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Tissue Distribution of Human Interferons After Exogenous Administration in Rabbits, Monkeys, and Mice

By

A. Billiau, H. Heremans, D. Ververken, J. van Damme, H. Carton, and P. de Somer

Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

With 2 Figures

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Summary

Preparations of human leukocyte (α) and fibroblast (β) interferon were given intramuscularly to rabbits and monkeys, and circulating interferon was measured. In rabbits, but not in monkeys, a marked difference between the two interferons was noted in that higher titers of circulating antiviral activity were obtained with leukocyte than with fibroblast interferon. In mice, injected interperitoneally, a similar difference could be noted. However, levels of antiviral activity in homogenates of spleens and lungs did not differ between mice injected with either interferon.

Fibroblast interferon that was injected intrathecally in monkeys was found to diffuse throughout the cerebrospinal canal and to reach the serum compartment. Some interferon could also be recovered from the pia mater surrounding the brain hemispheres, but none was found in the deeper layers of the brain.

Introduction

Preparations of human interferon are currently being injected in man in order to determine their prophylactic or curative potential against virus infections and cancer (for reviews see ref. 1, 12). Therefore, it would be valuable to have data on the tissue distribution, decay rate and metabolism of the injected material. The main reason for the current lack of sufficient information in this area is that interferons are available in very small quantities only, so that, even after injection of the highest possible dose, barely detectable concentrations are present in the tissues. In view of the host range restrictions to which the interferon system is subjected, ideally data on tissue distribution of human interferons should be collected in experiments on man. While it has already been possible to obtain useful information by studying blood levels in patients injected with interferon

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(see review in ref. 6), there remain questions which can so far be approached only by animal studies. In the present paper we report on experiments comparing the pharmacokinetics of fibroblast (β) and leukocyte (α) interferon in rabbits, monkeys and mice, with special reference to uptake by certain organ systems such as spleen, lungs and brain.

Materials and Methods

The experimental animals used in the present study were: mongrel rabbits weighing 1.5 to 2.0 kg, NMRI-mice weighing 20 to 25 g and *Cercopithecus aethiops* monkeys weighing 3 to 4 kg.

Human fibroblast interferon was prepared on diploid embryonic skin muscle cells and purified by adsorption on controlled pore glass (CPG) as described earlier (3). Human leukocyte interferon was kindly provided by Dr. K. Cantell (State Serum Institute, Helsinki, Finland). The approximate specific activities of the injected preparations were $10^{6.0}$ units/mg protein.

Blood samples for interferon titration were allowed to clot at room temperature for about 4 hours. Serum samples were clarified and stored at $-20°$ C before titration. Samples of cerebrospinal fluid were also stored at $-20°$ C. Lungs and spleens of mice were extensively washed in phosphate buffered saline, blotted, weighed and homogenized in 9 ml of regular cell culture medium per g of tissue, using a motor-driven Teflon-pestle tissue homogenizer. The homogenates were centrifuged at 3000 rpm for 20 minutes and stored at $-20°$ C. Before titration the samples underwent additional clarification by centrifugation at 3000 rpm for 30 minutes.

The brain hemispheres of monkeys that were injected intrathecally were carefully removed and extensively washed with phosphate buffered saline containing a 1/100 dilution of antiserum directed against fibroblast interferon (this serum had been prepared by injecting a goat with CPG-purified fibroblast interferon and had a neutralizing titer of 1/100,000 against 10 units/ml of fibroblast interferon). The brain was then given a final wash with saline whereafter various portions were dissected to prepare homogenates.

Serum interferon was titrated by inhibition of Semliki Forest or vesicular stomatitis virus cytopathic effect in human diploid cells, using a dye uptake method (5). Both interferons were calibrated against internal laboratory standard preparations. The unitages were adjusted to those of the international standards for either leukocyte or fibroblast interferon made available by the National Institutes of Health, Bethesda, Md.

