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Interferon: Pharmacokinetics and Toxicity [and Discussion]

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Interferon: pharmacokinetics and toxicity

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Interferons disappear rapidly from the serum of animals and man, and the kidney may be the major site of interferon destruction. The relevance of serum levels of interferons to their therapeutic activity has not been clearly established, particularly as the stimulation of host defence mechanisms by interferons may be important. Relatively low serum levels of antiviral activity are seen after intramuscular injections of fibroblast interferon compared with those after the same dose of leucocyte interferon.

Injections of very pure leucocyte and lymphoblastoid interferons from several sources cause fever, headaches, malaise and myalgia associated with a corticosteroid response and probably with inflammatory prostaglandin synthesis. These reactions become less with repeated dosing but very large doses of lymphoblastoid interferon have been shown to cause liver damage and serious metabolic disturbances. Treatment with moderate doses of exogenous interferons may occasionally be associated with the development of neutralizing antibodies.

Pharmacokinetics

Introduction

In recent years, it has been discovered that human interferons are an extensive family of different proteins or glycoproteins. Clear differences in the pharmacokinetic behaviour of leucocyte and fibroblast interferons have been observed but the clinical relevance of these differences is not yet clear. Very large doses of lymphoblastoid and cloned leucocyte interferons have been given to cancer patients, but such doses of fibroblast interferon have not yet been tested and no patient or volunteer has yet been given exogenous IFN-γ.

Interferons have been administered to man by virtually every available route. Pharmacokinetic studies have, in general, been performed as adjuncts to the clinical testing of interferons because, until recently, material has been scarce. Because the relation of serum interferon levels to antiviral activity in tissues *in vivo* appears to be tenuous, ascending or descending schedules of interferon have been tested to determine minimum effective regimens. This is illustrated by a series of studies of Merigan *et al.* (1978) who demonstrated that leucocyte interferon at 5.1×10^5 u kg^{-1} d^{-1} showed activity in the treatment of herpes zoster in cancer patients, whereas 1.7×10^5 u kg^{-1} d^{-1} was not active. (Units are by reference to MRC 69/19 standard.) The decision as to what dose to give in clinical trials has been governed almost entirely by the availability of material. In an attempt to find an effective lower dose scheme to conserve material, Merigan *et al.* (1981) shortened the course of treatment for herpes zoster, and found the effect of interferon to be less impressive. Thus the minimum dose level was determined by clinical activity rather than by reference to blood levels. It may be possible to assess *in vitro* the antiviral state induced by interferon *in vivo*; for example, Greenberg *et al.* (1977) have demonstrated the dose and time requirements for topical interferon to render nasal mucosal explants resistant to infection with vesicular stomatitis virus (see below).

Problems may occur when measuring low titres of interferon in serum or urine. Serum may interfere with assays either by mimicking or antagonizing the action of interferon. When Ennis *et al.* (1981) wished to measure plasma interferon after lymphocyte separation, they found that the heparin interfered with the interferon assay to such an extent that no meaningful results could be obtained. Furthermore, incubation of exogenous interferons with serum, urine, bile (Cesario *et al.* 1973; Cesario & Tilles 1973; Rossman & Vilček 1970) or nasal secretions (Harmon *et al.* 1976) caused various degrees of inactivation or blocking. We have observed that human serum enhances Semliki Forest virus RNA synthesis in human fibroblasts, clearly detracting from the value of this as a method of measuring low titres of interferon in serum (Scott *et al.* 1982*b*, *c*). In a recent study comparing the effect of two preparations of leucocyte interferon in volunteers, lower titres of antiviral activity were seen in the serum after the administration of highly purified interferon although the doses given were similar (Scott *et al.* 1981). However, when these sera were examined by immunoradiometric assay similar titres were seen (Walker *et al.* 1982) and the reasons for this discrepancy between immunoactivity and biological activity have not been clarified.

Routes of administration

1. *Gastrointestinal tract*

There is no evidence that interferons are or would be bioavailable if taken orally. Interferons are likely to be digested by the proteolytic enzymes in peptic and pancreatic juices. However, consistent with the observation that immunoglobulins may be absorbed from the immature gut, Schafer *et al.* (1972) have shown the absorption of mouse and rabbit interferons given orally to mice 1 week old. Interferon suppositories have been given by Yugoslavian workers (Vlatković *et al.* 1981) in controlled trials of acute hepatitis B, but the results do not indicate that interferon was absorbed.

