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TRANSIENT INCREASE OF HBsAg LEVELS FOLLOWING HUMAN IFN ALPHA TREATMENT SIGNALISES THE PATIENT'S RESPONSE IN CHRONIC ACTIVE HEPATITIS B

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Summary. — Eleven patients in early stages of chronic active hepatitis B (CAH-B) were treated for weeks or months with a natural or recombinant human interferon alpha (Hu IFN alpha). Changes of serum levels of selected hepatitis B virus (HBV) markers were observed after Hu IFN alpha administration. Increase of HBsAg level accompanied by more or less simultaneous HBeAg level depression was the most interesting observation. These changes were well expressed in 5 reactive patients only; they usually ceased after withdrawal of IFN therapy. Reaction of the remaining 6 patients was either poor or not demonstrable. The possible mechanism for HBsAg/HBeAg serum level changes during the IFN therapy of CAH-B is discussed.

Key words: *chronic hepatitis B*; *interferon therapy*; *combined CAH-B therapy*; *HBV markers*; *immunomodulation*

Introduction

Treatment of viral hepatitis B (VH-B) still depends on empirical approaches rather than on reproducible and efficient therapeutical applications. Adenine-arabinoside and acyclovir were the most frequently used with variable and often controversial results (Pollard *et al.*, 1978; Scullard *et al.*, 1981; Hoofnagle *et al.*, 1984; Weller *et al.*, 1985). These differences seem to come from the individual immunoreactivity of patients (Novick *et al.*, 1984). Similar results were obtained when various classes of natural or recombinant IFN preparations were used. In the majority of published trials they also exhibited rather partial and transient therapeutical effect on CAH-B infection (Greenberg *et al.*, 1976; Schalm and Heijting, 1982; Dooley *et al.*, 1986; Sotto *et al.*, 1986; Carreno *et al.*, 1987; Porres *et al.*, 1988; Suzuki, 1988; Kumada and Ikeda, 1988; Thomas, 1988, Colombo, 1988; Stanček *et al.*, 1988).

It is well known that spontaneous reactivations or seroconversions may occur during CAH-B infection (Liaw *et al.*, 1987; Tong, 1987). All these

changes reflect more or less efficient immunological capability of the infected organism to recognize foreign antigens and combat the disease. In the case of HBV infection immune mechanisms are often facing not-quite-non-self antigens, e.g. due to antibody formation against HBsAg-bound serum albumin receptors which may cause "HBsAg masking" (Alberti *et al.*, 1984; Machida *et al.*, 1984; Pontisso *et al.*, 1985; Gerken *et al.*, 1987).

In our previous work (Stanček *et al.*, 1988) we demonstrated well expressed oscilations of HBsAg serum levels without significant changes of other aetiological CAH-B markers. Here we present more data on serum marker changes during IFN therapy at early stages of CAH-B development comparing them with the previou s results obtained in IFN-treated patients in the course of well-established CAH-B.

Materials and Methods

IFN-alpha preparations and their dosage. Natural HuIFN alpha was prepared according to modification described by Fuchsberger *et al.* (1979); recombinant IFN were ROFERON (Roche, Switzerland) and REAFERON (USSR). Usual dosage of IFN was either 3×10^6 I.U. applied intramusculary three times a week or gradually in increasing doses from 5×10^4 to 10^6 I.U. daily for 2 weeks, then 10^6 I.U. 2—3 times weekly for the rest of the treatment. The total IFN dose was $3 \times 10^7 - 15 \times 10^7$ I.U. given either continuously for a period of 4 to 28 weeks or in once or twice repeated 4 to 12 weeks courses.

HBV serum markers. HBsAg, HBeAg, anti-HBc-IgM anti-HBc-IgG anti-HBe, and anti-HBs levels were estimated by commercially available tests (Abbott, Sorin, Boehring, and Sevac ELISA or RIA kits) and used according to manufacturers recommendations. For further details see also Stanček *et al.*, 1987.