Tissue homogenates of mice caused nonspecific inhibition of CPE. Therefore, the antiviral activity in these samples was titrated using a viral RNA reduction assay (8). Briefly, diploid skin fibroblasts were grown to confluency in small scintillation vials. The cells were incubated for 24 hours with serial dilutions of the tissue homogenates and then challenged with Semliki Forest virus at MOI $>$ 10 PFU/cell, in the presence of actinomycin D (1 μg/ml). After 3 hours incubation at 37° C tritiated uridine (5 μCi per vial) was added. The cells were further incubated for 3 hours at 37° C, washed with cold saline and extracted with cold trichloroacetic acid solution (5 per cent). Scintillant was then added to the vials and acid precipitable material, representing mainly newly synthesized viral RNA, was measured. Antiviral activity in the samples was expressed as the dilution of the sample that reduced the net incorporation (observed cpm minus "blank" cpm of cell control) to 50 per cent of control (without interferon). Each assay was calibrated by including internal laboratory standards of leukocyte and fibroblast interferon.

Results

One of the issues which can be resolved by experiments using human interferons in experimental animals is the comparative pharmacokinetics of different interferon types. In clinical studies on man it has been found that intramuscularly

injected fibroblast interferon fails to appear in the circulation (4), while similar injections of leukocyte interferon yield high serum values (6). Figure 1 shows the results of a series of experiments in which rabbits or monkeys were given intramuscular injections of either leukocyte or fibroblast interferon. Blood samples were taken at various time intervals for determination of circulating interferon. In rabbits, peak serum titers were more than 10-fold lower with fibroblast than with leukocyte interferon. Unexpectedly, the same preparations injected intramuscularly in monkeys failed to reveal a significant difference: high serum titers were obtained with either leukocyte or fibroblast interferon. This indicates, that contrary to what one would expect on the basis of phylogenetic relatedness, the monkey is not a good model for the pharmacokinetic behaviour of human interferon in man.

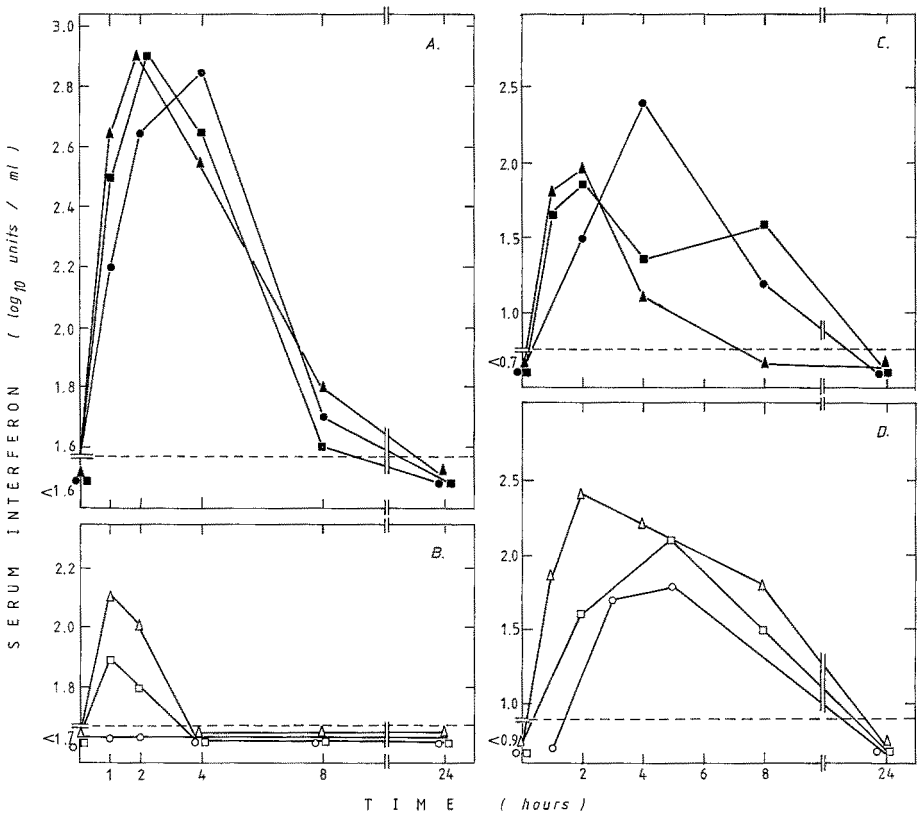

Fig. 1. Antiviral activity in the serum of rabbits (*A* and *B*) or monkeys (*C* and *D*) after intramuscular injection of 10^6 units of leukocyte interferon (*A* and *C*) or fibroblast interferon (*B* and *D*). Each curve represents data for one animal (Panels *A* and *B* taken from reference 2)