2. *Topical administration*

(*a*) *Eye drops*. Leucocyte interferons given as eye drops have activity in the treatment of established herpes simplex keratoconjunctivitis at concentrations of 3×10^6 u ml^{-1} (Sundmacher *et al.* 1976*b*) and $11–30 \times 10^6$ u ml^{-1} (Jones *et al.* 1976) but not at 6.25×10^4 u ml^{-1} (Sundmacher *et al.* 1976*a*). Fibroblast and leucocyte interferons were compared in clinical herpes keratitis at a concentration of 10^6 u ml^{-1} (Sundmacher *et al.* 1978); no differences in healing rate were observed but no control group was studied so it is not possible to say whether this concentration of interferon had any effect. However, Neumann-Haefelin *et al.* (1977) compared human leucocyte and fibroblast interferons at 10^6 u ml^{-1} against placebo in experimental herpes in monkey eyes and clearly demonstrated activity. The efficacy of human leucocyte interferon against herpes simplex in rabbit eyes was found to be related to its concentration and not to the frequency of application (McGill *et al.* 1976). More recent work (Scott *et al.* 1982*a*) using a similar model showed that the minimal effective concentration of both fibroblast and leucocyte interferons given after herpes inoculation was $10^5–10^{5.3}$ u ml^{-1}. However, concentrations of either interferon of up to 10^7 u ml^{-1} given only before virus inoculation did not consistently inhibit subsequent ulcer formation. Kaufman *et al.* (1976) failed to show that regular treatment with leucocyte interferon at 6.4×10^4 u ml^{-1} could prevent recurrences of herpes keratitis. Although this could have been because the concentration was rather low, the animal experiments suggested that interferon may not penetrate the intact cornea. Rabbit aqueous humour

was assayed after eye drops of 6×10^5 units of fibroblast and leucocyte interferons. The activity reached a peak of 200 u ml^{-1} 3 h after dosing if the cornea had been traumatized, but only 32 u ml^{-1} if it had not. Blood interferon levels were not measured but it was possible to show that if interferon was absorbed it did not affect the herpes lesions in control eyes. These observations have clear implications in the use of regular interferon eyedrops to prevent recurrent herpes keratitis. Higher concentrations of interferon may be required for prophylaxis than for treatment.

(*b*) *Intranasal administration*. Merigan *et al.* (1973) showed that 14×10^6 units of leucocyte interferon given by repeated sprays over a period of 4 days could inhibit experimental rhinovirus infection in volunteers, when virus was given on the second day. A similar schedule of treatment continued for 1 day after influenza virus challenge delayed the symptoms slightly but did not prevent infection. Schedules of lower concentration interferon had no effect (Tyrrell & Reed 1973; Scientific Committee on Interferon 1965), and we were unable to prevent colds by using fibroblast interferon (total not less than 6×10^5 units) given by nose drops (Scott *et al.* 1978).

Greenberg *et al.* (1977) considered the reasons why such large amounts of interferon appeared to be necessary to prevent colds. After all, low titres of interferon are highly active in sensitive tissue culture. Three possible reasons were suggested: the interferon may be inactivated in the nose or it may fail to penetrate to the epithelial cells which are susceptible to infection, or these epithelial cells may be relatively resistant to the action of interferon. Nasal secretions did not significantly inactivate leucocyte interferon (although they did inactivate fibroblast interferon) (Harmon *et al.* 1976). If interferon was placed carefully on the inferior turbinate in chimpanzees and volunteers, interferon activity recovered by currettage of the epithelium was shown to decrease very rapidly with time (Johnson *et al.* 1976). Whereas 300–1000 units were recovered immediately, not more than 100 units could be demonstrated 5 min or more after the application of 5×10^4 units; this suggested that there was extremely rapid clearance of interferon from these sites within a few minutes. Furthermore, human nasal epithelial explants require exposure to 1000 units of leucocyte interferon *in vitro* for 4 h to protect these cells against vesicular stomatitis virus (VSV) (Harmon *et al.* 1977). When 8×10^4 units was placed locally on the inferior turbinate by repeated drops for one hour, no protection was seen against VSV in mucosal explants taken 3 h later (Greenberg *et al.* 1978). However, when the interferon was applied by a small saturated cotton pledget for 1 h, a significant reduction of VSV yield was seen even when only 2×10^4 units were applied. This antiviral activity was dose dependent, and was seen to fade in samples taken at 18 and 24 h after the start of application (Greenberg *et al.* 1978). More recently, protection of volunteers against rhinovirus has been achieved by using 3×10^6 units given in nasal mucosal pledgets or by aerosol generated by a DeVilbis nebulizer (Greenberg & Harmon 1981; Greenberg *et al.* 1982).