Biochemical and immunological examinations. Commonly used techniques based mostly on commercially available tests were employed. Majority of the tests were performed on the basis of regular services at the Department for Clinical Biochemistry, Faculty Hospital, Kramvre, Bratislava.

Patients selection. All selected patients suffered from VH-B infection in its early stages of chronic development with persistence of their initial, usually high HBsAg, HBeAg, alanine aminotransferase (ALT), and bilirubin serum levels. Duration of the illness before application of IFN only or combined IFN therapy was 4 to 6 months. The term "conventional" therapy is used for nonspecific symptomatic therapies usually including vitamins and hepatoregenerating substances (e.g. Heparegen, Legalon, Essentiale, Flavobion, etc.).

Results

Oscilations of HBsAg/HBeAg blood levels during acute hepatitis B (AH-B) infection in "conventionally"-treated patients

Blood levels of HBsAg, HBeAg, anti-HBc-total, anti-HBe, and anti-HBs were frequently monitored during acute course of HB-V infection or an early period of recovery of conventionally-treated patients. Some immunological parameters were also checked. Fig. 1 shows the most typical dynamics of the followed markers in an uncomplicated case (Fig- 1-I) and in a patient with somewhat prolonged recovery (Fig. 1-II). In the first case three well distinguished and reproducible peaks of HBsAg were seen before HBeAg/anti-HBe seroconversion which was then followed by HBsAg/anti-HBs seroconversions with final recovery from the disease. The first HBsAg depression

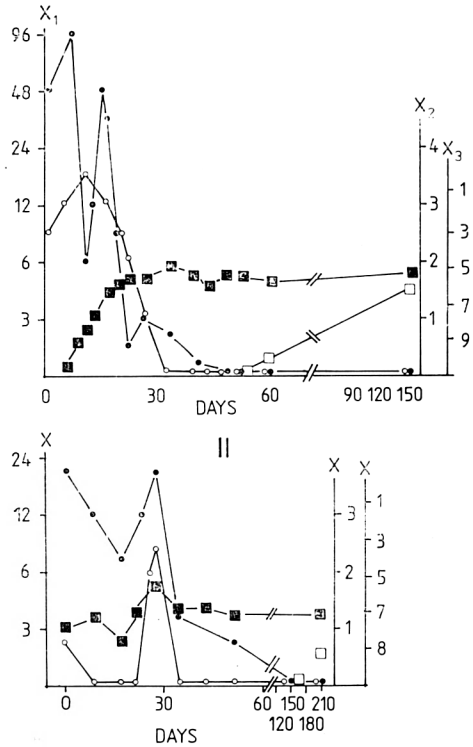
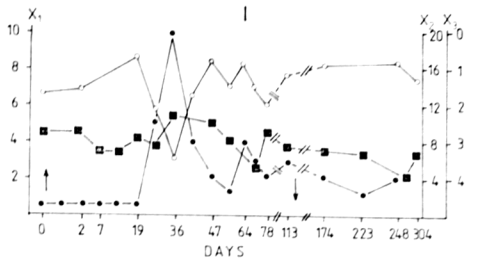


Fig. 1
Dynamics of HBV serum markers during AH-B infection of "conventionally" treated patients
X 1 — HBsAg titres × 10^3 ●——●
X 2 — HBeAg activity × 10^3 c.p.m. ○——○
anti-HBs activity × 10^3 c.p.m. □ – – □
X 3 — anti-HBc IgM + IgG levels (inhibition of c.p.m. × 10^2) ■——■

coincides with the maximum HBeAg level. Somewhat different picture can be seen in the second patient (II) with a prolonged recovery. Here we registered coincidental peaks of both HBsAg and HBeAg with disappearance and re-appearance of HBeAg/anti-HBe and final seroconversion to anti-HBs at recovery. Slight differences were observed also in the dynamics of the available immunological parameters.

