Circulating Interferon in Rabbits and Monkeys after Administration of Human Gamma Interferon by Different Routes

By K. CANTELL,* S. HIRVONEN, L. PYHÄLÄ, A. DE REUS 1 and H. SCHELLEKENS 1

National Public Health Institute, SF 00280, Helsinki 28, Finland and 1 Primate Center TNO, 2280 HV Rijswijk, The Netherlands

(Accepted 9 May 1983)

SUMMARY

Rabbits and rhesus monkeys were injected with $3 \times 10^5$ units of human gamma interferon (IFN) prepared in human leukocyte suspensions. Circulating IFN was detected up to 4 h after intravenous administration. Intramuscular injection maintained a relatively stable serum IFN level of about 50 units/ml for 7 to 9 h. The results in both species were similar. Little or no circulating IFN was detected after subcutaneous injection of $3 \times 10^5$ units, but $1.5 \times 10^6$ units maintained about 50 units per ml of serum for 30 h. Pharmacokinetically, human gamma IFN resembled human alpha interferons rather than human beta IFN.

Intramuscular (i.m.) or subcutaneous (s.c.) injections of different human alpha interferons (IFNs) give a long-lasting plateau of circulating IFN in man and several other mammalian species (Cantell et al., 1974; Cantell & Pyhälä, 1976; Priestman, 1980; Gutterman et al., 1982a). On the other hand, low or non-detectable levels of circulating IFN are obtained after administration of human IFN-β by the same routes (Edy et al., 1978; Quesada et al., 1982). Little information is available about the pharmacokinetics of human IFN-γ. Stewart & Wiranowska-Stewart (1980) reported that human IFN-γ derived from the leukocytes gave roughly similar blood levels as human IFN-α in mice after i.m. injection. However, recent experiments with natural human IFN-γ in man (Gutterman et al., 1982b) and with Escherichia coli-derived human IFN-γ in monkeys (Weck et al., 1982) indicate that very low or non-detectable levels of interferon are attained in the blood after i.m. injection. We now report pharmacokinetic studies in rabbits and monkeys with human IFN-γ prepared in leukocyte suspensions.

Leukocyte buffy coats from 100 to 150 blood donors were pooled and the leukocytes were purified by NH₄Cl treatment as described previously (Cantell et al., 1981). The purified leukocytes were suspended in Iscove's modified Dulbecco's medium supplemented with 6 mg of human gamma serum (Cantell et al., 1981) and 25 μg neomycin/ml to give a concentration of $3 \times 10^6$ cells/ml. Lens culinaris lectin (Rönnblom et al., 1982) was added to a concentration of 10 μg/ml. The lectin was prepared by Dr B. Ersson, Separation Center, University of Uppsala, Uppsala, Sweden. Aliquots of 200 ml of the cell suspension were transferred into 1-litre polycarbonate bottles which were incubated in a roller (1 rev/min) for 3 days at 36-5 °C. The cells were removed by centrifugation at 1300 g for 40 min; the supernatants were the crude IFN-γ and they contained about 3000 units/ml.

Polyethylene glycol (PEG) 4000 (Fluka, Buchs, Switzerland) was added to the crude interferon to a concentration of 25%. The suspension was stirred on a magnetic stirrer for 2 h and stored overnight at 4 °C. The precipitate was sedimented by centrifugation at 1300 g for 30 min at 4 °C. It was dissolved in 0.2 vol. phosphate-buffered saline (PBS) containing 20% (NH₄)₂SO₄. The dissolution was done on an orbital shaker (Bellco Glass, Vineland, N.J., U.S.A.) for 30 min and on a magnetic stirrer for 30 min at 4 °C. The NH₄SO₄ concentration was raised to 31% by slowly dropping 44% (NH₄)₂SO₄ during 3 h at 4 °C. The increase in the salt...
concentration separates PEG from the liquid phase. The separation took place in a separation funnel during 2 days at 4 °C. The lower liquid phase was collected and solid \((\text{NH}_4)_2\text{SO}_4\) was added to a final concentration of 50% and the suspension was kept on a magnetic stirrer for 2 h at 4 °C. The precipitate was sedimented by centrifugation at 13000 g for 60 min. It was dissolved in 1/40 vol. (in relation to crude IFN) of 0.1 m-phosphate buffer pH 8.0 on the magnetic stirrer for 3 h. The suspension was centrifuged at 13000 g for 2 h, dialysed against PBS and centrifuged at 31000 g for 1 h. The supernatant contained 30000 units of IFN-\(\gamma\) (mean of 25 assays) and 73 mg protein/ml. The specific activity was \(4 \times 10^2\) units/mg protein.