We have examined the recovery of interferon after intranasal sprays with high titre NK2-purified interferon (5×10^6 u ml^{-1}) (Scott *et al.* 1982*c*). After a single intranasal spray per nostril (2.5×10^6 units) by Risdon gun, the half-life of recoverable antiviral activity was 20 min in the first hour. In a rhinovirus protection study, the interferon recovered 2 h after completing a group of three sprays given over 40 min was that expected from a half-life of 20 min. Aoki & Crawley (1976) demonstrated the immediate half-life of radioactive albumin in the nose to be about 15 min. This suggests that a single dose of interferon (2.5×10^6 units) would result in undetectable levels of interferon $6\frac{1}{2}$ h after a dose. However, in rhinovirus protection experi-

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ments 10–100 u ml^{-1} of interferon were still found 24 h after inoculation in about half the volunteers, and in one volunteer 48 h afterwards. Merigan *et al.* (1973) also observed the occasional persistance of interferon in the nose. Whether interferon is absorbed to the mucosa and then released or is simply dried in the nasal vestibule remains a matter for conjecture. Although Aoki & Crawley (1976) showed that nose drops (given with patient reclining and neck extended) were superior to Risdon gun spray in terms of the distribution of radioactivity above the hard palate, the Risdon gun has continued in use and successful protection against rhinovirus infection has now been demonstrated with crude and NK2-purified α leucocyte interferons given by repeated sprays (Scott *et al.* 1982*c*).

For antiviral protection in the nose, therefore, it appears that prolonged contact of interferon with the mucosa is necessary. Antihistamines may slow down the clearance of interferon from the nasal cavity and enhance its activity (Greenberg *et al.* 1982). The minimal and optimal dose schedules have not yet been determined, but the antiviral state induced by a single effective application with a pledget has begun to fade by 18 h and is not detectable at 24 h. The success of the early rhinovirus trial (Merigan *et al.* 1973) was probably achieved because of repeated application of what would now be thought a rather low titre preparation (6×10^5 u ml^{-1}). Further trials are needed to determine whether the frequency of dosing can be reduced if higher interferon concentrations are used.

(*c*) *Topical administration* (*skin and mucous membranes*). No workers have demonstrated absorption of interferon through skin or mucous membranes. However, there are indications from therapeutic activity that leucocyte interferon may be absorbed. For example, an ointment containing about 4000 units leucocyte interferon per gram was shown in a double-blind trial to cure vulval warts (Ikić *et al.* 1975*a*). A similar preparation caused resolution of body warts, particularly if the skin was mildly traumatized, but this study was not properly controlled (Borzov *et al.* 1971). In a placebo-controlled (but not blind) trial, freeze-dried interferon was applied directly to lesions of primary herpes stomatitis in children (Ikić *et al.* 1975*b*), and these workers have also used interferon in the form of a pessary to apply treatment directly to cervical neoplasms (Ikić *et al.* 1981*a*). Although in both of these studies beneficial effects were claimed, there is no proof that interferon was absorbed.

3. *Parenteral administration*

(*a*) *Intradermal or intralesional routes*. Interferons have been injected locally into warts (Borzov *et al.* 1971; Scott & Csonka 1979; Ho *et al.* 1981) and into malignant melanomas and cutaneous metastases (Ikić *et al.* 1981*b*). Intradermal injections have been used to assess the inflammatory effects of interferons (DeSomer *et al.* 1977; Scott *et al.* 1977*a*, 1980*b*, 1981; Billiau *et al.* 1979; Carter *et al.* 1979) and to prevent experimental vaccinia infections (Scientific Committee on Interferon 1962; Scott *et al.* 1978). Interferon levels in the blood after intradermal injections have not been reported but generalized toxic reactions (see below) have been seen after both fibroblast (Scott, unpublished observations) and leucocyte interferons (Scott *et al.* 1981).

