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Induction of Mx-related protein in cat peripheral blood mononuclear cells after administration of recombinant human interferon hybrid

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Summary

A 78 kDa protein was induced in cat peripheral blood mononuclear cells after in vivo administration of recombinant human interferon-α hybrid (rHuIFN-α B/D). This protein was antigenically related to the IFN-induced human (78 kDa) and mouse Mx proteins. Quantitative immunoblot analysis indicated that the induction of the cat Mx protein was dose-dependent. There was a dissociation in time between plasma levels of IFN which were transient, and levels of cat Mx protein which remained elevated at least five days after dosing. Our results provide evidence that a human IFN-α hybrid may be active in cats. They also indicate that the Mx protein is a sensitive, quantitative, and stable marker to follow IFN activity in vivo in cats.

Interferon; Cat Mx protein; Feline viral disease

Introduction

Viral diseases are still very prevalent in the cat population world-wide: feline viral rhinotracheitis, feline leukemia, feline infectious peritonitis, feline panleukopenia. Recently, a feline T-lymphotropic lentivirus FTLV related to HIV has been isolated. Although these diseases may be controlled by vaccination, the first immunization must be made before natural exposure to the pathogen occurs.

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Therefore, the availability of an antiviral agent for treatment of cats with viral diseases or for prevention of the diseases after recent exposure to viruses would be most valuable.

The capacity of interferons (IFNs) to control virus replication within susceptible cells, in addition to regulating immune function, has been recognized for many years. A recombinant human interferon-α hybrid (rHuIFN-α B/D) has been found to have a broad host-range of antiviral activity in vitro (plaque reduction of vesicular stomatitis virus) in at least 12 animal cell species including cat embryonic lung diploid cells (Horisberger and De Staritzky, 1987).

We wanted to test the antiviral potential of this novel cross-species active, recombinant interferon hybrid in a feline model of viral disease, namely infection with the retrovirus feline leukemia virus. The hybrid IFN showed no efficacy in this particular model in preventing death from opportunistic infections. These negative results indicated that the hybrid IFN either had no efficacy against this particular viral disease, or had no biological activity when injected into the animal. To solve this dilemma we had to establish a test for monitoring biological activity of the hybrid in the cat.

The antiviral action of IFN-α can be related to the induction of specific cellular proteins: 2–5 A synthetase, protein kinase, and more recently, Mx proteins (Horisberger, 1988; Horisberger and Hochkeppel, 1987; Horisberger et al., 1983; Meier et al., 1988). The exact role of these proteins in the biologic activity of IFN is not yet clear, with the exception of the mouse Mx protein which has a direct activity against influenza virus (Staeheli et al., 1986). In this paper we demonstrate that an Mx-related protein can be induced in vivo in cat peripheral blood mononuclear cells (PBMc) after IFN treatment and that this protein marker may be used to monitor quantitatively biological responses of cats to IFN-α therapy.

Materials and Methods

Cats

Twelve specific pathogen-free kittens of 11 to 12 weeks of age, weighing 1 kg, were purchased from Ciba-Geigy. They were housed in an isolation unit to reduce the possibility of exogenous virus infection. The cats were fed twice daily on a commercial cat food and were allowed water ad libitum.

Interferon and cells

The recombinant human interferon-α B/D hybrid (rHuIFN-α B/D) was produced in yeast by recombinant DNA technology, and purified by immuno-affinity chromatography to a purity of >95%. It comprises amino acid 1 to 60 from HuIFN-αB, 61 to 92 from HuIFN-αD, and 93 to 166 from HuIFN-αB (and not 61 to 166 from HuIFN-αD as previously published; Horisberger and De Staritzky, 1987). The specific antiviral activity, as determined by plaque inhibition of vesicular stomatitis

virus (VSV) grown in human diploid cells, was 5×10^7 IU/mg. A similar specific activity was found on cat embryonic diploid cells (Horisberger and De Staritzky, 1987). Plasma IFN titers were assessed by an antiviral assay using reduction of cytopathic effect of VSV on MDBK cells as described elsewhere (Rubinstein et al., 1981). Cat embryonic cells were obtained from Flow Laboratories (Feline Embryo, No. 05-552).

Study design

Cats were assigned randomly to 4 treatment groups of 3 animals. Each test animal received either 3×10^4, 3×10^5 or 3×10^6 IU of IFN subcutaneously corresponding to 0.6, 6 and 60 μg respectively, on day 0 and 2 of the experiment. The cats of the control group received an equivalent volume (1 ml) of placebo.

The animals were examined clinically every day: 4 ml of blood was collected in heparinized tubes by venous puncture on day 0, 1, 2, 3, 4 and 7 after IFN treatment.

