DEMONSTRATION OF HIFN-β IN THE LIVER OF PATIENTS UNDERGOING HIFN-β TREATMENT

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1. Introduction

The various effects that interferon exerts in exposed cells are supposed to be triggered by its interaction with specific receptors in the cell membrane [1,2]. Measuring circulating interferon blood levels therefore has become a widely applied tool during systematically administered interferon therapy. Demonstration of interferon in tissues would provide more conclusive evidence that exogenous interferon indeed will reach the membranes of target cells. This question is of some clinical importance especially in cases when HIFN-β is used, since this type of interferon is rapidly inactivated and circulating interferon blood levels can be detected but hardly after subcutaneous or intramuscular application of HIFN-β in both men and animals [3,4]. We describe here the presence of HIFN-β in the liver tissue of 3 male patients with chronic active hepatitis B, who underwent HIFN-β treatment. HIFN-β was shown attached to the walls of sinosoids by means of indirect immunofluorescence using a monoclonal anti-IFN-β.

2. Materials and methods

The HIFN-β used was kindly provided by Dr Rentschler, Arzneimittel GmbH, Laupheim. The interferon preparation was intravenously administered in all 3 individuals for 6 months. The doses applied were 10 Mio U/day for 1 month and 10 Mio U every 3 days for 5 months. Liver biopsies were performed as in [5,6] at the last day of HIFN-β treatment immediately after a 10 min infusion of 10 Mio U of HIFN-β [5,6]. The specimens were embedded in tissue Tek II®, (Lab Tek Prod., Miles, Naperville IL) and were frozen in liquid nitrogen promptly thereafter. Subsequently unfixed 3.5 μm cryostat sections were prepared, rinsed with barbital buffer (pH 7.2) for 15 min and incubated with a 1:50 dilution of a monoclonal mouse-anti-HIFN-β at room temperature for 30 min.

The monoclonal mouse anti-HIFN-β antibody was obtained from a stable FO-myeloma hybrid line [7]. The antibody is specific for the protein moiety of IFN-β and completely neutralizes the antiviral activity of IFN-β in a plaque reduction assay on FS4 fibroblasts at a dilution of 1:30, and partly at a dilution of 1:100. For immunofluorescent studies the monoclonal antibody had been purified from ascitic fluid of Balb-c mice by a 35% ammonium sulfate and a subsequent Sephadex G-200 column step in a Tris–glycine–butanol buffer (0.2 M Tris–HCl, 0.5 M glycine, 0.2% *n*-butanol (pH 7.6)).

Staining of liver sections was performed by a 1:50 dilution of a FITC labelled goat anti-mouse IgG Cappel Lab., Cochranville PA) for 30 min after a washing procedure with barbital buffer (pH 7.2). Following a final washing with barbital buffer the sections were mounted in a solution of 9 parts glycerol and 1 part barbital buffer. The same staining procedure was applied in sections of liver specimen obtained prior to onset of HIFN-β treatment of each of the 3 patients and in sections of liver biopsies of 5 healthy subjects without any clinical or histological features of liver disease. Liver sections of patients with chronic