Low blood titers after intramuscular injection in rabbits or man (4) may be due to a number of factors: rapid destruction at the site of injection, rapid destruction in circulating blood, or rapid removal from the serum through uptake

by cells or organs. Clearance studies done in rabbits with the same preparations of leukocyte and fibroblast interferon as those used for the experiments shown in Figure 1, failed to reveal differences in clearance rate (2), a finding confirmed by VILCEK *et al.* (11). It is doubtful, however, whether these experiments were accurate and sensitive enough to reveal small differences that were sufficiently important to account for different blood values after intramuscular injection. Thus, these experimental results, while failing to support the idea of a more rapid clearance of fibroblast type interferon, did not refute this possibility either. Attempts to measure uptake of interferons in organs or tissues of rabbits injected with human interferon were unsuccessful in our hands. The obvious reason for this was that the injected amount of interferon was too small relative to the animals' weight.

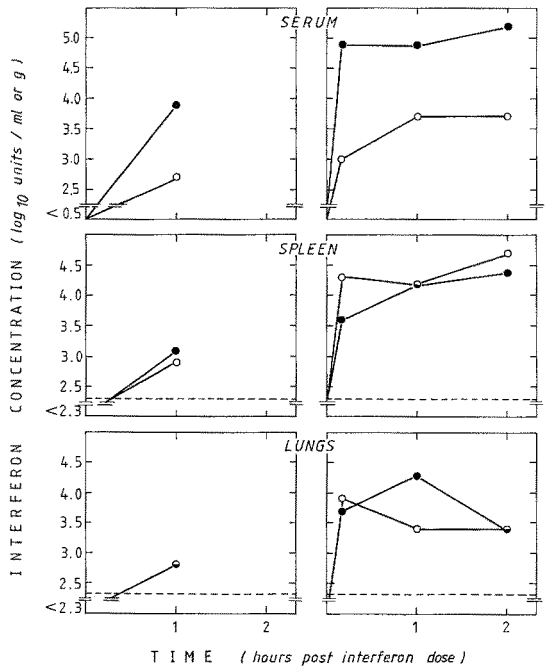

Fig. 2. Antiviral activity in serum, spleen and lungs of mice after intraperitoneal injection of interferon. Closed symbols: leukocyte interferon; open symbols: fibroblast interferon. Left panels: 10^5 units; right panels: 10^6 units

Therefore, it seemed reasonable to undertake experiments in mice in order to determine whether fibroblast interferon would be taken up more rapidly by tissues as compared to leukocyte interferon. Groups of three mice were injected intraperitoneally with 0.1 or 1×10^6 units of either leukocyte or fibroblast interferon and antiviral activities were determined in serum, lungs and spleens. Figure 2 shows the results of this experiment. A 10 to 30-fold difference was apparent in the serum levels reached by leukocyte and fibroblast interferon. In contrast, antiviral activities measured in the spleen and the lungs were similar, irrespective of which interferon was used.

An important organ to be protected during certain viral infections is the brain. Interferon administered by intramuscular or intravenous injection is not likely to reach the brain matter effectively. This can be inferred from studies in mice in which the concentrations of interferon in various organs were followed throughout the course of a fatal encephalitogenic virus infection (8). During the early pre-encephalitic phase of the disease high levels of interferon were found in the spleen and serum, with very little, if any, interferon detectable in the brain. Brain interferon was only detectable during the later (encephalitic) phase, as a result of local viral replication. This type of experiment indicates that there is a strong blood-to-brain barrier for interferon. Experiments with exogenous leukocyte interferon administered intramuscularly or intravenously to monkeys showed that some of the interferon crossed the barrier between the vascular and cerebrospinal fluid compartment (7). This does not necessarily mean that it did also reach the brain matter itself as there may exist a barrier between the cerebrospinal fluid and brain matter as effective as the blood-brain barrier itself. In order to examine this question, we injected fibroblast interferon intrathecally in monkeys and studied the appearance of antiviral activity in the cerebrospinal fluid, serum and brain biopsies (Table 1). Diffusion throughout the cerebrospinal fluid was found to occur rapidly: within one hour after injection in the lumbar region interferon could be recovered from fluid taken suboccipitally. Interferon could also be recovered from the serum, as was also reported for leukocyte interferon injected in the cerebrospinal canal (7). In initial experiments (not shown in Table 1) some interferon could be recovered from the brain homogenates. However, in view of the high concentrations of interferon in the cerebrospinal fluid it was considered that this might have been artifactual as a result of contamination by liquor at the time of tissue sampling. When great care was taken to remove all cerebrospinal fluid from the brain hemispheres before slicing, no interferon could be recovered from the white matter or cortex. Some activity could be recovered from the pia mater, scraped off together with superficial cortex. This interferon had probably penetrat-