Random depressions of HBeAg serum levels often coincidental with HBsAg elevations possibly due to IFN administration

In 5 out of our 11 IFN alone, or IFN combined with either transfer factor- (TF) or corticoids- or acyclovir-treated patients, periodical blood level oscilations of some HBV markers could be observed at different intervals after IFN administration (Fig. 2). No such oscilations were registered in the remaining 6 "non-reactive" patients treated accordingly. In some of the reactive patients the HBsAg level elevations were often accompanied by HBeAg level depressions and vice versa (e.g. Fig. 2-II). This phenomenon was usually closely related to a subsequent improvement of other biochemical, immunological, and clinical parameters and could not be seen in nonreactive patients. Nonetheless no final recovery from the disease was seen during several months of observation following IFN alone or combined IFN therapy



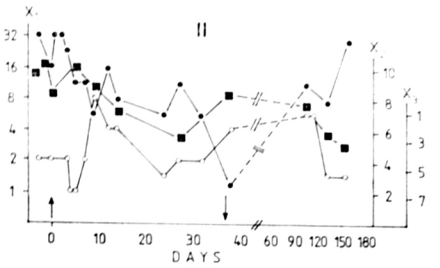


Fig. 2

Dynamics of HBV serum markers during early periods of CAH-B development in IFN treated patients
X 1 — HBsAg activity × 10^3 c.p.m. (patient I) or HBsAg titres × 10^3 (patient II) ●——●
X 2 — HBeAg activity × 10^3 c.p.m. ○——○
X 3 — anti-HBc IgM activity × 10^3 c.p.m. ■——■
Arrows on abscissas indicate Hu IFN therapy start (↑) or end (↓)

in either responsive or non-responsive patients. In some reactive patients substantially decreased serum levels of aminotransferases and bilirubin or even periodical disappearance of usually low HBeAg serum levels and milder clinical course could be observed. Short-lasting (10—15 days) depressions of immunoglobulins levels were commonly observed shortly after HuIFN alpha administration but they usually returned to original values regardless of the continuation of IFN therapy.

Influence of combined IFN, short-course TF, corticoids or acyclovir therapies on HBV serum markers expression

In two of our eleven patients HuIFN alpha therapy was combined with relatively short courses of corticoid (Praednison) therapy (Fig. 3). Several months after rather unsuccessful IFN administration, one of the patients (Fig. 3-I) was treated with usual gradually decresing dosage (80 to 1 mg/day) of corticoids by oral route. Sharp increase of HBeAg levels was observed shortly after corticoid administration which was then followed by decrease of both HBsAg/HBeAg and also anti-HBc-IgM levels. After reintroduction of HuIFN alpha a slight improvement of patient's disease could be registered following simultaneous fluctuation of HBsAg/HBeAg serum levels.

Another of the partially IFN-responsive patients (Fig. 3-II) in an early stage of CAH-B development received two injections of transfer factor (SE-

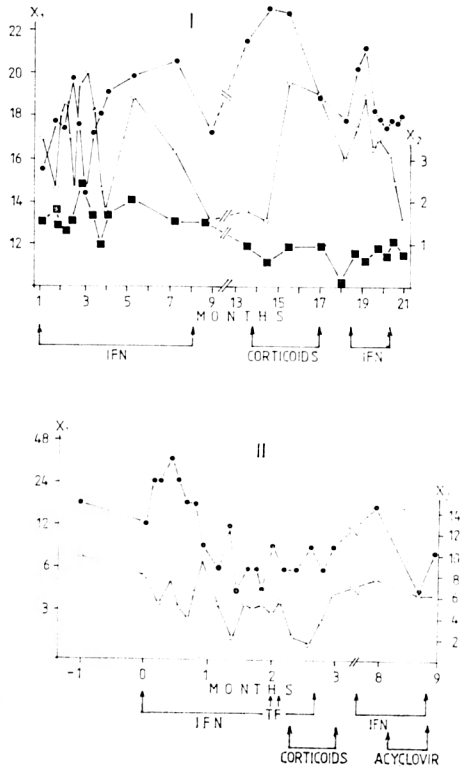


Fig. 3

Dynamics of HBV serum markers during early periods of CAH-B development in patients treated with combined IFN therapy

