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HUMAN INTERFERON ALFA THERAPY AND HEPATITIS B VIRUS MARKERS IN THE SERUM OF PATIENTS WITH CHRONIC COURSE OF ILLNESS

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Summary. — Dynamics of serum levels of HBsAg, HBeAg and anti HBc were followed during human interferon alpha (Hu IFN alpha) therapy of patients with chronic active or chronic persistent hepatitis B. More or less expressed oscilations of HBsAg serum levels seen in two out of our six treated patients seemed to occur due to IFN effect. Little and seldom changes were observed in HBeAg and anti HBc serum levels. The profiles of HBsAg serum levels of interferon-treated patients compaired with the profiles of "conventionally" treated patients disclosed occurrence of spontaneous or perhaps interferon-induced cyclic elevations and depressions of HBsAg blood levels. The possible significance of this phenomenon is discussed.

Key words: chronic hepatitis B; interferon therapy; HBV serum markers

Introduction

Natural Hu IFN alpha is used in experimental therapy of hepatitis B since 1976 (Greenberg *et al.*, 1976; Merigan *et al.*, 1980; Smith and Merigan, 1982; Gregory, 1986). More recently recombinant Hu IFN alpha A is most frequently used (Omata *et al.*, 1985; Hess and Meyer, 1986). Results obtained in these studies point on a versatile effect of IFN on hepatitis B infection. In our work we aimed at a better understanding of the effect of natural Hu IFN alpha on hepatitis B virus (HBV) serum markers through frequent and long-lasting monitoring before, during and after Hu IFN alpha therapy or conventionally treated patients with different forms of hepatitis B infection.

Materials and Methods

Hu IFN alpha preparation. A slight modification of commonly used method was used (Fuchsberger *et al.*, 1979). Specific activity of our NDV-induced human leukocyte IFN preparation was about 10^6 international units per 1 mg protein.

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HBV serum markers. HBsAg, HBeAg and anti HBc levels were checked by commercially available tests (Abbott, Behring and Sevac RIA or ELISA kits). For further details see Stanček *et al.*, 1987.

Immunofluorescence. Cell smears from liver biopsies were prepared on coverslips, dried and fixed in cold acetone. FTTC labelled anti-HBs conjugate was used for staining; Fluoval microscope (Carl Zeiss, Jena, GDR) with appropriate filters was employed for viewing.

Hu IFN alpha therapy. Patients were given i. m. 5×10^6 units of IFN 1—3 times weekly up to the total of $18—100 \times 10^6$ IFN units. In two patients (A. V. and H. S.) the treatment was divided into two periods (Fig. 1).

Results and Discussion

Influence of Hu IFN alpha on HBsAg, HBeAg and anti HBc blood levels in patients with VH-B was followed.

About 18—100 milion international units (IU) of partially purified Hu IFN alpha divided into several doses (5×10^6 IU each) was given intramuscularly to 5 patients suffering for more than 1 year of chronic active (CAH) or chronic persistent (CPH) hepatitis B. The Table 1 summarizes observed clinical and biochemical results. A partial effect seen in 3 patients was ma-

Table 1. Experimental IFN therapy in patients with hepatitis B: Clinical and biochemical observations

Patient, age, sex	Clinical diagnosis	Duration of treatment. Total IFN dose	Tolerance of IFN	Therapeutic effect
E.N. 21 y. female	HBsAg positive CAH/CPH	7 weeks 38×10^6 IU	slight ECG changes; elevated temperature	temporal decrease of cytoplasmic HBsAg levels (IF); otherwise no effect
H.S. 24 y. female	HBsAg positive CAH	4 weeks 60×10^6 IU	erythema in the site of injection	temporal decrease of serum HBsAg levels; clinical course unchanged; persistent ALT, bilirubin positivity
A.V. 25 y. female	HBsAg positive CAH	7 weeks 100×10^6 IU	intermitent erythema in the site of injection	temporal decrease of serum HBsAg, HBeAg, ALT, bilirubin; clinical course slightly improved
M.D. 67 y. female	HBsAg positive CPH	3 weeks 30×10^6 IU	local skin reaction after testing	clinical and biochemical signs unchanged
Š.Š. 52 y. male	HBsAg positive subacute hepatitis	2 weeks 12×10^6 IU	no adverse reactions	steady improvement before IFN therapy; recovery
H.S. 24 y. female	HBsAg positive fulminant hepatitis	1 1/2 week 18×10^6 IU	no adverse reactions	clinical and biochemical course unchanged; exitus

nifested by improved aetiological, biochemical and physiological parameters. It was interesting to compare this data with the dynamics of HBV serum markers. Figure 1 shows profiles of the HBV markers in 3 CAH/CPH patients. The most interesting was the cyclic appearance of HBsAg levels in A. V. and H. S. female patients. Following the first Hu IFN alpha injections, a several--fold increase of HBsAg blood levels was observed. At the same time the level of transaminases (not seen in the Figure) and HBsAg in liver biopsies increased. The medical staff decided to discontinue the IFN treatment. This was followed then by a sharp drop of HBsAg serum levels. Under these conditions soon after the first sign of HBsAg serum elevation the IFN treatment was reintroduced. It was followed by a repeated cycle of fluctuating serum HBsAg level. No such dramatic changes could be observed concerning HBeAg or anti-HBc blood levels. Exception were the coincidental cyclic elevations and depressions of HBsAg and HBeAg levels registered in the patient A. V. at the second series of IFN injections. The situation of the third patient (E.N.) was rather different perhaps due to CAH/CPH form which in all followed parameters differs from highly active chronic forms of the first two patients.

