	Range	No. of patients >2.4
CPH	11	7	4	–	–	8	3	–	–	1.5–4.9	5 (45%)
CAH	13	3	6	3	1	–	9	4	–	1.3–5.0	4 (30%)
Cirrhosis	10	2	8	–	–	–	5	5	–	0.3–2.8	5 (50%)

cant differences were observed at week 4 and week 8 between treated patients and control patients ($P<0.05$). No significant differences, however, were observed between responders and non-responders. Serum transaminases followed the same pattern with significant differences between treated and non-treated patients at week 4 and week 8 ($P<0.05$) but not between responders and non-responders. Renal function did not change significantly during therapy ($P>0.10$). Because of the observations of Pignatelli et al. (1986) we also tested the serum of seven responders and eight non-responders after 3, 7, 14, 21 and 28 days of treatment. β_2-microglobulin in responders showed a mean increase of 20, 99, 74, 82 and 52% at 3, 7, 14, 21, 28 days, respectively. In non-responders the mean increase in β_2-microglobulin was 177, 121, 133, 104 and 82%, respectively. Four of seven responders and six of eight non-responders showed a rise in β_2-microglobulin at two weeks (Fig. 2).

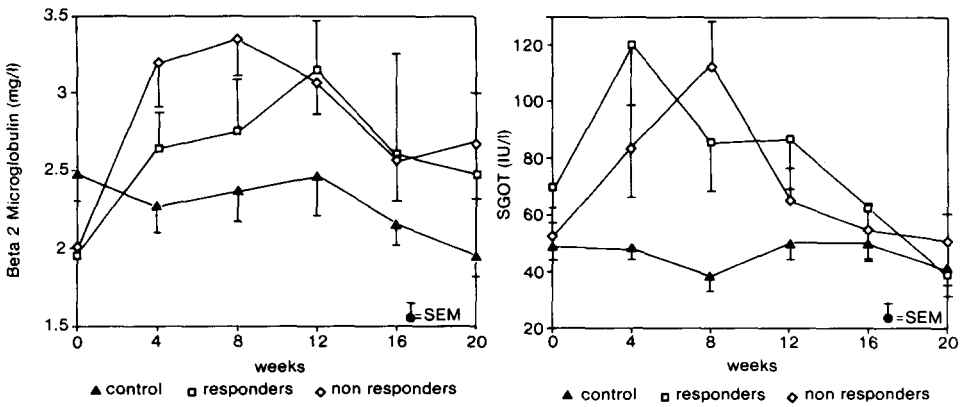

Fig. 1. β_2-Microglobulin (mean) and SGOT (mean) in responders (□), non-responders (◊) and control (▲). Treatment was given from week 0 to week 16.

