Circulating Interferon in Rabbits after Administration of Human Interferon by Different Routes

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SUMMARY

Human leucocyte interferon was rapidly cleared from the circulation of rabbits receiving 3 million units intravenously, but the clearance rate was greatly decreased after 1 h. The early half-time was 13 min and the late half-time 73 min. No circulating interferon was detected beyond 6 h. Repeated injections did not affect the clearance rate.

Intramuscular injection of 3 million units of human or rabbit interferon maintained a relatively stable interferon level in the serum for 12 h. Higher doses raised the level and prolonged the persistence of circulating interferon. A single intramuscular injection of 30 million units of human interferon maintained a detectable interferon level in the serum for 48 h. Subcutaneous injections resulted in even longer persistence of measurable interferon in the blood.

No interferon was detected in the serum after oral administration of 6 million units of human interferon.

INTRODUCTION

Interferons are rapidly cleared from the blood after intravenous injection. The half-time of mouse (Baron et al. 1966; Finter, 1966; Subrahmanyan & Mims, 1966), rabbit (Ho & Postic, 1967; Bocci et al. 1968), and rat (Billiau, 1969) interferons in the sera of their homologous hosts are of the order of only a few minutes. However, multiple injections of mouse interferon have been reported to decrease the clearance rate (Nuwer, De Clercq & Merigan, 1970. There are a few brief reports on the detection of circulating interferon following injections by routes other than intravenous. Finter (1966) found a low level of interferon in the blood of mice 1 h after intramuscular injection of concentrated mouse interferon. Gresser et al. (1967) demonstrated a low but fairly constant level of circulating interferon up to 5 h after intraperitoneal injection of mouse interferon.

To our knowledge, comparable studies have not been made with human interferon. The availability of potent preparations of human leucocyte interferon (Cantell et al. 1968; Cantell, 1970) prompted us to start animal experiments to obtain some guide-lines for a suitable route of administration, dosage and timing of interferon in the systemic treatment of human diseases. The rabbit seemed an appropriate experimental animal for these studies, because human leucocyte interferon exerts antiviral activity in rabbit cells both in vitro (Levy-Koenig, Golgher & Paucker, 1970a) and in vivo (Pinto et al. 1970). Interferon levels in rabbit sera after administration of concentrated human interferon by various routes are presented below.
**METHODS**

**Preparation of human leucocyte interferon.** The interferon was prepared essentially as described previously (Cantell, 1970), with the following modifications: (1) The medium consisted of Eagle’s minimum essential medium supplemented with 5% (NH₄)₂SO₄-treated human serum, 3 mg/ml of Tricine (Calbiochem, Los Angeles, California) and 25 µg/ml of neomycin. The (NH₄)₂SO₄-treated serum was prepared as follows: pooled human serum was precipitated slowly at 35% saturation of ammonium sulphate at 0 to 4 °C. After 1 h the precipitate was sedimented by centrifuging at 16000 g for 60 min at −1 °C. The supernatant fluid was thoroughly dialysed against phosphate-buffered saline, pH 7·3, and the dialysed (NH₄)₂SO₄-treated serum was filtered through a Seitz EKS plate 14 cm in diam. (2) The leucocyte suspension was ‘primed’ by adding about 100 units of human leucocyte interferon 2 h before induction with 300 H.A.U. of Sendai virus/ml. (3) The incubation temperature was 37·5 °C.

The interferon was precipitated in the presence of 0·5 M-KSCN at pH 4·0, and the sediment dissolved in about ½-th vol. of 0·1 M-sodium acetate, pH 7·4 (K. H. Fantes, personal communication). The concentrated interferon was dialysed against 0·1 M-phosphate buffer at pH 7·3 and centrifuged at 25 000 g for 1 h. The supernatant fluids were used in the experiments described below. They contained 350 000 to 2 million interferon units/ml and had a specific activity in the order of 10 000 units/mg protein.

**Assay of human interferon.** Human leucocyte interferon was assayed by VSV plaque reduction in a line of human amnion cells (U cells) as described by Strander & Cantell (1966, 1967). All assay results are given in terms of the unit which has been assigned to the research standard preparation 67/87 (International Symposium on Standardization of Interferon and Interferon Inducers, London, 1969). All interferon titres are given per ml of rabbit serum. The human leucocyte interferons exhibited about the same activity in U cells and in primary rabbit kidney cells, while the titres in rabbit embryo fibroblasts were about one-third as high.

**Preparation of rabbit interferon.** Rabbits weighing about 3·5 kg were injected intravenously with 5 ml of Newcastle disease virus (NDV, Hertfordshire strain, kindly supplied by Bosko Postic) which had been propagated in the allantoic cavity of 11-day-old chick embryos and contained 256 H.A.U. per ml. Blood was collected 4 to 5 h later by cardiac puncture; the sera were dialysed against pH 2 for 6 days at 4 °C and then dialysed back to neutrality.