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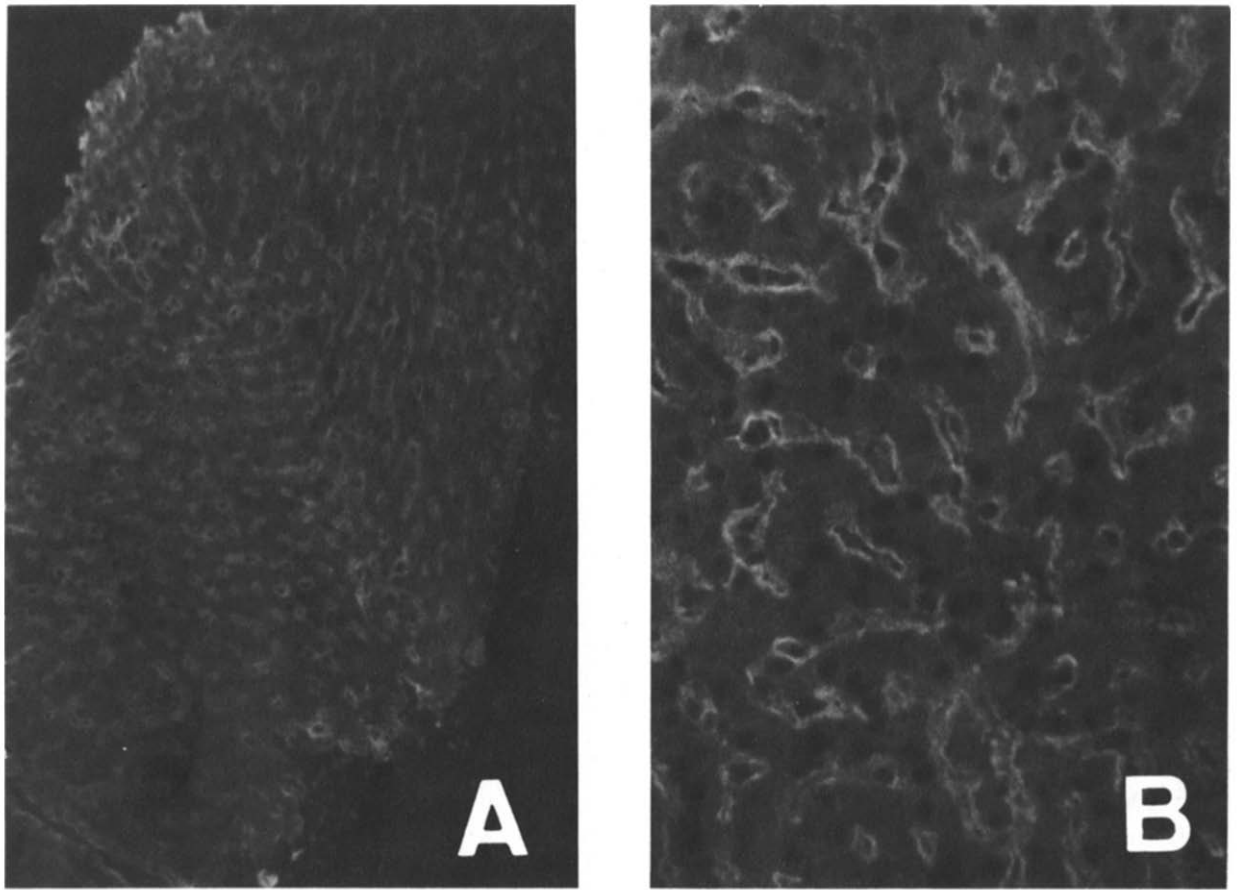

Fig.1. Demonstration of HIFN-β in cryostat liver sections by indirect immunofluorescence using a monoclonal anti-IFN-β: (A) × 333; (B) × 833

active hepatitis B who were not treated with HIFN-β did not appear to be appropriate controls, since small amounts of autologous interferon may be detected in some livers of such subjects by indirect immunofluorescence using the monoclonal mouse anti-HIFN-β applied. Moreover replicate slides of all livers were also stained with normal human immunoglobulin. Examination of all sections was performed by the following optical equipment: fluorescence microscope Leitz Ortholux 2, super pressure mercury lamp HBO 200 W, Ploemopak 2.2 fluorescence incident light illuminator, filter block: excitation filter I 2 BP 450–490 nm, dichroic filter RKP 510 nm, barrier filter LP 515 nm, objectives NPL Fluotar 10/0.30, 25/0.55 and 40/0.70.

3. Results and discussion

A small fringe of bright fluorescence attached to the membranes of hepatocytes confining the walls of sinusoids were observed throughout the liver lobules in the sections of the 3 patients after HIFN-β treatment, but were not found in liver sections of the same subjects prior to onset of interferon treatment. In addition all sections of the 5 individuals with normal livers and those of all patients investigated and stained with normal immunoglobin were found to be completely negative. These data suggest that HIFN-β does indeed reach the liver, and can easily be demonstrated by means of indirect fluorescence when given intravenously in the above dose.

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