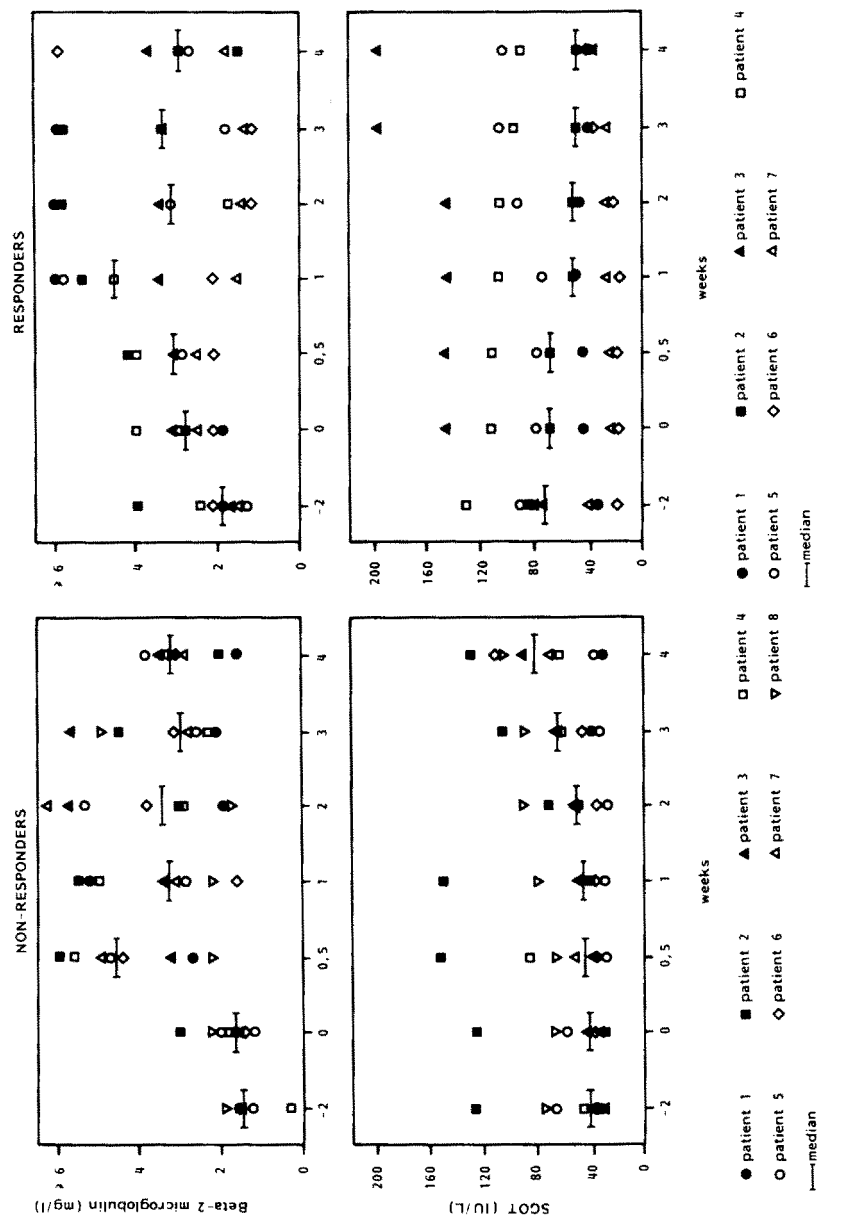

Fig. 2. β_2-Microglobulin and SGOT in non-responders (left panel) and responders (right panel) during the first 28 days of treatment.

Discussion

Recent studies suggest possible therapeutic efficacy of long-term interferon therapy for chronic hepatitis B. However, HBe seroconversion with development of antibodies occurred in only a minority of the treated patients (Dusheiko et al., 1985; Lok et al., 1985; Schalm et al., 1985, 1986; Dooley et al., 1986; Perillo et al., 1986). Early identification of patients who could benefit from treatment is therefore important. Various factors related to the outcome of treatment have been proposed retrospectively: oriental race, positive HIV antibody status and homosexuality are characteristics of those who show little response whereas an elevated SGOT level or a histology compatible with chronic active hepatitis is said to identify patients who could benefit from treatment (Thomas et al., 1987). In a matched controlled study a mean increase in β_2-microglobulin after 14 days of interferon therapy was reported to be indicative of a favorable response to therapy, the mechanism being enhanced expression of HLA class I antigens leading to increased presentation of infected hepatocytes to the immune system (Pignatelli et al., 1986).

Our study indeed showed a mean increase in β_2-microglobulin levels in patients during interferon therapy. This rise in β_2-microglobulin could not be explained by age or HIV status because both were used as covariates in our analysis, nor could it be attributed to either decreased renal tubular function related to descyclovir therapy or other viral infections (Berk et al., 1988). Possibly concomitant cell lysis induced by interferon after 4 weeks of therapy can explain the rise in β_2-microglobulin after 4 weeks. However, elevation of β_2-microglobulin in our studies was not related to imminent HBe seroconversion, but probably reflects interferon-induced hepatitis. Lok and colleagues also found interferon therapy-related hepatitis (Lok et al., 1985). After two weeks of interferon therapy β_2-microglobulin increased in 73% of patients without discrimination between responders and non-responders (Fig. 2).

We conclude therefore that exogenous α-lymphoblastoid interferon increases serum levels of β_2-microglobulin, possibly by enhanced HLA class I expression. However, we could not distinguish responders from non-responders by means of β_2-microglobulin serum levels alone, even if assessed in the first 4 weeks of therapy before SGOT rises.

In the complex interaction between the virus, the host and the antiviral agent β_2-microglobulin reflects HLA antigen expression. We could not use β_2-microglobulin alone for the selection of individual patients for antiviral therapy but it may be of use in a multifactorial model which describes this interaction.

Acknowledgement

This study was supported by a grant from the Wellcome Research Laboratories. The authors thank their colleagues Dr. R.A.F.M. Chamuleau, Dr. H.W. Reesink, Dr. R. Grijm, J.T.M. van der Heyden, J. den Ouden and Dr. M. de Jong for par-

ticipating in the trial on antiviral combination therapy. We thank Ir. P.F.M. Schmidtz for his help with the statistical analysis; N. Vink-Wendels and C.A. de Leeuw for their help in the laboratory.

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