X 1 — HBsAg activity × 10^2 c.p.m. (patient I) ●——● or HBsAg titres × 10^2 (patient II) ●——●
HBeAg activity × 10^2 c.p.m. (patient I) ○——○

X 2 — anti-HBc IgM activity × 10^3 c.p.m. ■——■ HBeAg activity × 10^3 c.p.m. (patient II) ○——○

VAC, Praha) together with the usual dosage of recombinant HuIFN alpha (ROFERON-A, Roche). Since the effect achieved was again partial, the patient was given 90 mg/day of Praednison with decreasing dosage during 14 days to final 35 mg/day followed by sudden interruption of the corticoid therapy and IFN readministration. Gradually increased HBsAg/HBeAg levels were seen after corticoid application but no beneficial therapeutic effect was achieved. Final attempt was then made by combined HuIFN alpha and acyclovir treatments. Neither this combination lead to recovery from the disease. It has to be stressed, however, that during all therapeutic approaches gradually decreasing pathogenetic activities were observed in both patients and at the final release from the hospital their disease was qualified as being changed to a less aggressive form.

Changes of HBeAg/anti-HBc-IgM ratio during immunomodulation therapy of CAH-B infection

To follow the immunosuppressive influence of corticoids, the immunomodulating effect of IFN and also to be able to judge more accurately the putative danger of possibly corticoid-related HIV replication, we checked

Table 1. HBeAg/anti-HBc-IgM index and its changes during immunomodulation therapy of CAH-B infection

Stage and type of therapy	HBeAg/anti-HBc-IgM index (1)	Activity of infection
Before immunomodulation therapy	9.84	relatively steady state
During primary HuIFN alpha therapy	13.42 13.25 12.40 10.71 12.73 14.51	HBeAg stimulation (increased namely NK cell activity)
	8.94 8.96 9.91 7.86 8.33	anti-HBc-IgM reactive phase
Without specific therapy	10.46 8.84 11.11 11.03 10.37	relatively steady state
During corticoid therapy	14.01 16.48 13.76 16.55 14.69 14.86 13.94 12.71 (!)	HBeAg superstimulation phase (increased HBeAg translation only ?)
During repeated HuIFN alpha therapy	12.65 14.17 11.59 11.39 11.17 11.26	anti-HBc-IgM reactive phase

1 — values in individual serum samples

the changes of HBeAg/anti-HBc-IgM index, i.e. the ratio between HBeAg (e.i. an integral part of the replicating HBV nucleoprotein) and anti-HBc-IgM (i.e. antibody reflecting actual HBV nucleoprotein production). As documented in Table 1, HBeAg/anti-HBc-IgM index increased for a short time after HuIFN-alpha introduction but dropped down again during the following days apparently due to the reactive anti-HBc-IgM antibody formation. Different situation was observed after replacement of IFN by corti-

Table 2. General conclusions on the effect of HuIFN alpha administration on some aetiological, immunological, and biochemical serum markers in developing CAH-B infection

Serum markers	Changes observed after IFN administration	Remarks
Aetiological	HBsAg: stimulation/inhibition or no effect HBeAg: inhibition/stimulation or no effect anti-HBc-IgM: close to HBeAg changes	HBsAg serum level oscilations. HBsAg-indirectly-related oscilations
Biochemical	ALT: stimulation/inhibition or no effect Bilirubin: close to ALT (total) changes	almost parallel with HBsAg dynamics
Immunological	Immunoglobulins: initial depression, then normalization T ly (T_a, Tt), B ly, PA, PI, C3, C4, CH50, Ap50, LTT-PHA, IRI (OKT4/OKT8): stimulation or inhibition preceeding frequently observed normalization CIC: depression when PI and PA increased elevation when Igs were increasing depression when Igs were decreasing	

ly = lymphocytes; PI, PA = phagocytic index, p. activity; C = complement; CH, AP 50 = classical or alternative C pathway; LTT-PHA = paytohaemaglutinin-lymphocyte-transformation test; CIC = circulating immune complex; IRI = immunoregulation index; Igs = immunoglobulins

coids: HBeAg/anti-HBc-IgM index changed considerably and reached the highest observed level (value 42) signalizing a substantial stimulation of HBeAg production. After re-introduction of HuIFN alpha this phenomenon ceased and HBeAg/anti-HBc-IgM index returned close to its initial values. Similar picture was seen in other 2 patients treated accordingly.

