Pharmacokinetic Properties of Human Fibroblast and Leukocyte Interferon in Rabbits

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When rabbits were given intramuscular injections of the same quantities of human leukocyte or fibroblast interferons, the former produced moderately higher levels of circulating interferon. Fibroblast interferon was not cleared faster from circulation, nor was direct inactivation by rabbit blood responsible for this difference.

Interest is growing in the clinical applications of interferons in viral infections and some malignancies. Most clinical trials have been done with interferon induced by Sendai virus in buffy coat cell (leukocyte) cultures (20). These preparations contain Le interferon as the major component (11). It was demonstrated that after intramuscular injection much of the administered leukocyte interferon enters the circulation and can be recovered from the blood in biologically active form (3, 4, 16).

Human diploid fibroblasts stimulated with polyinosinate-polycytidylate serve as another source of interferon. High yields of interferon can be derived from this source by judiciously timed treatment with metabolic inhibitors, termed “superinduction” (2, 12, 14). The active component of fibroblast interferon preparations is F interferon, distinct from Le interferon in antigenic, physicochemical, and biological properties (11, 23). Fibroblast interferon has only recently become available in quantities sufficient for limited clinical trials. It was reported that, unlike leukocyte interferon, fibroblast interferon failed to produce measurable levels of activity in the serum of patients after intramuscular administration of about 50,000 U/kg (1, 9).

Failure of intramuscularly administered fibroblast interferon to produce adequate blood levels would limit the therapeutic usefulness of this interferon. Interferon is not likely to show systemic activity if it does not reach the site at which it is supposed to exert its action. Alternative routes of administration of fibroblast interferon would have to be used.

Fibroblast interferon might fail to produce the expected levels of activity in the blood for some of the following reasons: (i) interferon is either bound or inactivated at the injection site before it can enter the circulation, (ii) interferon enters the circulation but is rapidly inactivated in the blood, or (iii) interferon enters the circulation but is very rapidly cleared from the blood. In the present study some of these possibilities were tested experimentally in rabbits.

Rabbits and monkeys were previously used for pharmacokinetic studies with human interferons. There is reason to believe that results from such animal experiments are generally applicable to humans. This view is supported by the good correlation between results obtained with leukocyte interferon in experimental animals and in patients (3, 4, 16, 18).

Fibroblast interferon employed in the experiments was produced in FS-4 cells by stimulation with polyinosinate-polycytidylate under conditions of superinduction as described (22). Human serum albumin (0.1%) was used in the production medium. Crude interferon was concentrated about 10-fold by ultrafiltration with the aid of a Millipore Pellicon apparatus (using a membrane with a 10,000-molecular-weight cutoff point). Leukocyte interferon (20) was the kind gift of Kari Cantell. Residual virus was inactivated by adjusting the pH to 2 and storing the material for 5 days at 4°C. Both interferon preparations were exhaustively dialyzed against phosphate-buffered saline (pH 7.4). The preparations used in the experiments were carefully calibrated to contain 50,000 interferon reference units per ml. Protein concentrations (17) were 2.5 mg/ml (leukocyte) and 12 mg/ml (fibroblast).

Interferon titrations were done by a semi-micromethod in 96-well plastic tissue culture plates, based on the inhibition of vesicular stomatitis virus cytopathic effect. For assaying the original interferon samples we employed the FS-7 cell strain, and all titers were corrected to the value of the international human leukocyte interferon standard G-023-901-527 as described (12). A slightly modified procedure was employed for the assay of interferon in samples of rabbit serum. To enhance the sensitivity of the assay, we employed the GM-258 strain of human fibroblasts, trisomic for chromosome 21 (21, 24),
received from the Human Genetic Mutant Cell Repository, Camden, N.J. Furthermore, after cells were incubated with the test dilutions of sera overnight, the serum dilutions were drained off and fresh maintenance medium containing the desired dose of vesicular stomatitis virus was added. Removing the dilutions of rabbit serum before virus inoculation is essential because many animal sera exert inhibitory activity on vesicular stomatitis virus multiplication (3). The presence of this inhibitory activity may be the cause of false-positive interferon titers. In the modified assay in GM-258 cells, none of the control ("prebleed") sera from rabbits used in the experiments inhibited vesicular stomatitis virus cytopathic effect at a dilution greater than 1:16. Experimental results are expressed in terms of actual assay units without correction to the reference standard. Each assay unit equals 2 to 4 reference units.