Preparation of PBMc

Blood samples were centrifuged at $1000 \times g$ for 20 min at 4°C. The plasmas were removed frozen at −20°C for subsequent IFN titer determination. The peripheral blood mononuclear cells (PBMc) were isolated from the buffy coats by means of Ficoll Hypaque (Pharmacia Fine Chemicals) density gradient centrifugation. Each PBMc sample was stored at −70°C as a pellet of 2×10^6 cells until the IFN-induced protein assay could be performed.

Quantitative determination of IFN-induced Mx proteins

PBMc (cell lysates) were separated by one dimensional polyacrylamide gel electrophoresis, and blotted onto nitrocellulose using a semidry electroblotter SM 17556 (Sartorius GmbH). The ELISA procedure was then performed as described elsewhere (Horisberger and Hochkeppel, 1987) using monospecific polyclonal mouse antibodies directed against the human homolog of the mouse Mx protein. Quantitative measurement of cat Mx protein was determined by densitometry tracing obtained by reflection-absorption.

Results

Fig. 1a illustrates that a protein related both antigenically and by size to the IFN-inducible human 78 kDa protein, the human homolog of mouse Mx protein (Horisberger and Hochkeppel, 1987; Goetschy et al., 1989), could be induced in cat embryonic cells by rHuIFN-α B/D. The polyclonal antibodies raised against the IFN-induced human 78 kDa protein (Horisberger and Hochkeppel, 1987) cross-reacted with the cat IFN-induced protein (Fig. 1a) and they cross-react also with

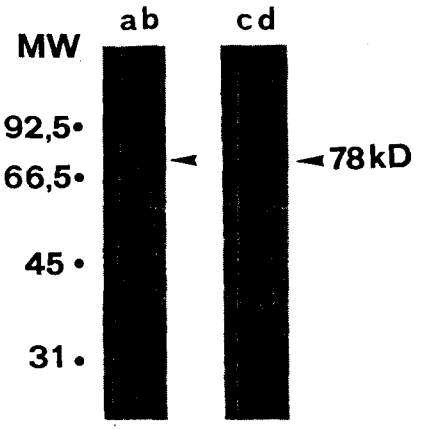

Fig. 1. Immunoblot detection of the feline Mx protein induced by IFN. Immunoblots of proteins extracted from uninduced (a) or IFN-induced (b) cat embryonic cells, and from uninduced (c) and IFN-induced (d) human embryonic lung cells. Cells in culture were induced for 18 h with 1000 IU/ml of rHuIFN-α B/D. Cellular extracts were prepared and analyzed by the procedure described earlier (Horisberger and Hochkeppel, 1987) using polyclonal antibodies to the IFN-induced human Mx protein (78 kDa). Position of molecular-weight markers: phosphorylase b (92 500), bovine serum albumin (66 200), ovalbumin (45 000) and carbonic anhydrase (31 000).

the mouse Mx protein from influenza-resistant mice and with the Mx-related bovine proteins induced by IFN-α (Horisberger, 1988). The cat protein was further analyzed by 2-D gel electrophoresis and found to be comparable in size and pI to the homologous proteins in human, mouse, and bovine cells (data not shown).

We then monitored the induction of Mx protein in PBMc from cat treated with various doses of rHuIFN-α B/D hybrid. Quantitative densitometric determina-

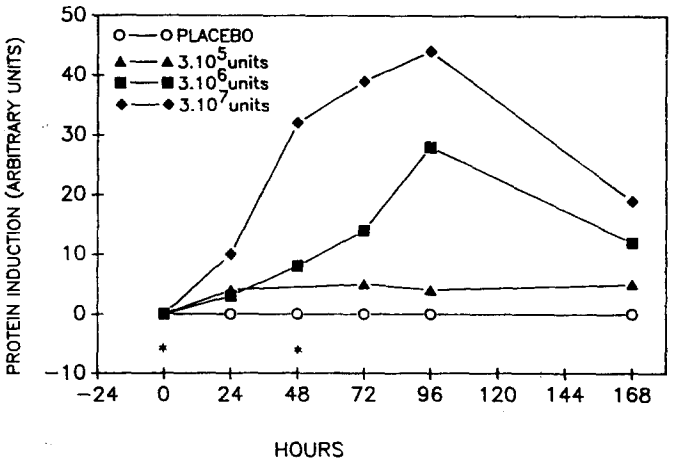

Fig. 2. Kinetic and dose-dependent effect of rHuIFN-α B/D of Mx protein induction in feline PBMc in vivo (asterisk indicates the times of injection of IFN). The increasing doses correspond to 3×10^4, 3×10^5, and 3×10^6 IU of IFN injected subcutaneously per animal.