(*b*) *Intravenous route*. Following fears about the safety of intravenous bolus doses of partly purified leucocyte interferon in man (Strander *et al.* 1973; Emödi *et al.* 1975*a*), virtually all the important clinical studies have employed the intramuscular route of administration. However, early studies showed that high levels of interferon in serum could be achieved immediately after intravenous (i.v.) injection, with rapid clearance of the antiviral activity from the circula-

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tion. For example, after i.v. injection of 4.5×10^5 units of leucocyte interferon, the serum level was 200 u ml^{-1} immediately, and fell to 65 u ml^{-1} after 15 min (Strander *et al.* 1973). After 3×10^7 units i.v. on two occasions in one patient, serum concentrations of 7×10^3 u ml^{-1} were found immediately, and the half-life of serum antiviral activity in the first hour was 15 min, rising to 90 min between 1 and 4 h after injections (Emödi *et al.* 1975*a*). Interferon was not detectable in the serum 6 h after injection, but after continuous infusion of the same total dose over 8 h, a peak of 510 u m^{-1} was achieved by the end of infusion and interferon was still detectable 16 but not 28 h later.

Jordan *et al.* (1974) gave sustained i.v. infusions of up to 8×10^7 units over 12 h. The peak serum concentrations ranged from 240 to 1500 u ml^{-1} achieved 4–16 h after the start of the infusion. At the end of the infusion, the serum half-life was 2–3 h. Levels over 100 u ml^{-1} in serum were achieved more rapidly and persisted longer after a single intramuscular injection of the same dose, but the areas under the serum level-time curves were similar for both routes of administration.

A. Rohatiner & A. Lister (personal communication 1982) have administered up to 2×10^8 units of lymphoblastoid interferon per square metre of body surface area to adult patients with leukaemia. Continuous infusion over several hours was employed and the results of these kinetic studies are awaited with interest.

(*c*) *Intramuscular and subcutaneous routes.* Despite the observation that after subcutaneous injections in rabbits (Cantell & Pyhälä 1973) and in man (Emödi *et al.* 1975*a*), the blood levels peaked later and persisted for longer than after intramuscular (i.m.) injections, the former route has not been widely adopted for clinical trials. This may be because subcutaneous injections are sometimes associated with local inflammatory reactions (Kingham *et al.* 1978).

After i.m. injections of about 2.5×10^6 units of leucocyte interferon in patients with Hodgkin's disease, peak serum levels of 60 u ml^{-1} were achieved by 2–4 h and antiviral activity was still detectable 24 h after injection (Cantell *et al.* 1974). Jordan *et al.* (1974) found similar peak levels after 6×10^6 units i.m. but noted that they occurred later (10–14 h). With higher doses, proportionately higher levels were seen, but the peak level occurred at rather variable times from 4 to 12 h after injections. The maximum titres of interferons reported in the literature are shown against the total i.m. dose given in figure 1. Approximations are made where the dose administered was proportional to body weight (assuming 70 kg) or body surface area (assuming 1.7 m^2) (Cantell *et al.* 1974; Jordan *et al.* 1974; Emödi *et al.* 1975*a*, *b*; Greenberg *et al.* 1978; Merigan *et al.* 1978; Cheeseman *et al.* 1979; Pazin *et al.* 1979; Scott *et al.* 1981). At 24 h after i.m. injection, interferon may still be detectable but injections need to be repeated at 12 h intervals to maintain persistent high serum antiviral activity (Merigan *et al.* 1978).

Although three intramuscular injections of 8×10^7 units at 24 h intervals resulted in progressively higher peak levels, the rate of clearance was the same as after one dose (Jordan *et al.* 1974). At the highest dose schedule used by Merigan *et al.* (1978), trough levels before repeated injections were higher than expected from results with lower doses, also suggesting that some accumulation may occur with repeated dosing.

We have examined serum interferon levels in volunteers after i.m. injections of partly purified (PIF), monoclonal antibody (NK2-) purified and cloned α2 interferons (see Scott *et al.* 1981). Although Cantell & Pyhälä (1976) showed that a tenfold purified human leucocyte interferon preparation could enter the bloodstream of rabbits more rapidly than a crude preparation, no clear differences of this nature were seen in man. Serum levels of immuno-

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logically active interferon after NK2–IFN and PIF were the same after the same antiviral and immunological dose, but the serum antiviral activity after NK2–IFN was less than half that of PIF (Scott *et al.* 1981; Walker *et al.* 1982). Volunteers were given single intramuscular doses of cloned IFN–α2 (Schering-Plough) (G. Scott, unpublished). After an i.m. dose of 3×10^6 units, mean serum levels of 30 u ml^{-1} were achieved but not until 8 h after injection. This interesting difference in pharmacokinetics from NK2–IFN (which peaked at 2 h) may reflect slight differences in the molecular structures of these interferon preparations.