To clarify whether the observed HBsAg elevations were due to Hu IFN alpha treatment or they were rather a natural phenomenon, the HBsAg blood levels with different forms of hepatitis B were monitored under "conventional" therapy for several weeks. The Figure 2 shows repeatedly checked well distinguished 2—3 peaks of HBsAg blood levels before complete HBsAg elimination from the patients circulation (patients A. C., L. Š., A. B., and V. K.). All patients were with acute hepatitis B proceeding to

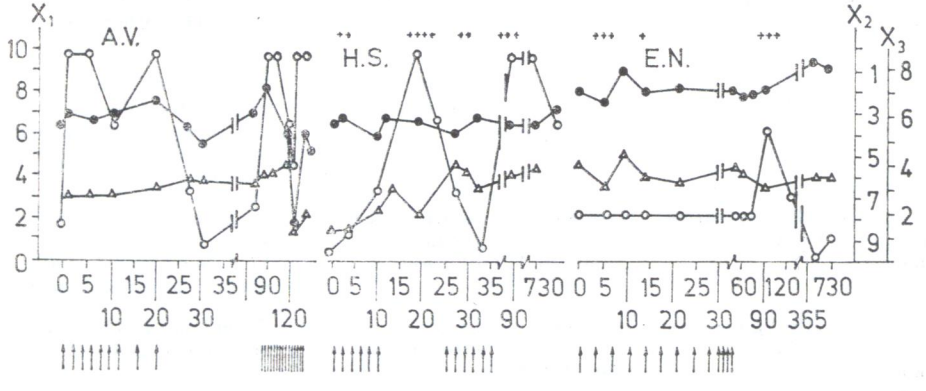

Fig. 1

Influence of Hu IFN alpha therapy on HBV markers in patients with CAH or CPH.
X_1 — HBsAg titres $\times 10^3$ ○——○
X_2 — anti HBc levels (inhibition of c.p.m.) $\times 10^2$ △——△
X_3 — HBeAg activity $\times 10^3$ c.p.m. ●——●
Abscissa: days Arrows: Hu IFN alpha injections
+ = immunofluorescence (hepatal biopsies)

recovery within 1—3 months of the illness. The remaining two HBsAg profiles (patients A. K. and N. N.) were found in patients suffering from prolonged courses of hepatitis B which eventually led to CPH (A. K.) or CAH (N. N.).

Based on these findings it seems plausible that IFN given to certain hepatitis B patients stimulates immunopathogenetic events during the natural antigenic boost (HBsAg, HBeAg, HBcAg) which in individuals with appropriate immune reactivity leads to antigen-antibody seroconversion, elimination of HBV-infected cells and recovery. In the case of non-reactivity, more or less prolonged struggle for HBV clearence may occur. In our two described patients (A. V., H. S.) Hu IFN alpha treatment alone did not provide the efficient stimulation for a reversion of the unfavourable course of the illness. Thus, the search for more efficient combined therapy is fully substantiated and promissing (Schalm *et al.*, 1985; Sculard *et al.*, 1981). In this relation it was interesting to follow the depression of spontaneous HBsAg production in vitro by PLC/PRF/5 human hepatoma cells in response to exogenous Hu IFN alpha treatments as reported elsewhere (Hajnická and Stanček, 1987).

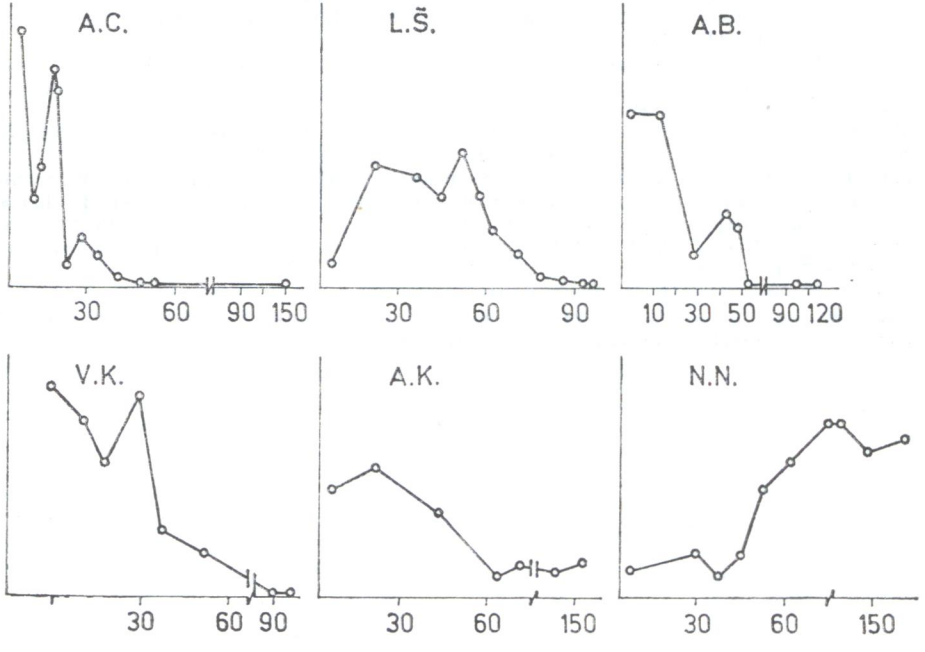

Fig. 2
Dynamic of HBsAg serum levels during conventional therapy of VH-B.
Ordinate: relative levels of HBsAg
Abscissa: days

In conclusion, Hu IFN alpha therapy in CPH or CAH patients may positively influence naturally occuring aetio-immunopathogenetic processes as reflected by cyclic changes of serum HBsAg levels. Administration of exogenous IFN alone is often not enough to discontinue HBV replication in patient's hepatocytes (or white blood cells). Other effective immunostimulating or modulating and antiviral factors are necessary for successful and efficient treatment of hepatitis B infections.

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