Table 1. *Recovery of interferon from liquor, serum and brain of monkeys injected intrathecally with fibroblast interferon*

Sample	Time (hours after injection)	Interferon level ($\log_{10}$ units per ml or per g)	
		Expt. 1 (1×10^6 units)	Expt. 2 (3×10^6 units)
Liquor	0	<1.0	<0.9
	1	3.3	—
	2	2.2	4.7
Serum	0	<1.0	<0.9
	1	<1.0	1.4
	2	<1.0	2.3
	4	1.5	—
	8	1 5	—
White matter (+ ganglia)	3	—	<0 9
Cortex	3	—	<0.9
Pia mater + superficial cortex	3	—	2.3

ed the tissue as it was not inactivated by washing the brain with a saline solution containing strong antibody to fibroblast interferon. From these results it would appear that, in spite of high levels of residual interferon in the cerebrospinal fluid very little, if any, had penetrated in to the deep layers of brain matter.

Discussion

The data presented in this paper show that human leukocyte and fibroblast interferon, after intramuscular injection in monkeys gave rise to virtually superimposable curves of antiviral activity recovered from the serum. This is in contrast to earlier findings in rabbits (2, 11), where intramuscular injection of fibroblast interferon yielded blood titers that were several-fold lower than those obtained with leukocyte interferon. Weinman and Hilfenhaus (personal communication) used cynomolgus monkeys to compare blood levels of interferon after intramuscular or intravenous injections. After intramuscular injection they found only small differences between leukocyte and fibroblast interferon, blood levels were not much higher with leukocyte than with fibroblast interferon. Studies in human patients showed that intramuscular injection of fibroblast interferon yielded very low to undetectable levels of antiviral activity in serum. Thus, the rabbit should be considered as a better model than the monkey to study the pharmacokinetic behaviour of human interferons in man.

In the present study a similar difference in pharmacokinetic behaviour was also found in mice injected intraperitoneally. Yet, determinations of the amount of interferon present in the spleen and lungs of these animals revealed uptake of interferon by these organs. Despite lower blood titers with fibroblast interferon, antiviral activity recovered from the organs was as high as that in mice injected with leukocyte interferon. These data indicate that both interferons did reach certain internal organs with equal efficiency. This view is also supported by the observations of Lucero *et al.* (9), who determined activation of the natural killer (NK) cell system in patients given intramuscular injections of human interferon. Despite the lower blood titers obtained with fibroblast interferon, the NK-cell system was activated to about the same extent as in patients receiving leukocyte interferon. These observations make it unlikely that the observed differences in serum titers between the two interferon types are related to destructive processes. Whether they are related to the more rapid vascular clearance of fibroblast interferon also remains questionable as experiments designed to test this possibility failed to reveal differences in clearance rates between the two interferon types (2, 11).

Fibroblast interferon injected in the cerebrospinal canal of monkeys led to detectable serum levels. Some interferon activity could also be recovered from the pia mater and superficial cortex. Despite high concentrations in the cerebrospinal fluid interferon remained undetectable in deeper layers of the brain. These data are in line with those obtained in studies using radioiodinated albumin (10), which was also found to rapidly diffuse from the cerebrospinal fluid into the blood and to penetrate barely if at all into the brain matter. In conclusion, it seems reasonable to assume that intrathecally administered fibroblast interferon can effectively reach the membranes surrounding the central nervous system but offers little chance for reaching the deep layers of white and gray matter.

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Authors' address: Prof. Dr. A. Billiau, Rega Institute of Medical Research, University of Leuven, B-3000 Leuven, Belgium.

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