Gutterman *et al.* (1982) have examined the effect of cloned IFLrA, which is very similar to IFN-α2, in cancer patients. Where the IFLrA was compared with natural leucocyte interferon, very similar kinetics were seen at 3×10^6 u ml^{-1}, but at 9×10^6 u ml^{-1} significantly higher peak serum titres were seen after the natural leucocyte interferon.

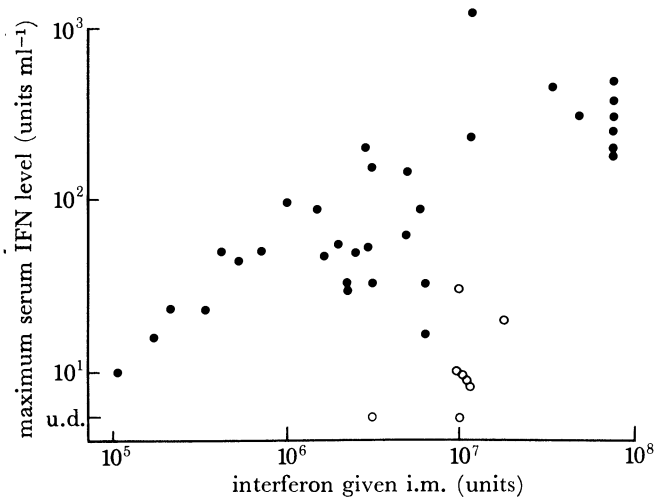

FIGURE 1. Relation between the dose of leucocyte (●) and fibroblast (○) interferons given and the peak serum levels for individuals or groups, abstracted from the literature (see text). U.d., undetectable.

After i.m. doses of 3×10^6 units in renal transplant recipients, serum levels ranged from 17 to 200 u ml^{-1} at 4–12 h and from 15 to 20 u ml^{-1} at 18–24 h (Cheeseman *et al.* 1979). Details of the kinetics were not given so it is not possible to tell whether renal disease affected distribution. Similarly, in chronic active hepatitis (Greenberg *et al.* 1978), no evidence for differences in kinetics was shown.

In one study performed with Searle fibroblast interferon (Kingham *et al.* 1978), very low levels were seen in blood samples taken frequently on three separate treatment days from two patients with chronic active hepatitis. Similar results were seen by Edy *et al.* (1978), and recent papers regarding the clinical use of fibroblast interferon have been notably lacking in pharmacokinetic data (Carter & Horoszewicz 1979; Billiau *et al.* 1979).

After i.v. injections of human leucocyte and fibroblast interferons in rabbits, high levels of interferon in serum were achieved, rapidly falling to low levels by 8 h (Edy *et al.* 1976; Billiau *et al.* 1979; Vilček *et al.* 1980). Human interferons are cleared as rapidly from the circulation of rabbits as in man. Edy *et al.* (1976) could not demonstrate a difference in serum interferon levels after i.m. injections of crude leucocyte and fibroblast interferon in rabbits but, after the observations of low titres of fibroblast interferon in man, the model was re-examined (Billiau *et al.* 1979; Vilček *et al.* 1980). These studies confirmed that lower levels of interferon in serum

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were seen after the injection of fibroblast than of leucocyte interferon in rabbits, and similar observations were made after intraperitoneal injections in mice (Billiau *et al.* 1981). However, in these mice, levels of antiviral activity in spleen and lung homogenates were similar. Differences between the behaviour of leucocyte and fibroblast interferons have not been fully explained. Since the kinetics after intravenous injections are similar, fibroblast interferon may not be released as freely from intramuscular injection sites; differences in molecular structure may account for increased binding (Edy *et al.* 1976), but in addition fibroblast interferon is readily inactivated by crude muscle extracts (Stewart & Wiranowska-Stewart 1979). It is not known whether lower levels of fibroblast interferon in serum are of clinical significance. In some diseases fibroblast interferon may be less active than leucocyte interferon (Weimar *et al.* 1979), although no significant differences in activity have been shown in rabbit, monkey or human eyes infected with herpes simplex virus (Sundmacher *et al.* 1978; Neumann-Haefelin *et al.* 1977; Scott *et al.* 1982*a*), or in monkeys infected with vaccinia when the interferons were given intradermally (Scott *et al.* 1977*b*) or intramuscularly (Weimar *et al.* 1980).