The gamma interferons were routinely assayed by vesicular stomatitis virus (VSV) plaque reduction in human HEp2 cells. A crude preparation of human IFN-\(\gamma\) containing 3000 units/ml served as a laboratory standard. All titres are expressed in terms of this laboratory standard.

The concentrated human IFN-\(\gamma\) had about the same activity in human HEp2 and in two monkey cell lines (GMK and Vero). It had no detectable activity (<60 units/ml) in bovine NBL-1 and rabbit RK3 cells. Its activity was not neutralized by a sheep antiserum which contained 450000 neutralizing units against human leukocyte IFN-\(\alpha\) and 3000 neutralizing units against human IFN-\(\beta\) (Mogensen et al., 1975). The treatment of the concentrated human IFN-\(\gamma\) at pH 2 for 24 h decreased its titre by more than 95%.

Outbred rabbits (weight 2.9 to 3.3 kg) of a local albino strain and rhesus monkeys (2.0 to 2.8 kg) were used. The animals received 10 ml of the concentrated human IFN-\(\gamma\) as an intravenous injection. The i.m. and s.c. injections were given into ten different sites, 1 ml to each site. A rabbit was injected s.c. with 5 ml IFN-\(\gamma\) into 10 different sites.

Fig. 1 shows the clearance of IFN-\(\gamma\) from blood after intravenous injection of \(3 \times 10^5\) units. The results in rabbits and monkeys were similar. The early clearance rate was greatly decreased after 1 to 2 h. Circulating IFN was detected up to 4 h.

Intramuscular injection of \(3 \times 10^5\) units of human IFN-\(\gamma\) resulted in detectable amounts of interferon in the blood and a fairly stable level of circulating IFN was maintained up to 9 h in rabbits and 7 h in monkeys (Fig. 2). Several rabbit serum samples collected during the plateau phase were assayed in bovine and rabbit cells. No interferon activity was detected. Hence, the antiviral activity in the rabbit sera must have been due not to rabbit IFN but to the i.m. injected human IFN-\(\gamma\).

Subcutaneous injection of \(3 \times 10^5\) units of human IFN-\(\gamma\) did not yield readily detectable levels of circulating interferon. However, a slight reduction of VSV plaque number and size in the IFN assay suggested that the serum samples contained a low level of IFN activity. Therefore, a rabbit was injected with a fivefold higher dose than in the previous experiments. Circulating IFN was now detected and a fairly stable level of about 50 units/ml was maintained up to 30 h.

The IFN injections did not show any overt toxicity to the rabbits or the monkeys.
The present pharmacokinetic results after i.m. injection of human gamma interferon are similar to those obtained earlier with human alpha interferons. The IFN-γ preparation used in the present experiments was impure. However, studies with alpha interferons indicate that the degree of purity of the preparation does not essentially affect the IFN level in the blood after i.m. injections.

In earlier rabbit experiments, human IFN-α gave similar levels of circulating IFN after i.m. and s.c. routes of administration (Cantell & Pyhälä, 1976). In the present study, human IFN-γ gave lower blood levels after s.c. than after i.m. injection. However, the dose of IFN-γ was only about one-tenth of the dose of IFN-α.

The i.m. injection of human IFN-γ derived from E. coli gave very low or non-detectable levels of circulating IFN in monkeys (Week et al., 1982). Our natural IFN-γ gave readily detectable IFN levels in the blood after i.m. administration. The natural IFN-γ is a glycoprotein, but IFN-γ from E. coli is non-glycosylated. It seems likely that the glycosylation of the IFN-γ plays a role in its pharmacokinetics. It is more difficult to reconcile our findings with those of Gutterman et al. (1982b) who detected no IFN in the blood of patients after i.m. injection up to 10 million units of natural human IFN-γ. Comparative pharmacokinetic studies with human gamma interferons from different sources are needed.

REFERENCES


Short communication


(Received 11 February 1983)