**Assay of rabbit interferon.** The rabbit serum interferons were assayed by essentially the same VSV plaque reduction method, but the primary fibroblasts used were derived by trypsinization from 18- to 20-day-old rabbit embryos. The titres are given in terms of the unit assigned to the research standard for rabbit interferon (International Symposium on Standardization of Interferon and Interferon Inducers, London, 1969). Different batches of rabbit serum interferon was assayed separately and the most potent ones were pooled to give a preparation containing 300 000 units/ml. It did not show any antiviral activity in U cells.

**Administration of interferon.** The weight of the rabbits used ranged between 3·3 and 3·6 kg. In most experiments the vol. of the interferon injections was 5 ml. The intravenous injections were given into the marginal ear vein, and the intramuscular injections into the gluteal muscles. Oral administration was performed through a plastic stomach tube which was slowly passed for a distance of 12 to 18 cm from the incisors.

**Blood samples.** Blood samples of about 5 ml were collected by incision of the marginal ear vein before and at intervals after the interferon injections. The serum was separated and
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Fig. 1. Serum interferon levels of three individual rabbits (○, △, ■) at different times after intravenous injection of 3 million units of human interferon.

Fig. 2. Serum interferon levels at different times after the first or fourth intravenous injection of 1.75 million units of human interferon. ○—○, △—△, interferon clearance in two rabbits after the first injection. ○—○, △—△, interferon clearance after the fourth injection. The interval between the repeated injections was (A) 0.5 h, (B) 2 h, (C) 24 h.
assayed for interferon activity. Normal rabbit sera reduced the VSV plaque counts at low dilutions. No such inhibitory activity was ever seen when normal sera were diluted 1:20 or more.

RESULTS

Intravenous injections

Fig. 1 shows that human interferon was rapidly cleared from the blood of rabbits during the first hour after an intravenous injection of 3 million units. During this period the half-time was of the order of 13 min. There was a marked tailing effect in the disappearance curves. Thus, during the period from 1 to 6 h after injection, the half-time of the circulating interferon was about 73 min.

The effect of repeated interferon injections on the clearance rate was studied in the experiments illustrated in Fig. 2. Two rabbits in each group (A, B, and C) were injected with 1.75 million units of human interferon either once or four times, at intervals of 0.5 h (A), 2 h (B) or 24 h (C). The Fig. indicates that the clearance rates obtained after the first and the fourth injection were essentially the same.

Intramuscular injections

Intramuscular injection of 3 million units of human interferon resulted in the appearance of readily detectable amounts of interferon in the blood (Fig. 3). The peak level was reached in about an hour and a fairly stable level of circulating interferon was maintained up to 12 h.

The following tests were done to ascertain that the antiviral activity found in the sera of the rabbits receiving intramuscular injections of human interferon was due to human interferon. Centrifuging sera at 10,000g for 4 h and dialysis against buffer of pH 2 for 6 days did not affect the activity. The antiviral activity could not be demonstrated in chick embryo fibroblasts. In accord with earlier findings (Desmyter, Rawls & Melnick, 1968; Levy-Koenig et al. 1970a), potent rabbit serum interferons failed to show any activity in U cells. Hence, the antiviral activity in the rabbit sera must have been due not to rabbit interferon but to human interferon.

Two rabbits were injected intramuscularly with 3 million units of human interferon on
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Fig. 4. Serum interferon levels in rabbits after intramuscular injection of 30, 10, 3, 1 or 0.3 million units of human interferon. ▲, ■, ○, geometric mean values of three rabbits, ●, △, individual rabbits.

Fig. 5. Serum interferon levels in rabbits after intramuscular injection of 10 (A) or 3 (B) million units of rabbit or human interferon, respectively. ●— ●, ▲— ▲, human interferon. ○— ○, △— △, rabbit interferon.

five successive days. The repeated injections did not affect the pattern of the circulating interferon in any significant way.

A potent human interferon preparation containing 2 million units/ml was used to study the effect of the interferon dose on the appearance and level of the circulating interferon. A rabbit received 15 ml of the preparation divided into three doses of 5 ml given in rapid succession. Other rabbits were injected with 5 ml of the same undiluted material or with 5 ml...
Fig. 6. Serum interferon levels in rabbits at different times after subcutaneous injection of 3 million units of human interferon. Two rabbits (●●●●, ▲▲▲▲) received an injection of 5 ml. The other two rabbits (■■■■, ○○○○) received the same total dose divided in five injections each one ml.

of the same preparation diluted 3, 10 or 30 times in phosphate-buffered saline. In all cases the maximum level of circulating interferon was reached within 1 h after the injection (Fig. 4). The interferon dose affected both the level in the blood and the time during which circulating interferon could be demonstrated, but the interferon levels were not directly proportional to the amount injected. An increase in dose from 0.3 million to 30 million units gave only about 20-fold increase in the level of circulating interferon. The highest dose given maintained a detectable interferon level in the blood for 48 h.