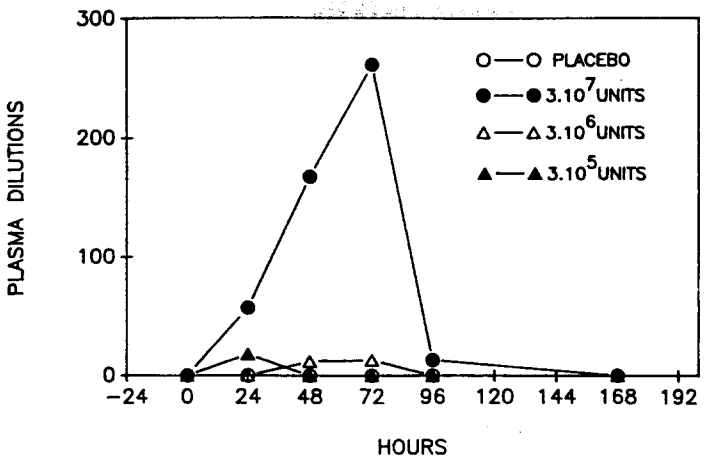

Fig. 3. Plasma IFN levels from cats treated with different doses of rHuIFN-α B/D or placebo. Results are expressed as the highest dilution of plasma that protected MDBK cells against the cytopathic effects of VSV (serial dilution 1:3).

tions on Western blot ELISA indicated that the induction of the cat Mx protein in PBMc was dose-dependent (Fig. 2). With the highest dose of rHuIFN-α B/D there was a rapid accumulation of the protein during the first 48 hours following a single injection. In all but two treated animals a significant increase of the protein could be seen by 24 hours post injection. After a second administration of IFN, the levels of Mx protein continued to rise, peaking at day 4 of the experiment. Although the level declined thereafter, significant amounts of Mx protein were still detectable on day 7.

No adverse effects were observed immediately after treatment with rHuIFN-α B/D and none occurred throughout the course of the experiment. The plasma levels of IFN-α before and after treatment of cats are shown in Fig. 3. No IFN was detectable in the plasma of the 12 cats before treatment, and IFN plasma levels were measurable only in animals treated with the highest dose of IFN. The IFN plasma levels were transient, in contrast to the Mx protein levels in PBMc, which were relatively stable.

Discussion

Proteins induced by IFN are important indicators of cell responsiveness to IFN action. In the present work we have identified a protein induced by IFN-α in feline embryonic cells in vitro. This protein is antigenically related to proteins described in three distant species, namely the human 78 kDa protein (Horisberger and Hochkeppel, 1987), the Mx protein from influenza-resistant mice (Horisberger et al., 1983), and the bovine Mx-related proteins (Horisberger, 1988). All these pro-

teins are induced by IFN-α, and they have comparable molecular weight and pI.

We have shown that induction of cat Mx protein was easily detected by immunoblot analysis of PBMc extracts from cats treated with an IFN-α B/D hybrid. In control animals (no IFN treatment) the amount of cat Mx protein was below the detection limit of our sensitive assay. The induction was dose-dependent, and it remained significantly elevated during at least 5 days after dosing, indicating that the cat Mx protein is stable. Cat Mx protein levels did not parallel the serum IFN levels which were transient. Thus the IFN-α B/D hybrid was no longer detectable in serum 24–48 h after the injection. Moreover, induction and maintenance of cat Mx protein was identified at the intermediary dose of IFN in the absence of detectable IFN serum levels. Taken together, these results indicate that cat Mx induction can be considered as a good index of biological responsiveness of cats to IFN treatment.

The results found on IFN kinetics are consistent with those obtained with rHuIFN-α in healthy human individuals after intramuscular administration (Barouki et al., 1987). Particularly, the rHuIFN-α is not detectable in plasma at 24–48 h after injection. The dissociation in time between serum levels of IFN and biological activity has been observed in human volunteers using a 2–5 A synthetase assay (Barouki et al., 1987; Merritt et al., 1986) or a virus yield reduction assay (Barouki et al., 1987). Both studies suggest that the biological activity of IFN is detectable long after plasma IFN levels have fallen.

HuIFN-α has been used with limited success in various studies to prevent feline rhinotracheitis (Cocker et al., 1987). However, no results on the monitoring of IFN-α effects in cats have been published so far and an optimization of IFN therapy in the feline species is still needed. We have provided evidence that a human IFN-α hybrid may be active in cats. We have also established a sensitive and quantitative method to monitor biological activity of IFN in cats. It is therefore now possible to characterize more accurately IFN efficacy in vivo during the attempted therapy of various feline diseases.

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