Condensed account of our aetiological, biochemical, and immunological observations is given in Table 2. In general, it can be concluded that only partial effects were observed regardless of the type of either IFN alone or combined therapy applied to our 11 patients suffering from early stages of CAH-B development.

Discussion

Our data presented here differ from the previously published ones (Stanček *et al.*, 1988) in the effect of IFN administration on the HBeAg serum levels. The previous therapy was applied to patients with well established, long-

lasting CAH-B while for the work presented here a group of patients in early stages of CAH-B development was selected. At this stage there still might be a good chance to reverse an unfavourable course of the disease by immunomodulating therapy. HBsAg oscilations during AH-B infection followed by HBeAg depression and its seroconversion on one side (Fig. 1-I), the absence of well expressed dynamics of HBsAg "antigenic stimulus" together with related disturbances in HBeAg/anti-HBe seroconversion (Stanček *et al.*, 1988 and Fig. 1-II) on the other side, indicate a shortage or absence of the antigenically potent HBsAg stimulus for cellular/humoral defense mechanisms which would recognize and eliminate the HBV genome-harboring cells. This is further supported by our observation of HBsAg oscilation accompanied by HBeAg depression in exogenous IFN-responsive CAH-B patients and the absence of this phenomenon in non-responsive ones. It seems likely that only through an efficient booster of patient's immunoreactivity (e.g. by increased levels of fully reactive antigens) can be achieved a substantial change in the unfavourable disease development as often seen at appearance of "the second jaundice" preceeding recovery (Liaw *et al.*, 1987). It looks plausible that exogenous IFN through stimulation of LAK/NK cell cytotoxic activities brings about an indirect increase of HBsAg serum levels — due to destruction of HBV-bearing cells — which in turn means another stimulus for the same or other sets of defense cells. In unresponsive cases exogenous IFN alone is just not efficient enough to start or efficiently support the necessary reaction chain. We hade to look either for better IFN application schemes, or for other more efficient single or combined therapies including efficient stimulation of endogenous IFN production and its action. Exogenous IFN itself can hardly directly influence replication of HBV in already infected cells with viral DNA well established inside cells (Vilček and Stanček, 1963; Stanček, 1965*a*). It has, however, some potential to prevent reinfections of those cells in which, due to feedback functions of endogenous intracellular IFN, replication of HBV was interrupted and cells became permissive again for both extracellular endogenous or exogenous IFN as well as for infectious virus (Stanček, 1965*b*; Stanček, 1966). When applying IFN therapy we presume that the endogenous IFN production and its antiviral or immunomodulating activities are disturbed (Fuji *et al.*, 1987) otherwise we would "lubricate a machine which is self-lubricated enough" and the solution of the problem is lying somewhere else. On the other hand, we have to be aware of the possibility of exhausting IFN receptors by inappropriate time schedule and/or IFN dosage (Pestka and Langer, 1987) allowing reactivation of HBV inside "once-cured" cells leading to an endless "vicious cycle".

In conclusion: we still have to look for more efficient and better controlled HBV infection therapy. One cannot say what is going to be more successful: modulation of cytotoxic cells and other cellular-humoral activities, stimulation of endogenous IFN and related defense mechanisms, application of non-toxic and effective chemotherapy or combination of two or more approaches. We should not overestimate, however, the therapeutical potential of exo-

genous IFN application. There are too many "not-so-clear" places in our understanding of in vivo mechanisms of IFN induction and action, its pharmacodynamic and links with other lymphokines and defense factors.

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