An experiment was made to compare the levels of circulating interferon obtained by intramuscular injections of rabbit and human interferons. Two rabbits were injected with 10 and 3 million units of rabbit interferon, respectively and the sera collected at intervals were assayed for rabbit interferon in primary rabbit embryo fibroblasts. Two other rabbits were injected with corresponding doses of human interferon and the sera were assayed for human interferon in U cells. The results obtained with the two interferons were similar, although the peak levels of human interferon were somewhat higher than those of rabbit interferon (Fig. 5).

Subcutaneous injections

Peak levels of circulating interferon were reached 3 to 6 h after subcutaneous injections of 3 million units of human interferon (Fig. 6). The titres in the blood then decreased slowly and the last traces of activity were detected 24 to 36 h after the injections. The shape of the curve was essentially the same, regardless of whether the total interferon dose was given as one injection or divided into five injections given at different sites.

Oral administration

A rabbit was fed with 5 ml of concentrated human interferon containing a total of 2.5 million units. Blood samples were taken before ingestion and 15 min, 1, 3, 6, 12 and 24 h afterwards. No interferon activity was detected in any of the samples. In another experiment the dose of interferon was 6 million units, but no interferon was found in any of the sera collected as above.
DISCUSSION

The clearance curves obtained for circulating human interferon after intravenous injection resemble those presented earlier for mouse, rabbit and rat interferons in their homologous hosts (Ho & Postic, 1967; Bocci et al. 1968; Billiau, 1969). Various explanations have been suggested for the tailing effect in the disappearance curves. The present findings on the ready passage of intramuscularly or subcutaneously injected interferon into the blood is in line with the idea that equilibrium between plasma interferon and extravascular interferon plays a role in the phenomenon.

In a recent study (Nuwer et al. 1971) the clearance rate of mouse interferon seemed to be slower after multiple intravenous injections of interferon. In spite of several efforts, no such effect could be seen in the present study. It must be pointed out that the interferon doses used by us were clearly higher than those used by Nuwer et al. 1971 (in relation to the weight of the animals). More studies with different doses and with other interferons and hosts are needed to throw additional light to this point, which has important implications.

Schafer et al. (1972) have recently demonstrated that mouse and rabbit interferons administered orally can reach the circulation of neonatal mice. However, no interferon could be detected in the sera of adult mice fed with interferon. The interferon doses administered per os in the rabbit experiments above were at least as high (on a weight basis) as those given by Schafer et al. (1972). Not surprisingly, the adult animals failed to show any circulating interferon in our per os experiments also.

The main finding of the present study is the demonstration of long-lasting interferon activity in the blood of rabbits receiving intramuscular or subcutaneous injections of exogenous human interferon. It is likely that the interferon plateau is maintained by continuous entry of interferon molecules from the injection site into the blood and clearance of interferon from the blood at about the same rate. The more interferon is injected, the longer the local depot supplies interferon. At high interferon doses the interferon levels attained in serum were not proportional to the number of units injected. It remains to be shown whether this is due to slower entry of interferon into the blood or to faster clearance of the circulating interferon.

How much of the intramuscularly injected interferon enters the circulation? Three million units diluted in the total plasma vol. of a rabbit would give about 20000 units/ml. With a half-time of 73 min, the interferon level in the serum would decrease to 200 units/ml in about 8 h. In fact, intramuscular injection of 3 million units maintained the interferon level in serum at 200 units for 12 h at least. This implies that practically all the intramuscularly injected interferon entered the blood and/or that this circulating interferon had a substantially longer half-time than intravenously injected interferon.

It may be argued that the significance of the present findings is weakened by the fact that the pharmacokinetics of human interferon was studied in a heterologous host. It is true that the rabbit is a heterologous host for human interferon: it is able to make antibodies against human interferon (Levy-Koenig, Mundy & Paucker, 1970b) and rabbit interferons fail to exert antiviral action in human cells (Desmyter et al. 1968). However, (1) rabbit interferon injected intramuscularly into rabbits gave a response resembling that of human interferon and (2) results essentially similar to those reported here were obtained when the kinetics of the clearance of human leucocyte interferon was analysed in gibbons (to be published). These lines of evidence suggest that the ‘heterologousness’ of the rabbit model did not essentially affect the results obtained and that similar findings may be expected when it is possible to study the pharmacokinetics of human leucocyte interferon in man. Accordingly,
it could be predicted that intramuscular injections of 10 to 20 million units of human leuco-
cyte interferon twice daily would continuously maintain 100 units of interferon/ml of
plasma in man.

REFERENCES


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