To compare activity levels in the blood of rabbits after intramuscular administration of fibroblast or leukocyte interferon, 500,000 reference units of either interferon preparation per kg of weight was injected into the gluteal muscles of female New Zealand white rabbits weighing 1 to 1.5 kg. Samples of venous blood were collected at frequent intervals from the ear. The blood was allowed to coagulate, and serum was isolated as soon as possible. Sera were kept frozen at −70°C until interferon was assayed. A total of three independent experiments was done, using two rabbits per interferon preparation in each experiment. The results of a representative experiment are shown in Fig. 1.

The results obtained with leukocyte interferon are qualitatively and quantitatively similar to those reported by Cantell and co-workers (3, 4). One slight difference is that in our experiments maximal levels in the blood seemed to be reached about 1 to 2 h earlier. This difference may be due to the fact that we used smaller rabbits and a larger volume of injected material than did Cantell et al. Compared with leukocyte interferon, fibroblast interferon titers in the sera were on the average about twofold lower. Despite some variation in the mean titers, this difference was seen consistently in all three experiments.

Next we compared the clearance of the two interferons after rapid intravenous administration into the ear vein. Blood specimens were collected from the opposite ear (Fig. 2). In this case, high levels of circulating interferon were rapidly established, but, in agreement with previous observations (3, 4, 13 19), clearance was also very fast, particularly during the first few minutes. There was no clear-cut difference in the clearance rate of the two interferons during the first 20 min. At later intervals, titers of fibroblast interferon tended to be higher. However, the reproducibility of this difference needs to be further examined.

As a control we also collected heparinized fresh blood from the rabbits before interferon administration. Samples of this whole blood were mixed with leukocyte and fibroblast interferon in vitro and incubated at 37°C to determine whether the two interferons are inactivated at different rates. With none of the many different blood specimens was there a marked inactivation of either interferon by the fresh blood or by control medium containing fetal calf serum. Representative results obtained with four specimens of rabbit blood (Table 1) suggest that fibroblast interferon tended to be marginally less stable. Other authors reported that various body fluids, including serum, exert a marked inactivating effect on human interferon, particularly fibroblast interferon (6, 10). The presence of inactivating factors in human and animal sera was recently examined by Cesario et al. (5). They reported that most of the examined sera exerted an inactivating effect on fibroblast inter-
interferons. Differences in titrations could be comparable eight times levels interferon properties detect differences Billiau particularly dramatic, the administration, blood producing cytokine between the affect components that takes several minutes from the interferon the in activity the volume of either control medium (Eagle minimal essential medium [MEM] with 5% fetal bovine serum [FBS]) or freshly collected blood from four different rabbits.

Numbers in parentheses denote percent of original interferon activity recovered after incubation.

Also be responsible for some variability. Further experiments will be needed to ascertain whether the pharmacokinetic behavior of fibroblast interferon is the same in rabbits and humans. The results of Billiau et al. (1) suggest that rabbit experiments provide a fairly accurate reflection of the pharmacokinetic behavior of fibroblast interferon in humans.

What might cause the less efficient penetration of fibroblast interferon into the blood stream after intramuscular injection? Our results virtually eliminate more rapid clearance from the circulation or faster inactivation in the blood stream as possible causes. It is much more likely that some of the intramuscularly injected fibroblast interferon binds firmly to the tissues at the injection site. It is known that fibroblast interferon has a much greater binding affinity for various surfaces than does leukocyte interferon, a property that may be due largely to the demonstrated hydrophobicity of the fibroblast interferon molecule. It is also possible that fibroblast interferon is inactivated at the intramuscular injection site by some other mechanism. Inactivators of interferon present in body fluids (5, 6) might play a role in this process. It is conceivable that either modification of the fibroblast interferon molecule per se or addition of substances that alter its binding properties would cause more efficient adsorption from the injection site and improve the blood levels produced on intramuscular administration.

In the meantime intravenous administration represents a possible alternative route of application for fibroblast interferon. Although bolus intravenous injection produces high concentrations of circulating interferon for a rather short period of time, such a regimen may be adequate. It is known that an antiviral state can be established after very brief contact with high doses of interferon and that a continuous presence of interferon is not required for persistence of the antiviral state (7, 19). For the treatment of herpes keratitis, less frequent topical administration of high-titered interferon in the eye proved far superior to more sustained treatment with lower doses (15). Some time ago Cantell and Pyhälä (3) concluded that “it is not known which route of administration is the most efficient for the systemic treatment of viral infections and malignancies.” The answer to this fundamental question is still not available for either fibroblast or leukocyte interferon.

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