(*d*) *Intrathecal route*. Leucocyte interferon was given intrathecally to a child with disseminated herpes simplex virus infection (DeClercq *et al.* 1975): 12–24 h after a dose of 6×10^5 units, between 1.3×10^4 and 5×10^4 u ml^{-1} was still present in the cerebrospinal fluid (c.s.f.). Jacobs *et al.* (1981) gave intrathecal fibroblast interferon three times a week to patients with multiple sclerosis. Occasional antiviral activity was detectable at very low titres in c.s.f. samples taken at the time of subsequent treatments in five out of ten patients.

Hilfenhaus *et al.* (1977) and Habif *et al.* (1975) gave intrathecal leucocyte interferon to monkeys. Peak levels of interferon in c.s.f. 1 h after injection were related to the dose given (2×10^3 u ml^{-1} after 10^5 units, 2×10^5 u ml^{-1} after 10^6 units and 10^6 u ml^{-1} after 10^7 units), and interferon was still detectable in the c.s.f. 48 h after injection. Antiviral activity was found in the pial tissue and superficial layers of the cortex but not in the deeper layers of the cortex in monkeys after intrathecal administration (Billiau *et al.* 1981).

Fate and metabolism of interferons

After intravenous injection, interferon activity in the serum at first falls rapidly with a half-life of several minutes and later of a few hours. After an infusion of leucocyte interferon over 12–24 h in rabbits and man, serum levels build up progressively and, when the infusion is stopped, they decay more slowly than after a bolus intravenous injection, suggesting that equilibration in extravascular pools probably occurs. There is some evidence (listed above) that suggests that interferon may accumulate in man but not in rabbits (Cantell & Pyhälä 1976). Interferons may be found in the urine but inconsistently and in low titre. There is no correlation between peak levels of serum interferon and the likelihood of finding it in the urine (Emödi *et al.* 1975*b*). Moderate urinary interferon levels (28–50 u ml^{-1} with serum levels of up to 440 u ml^{-1}) were seen in one patient with infiltrative renal disease (Jordan *et al.* 1974).

Interferons may enter the serum from c.s.f. When 10^7 units of leucocyte interferon were injected intrathecally in monkeys, prolonged levels of up to 600 u ml^{-1} were seen in the serum (Habif *et al.* 1975). One hour after the seventh intrathecal dose of 6×10^5 units of leucocyte interferon in a child with herpes encephalitis, a titre of 125 u ml^{-1} was found in the serum (DeClercq *et al.* 1975). On the other hand, high levels of interferon in the serum have not been shown to give rise to detectable levels in the c.s.f. (Emödi *et al.* 1975*a*; Jordan *et al.* 1974) except

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in monkeys (Habif *et al.* 1975). Interferon may be found in saliva in the same concentrations as in serum (Merigan *et al.* 1978), but interferon did not penetrate into respiratory secretions from the serum in mice (Finter 1968).

Can we assess the tissue penetration of interferons by their antiviral activity? Increasing intramuscular dosage schedules of leucocyte interferon first did not, then did show activity against herpes zoster (Merigan *et al.* 1978). Even at the lower dose schedules, levels of antiviral activity in serum were similar to those seen in natural infections. However, interferon needs to be where virus is replicating in the skin, and penetration to these sites was clearly not achieved. (A caveat here is that Schellekens *et al.* (1981) have suggested that i.m. interferons do not protect rhesus monkeys against intradermal vaccinia by direct antiviral activity but rather by the enhancement of host defence.)

There are several possibilities concerning the fate of endogenous or exogenous interferons, which have recently been reviewed by Bocci (1981). Interferons act by temporarily binding to cell-surface receptors and inducing rapid intracellular metabolic changes (Dianzani & Baron 1975; Tovey *et al.* 1981). It is not known whether this interferon is released unchanged, or altered so that it is inactive or more susceptible to degradation, or internalized and destroyed by activated cells. Early work suggested that interferon is not consumed during its antiviral activity (Buckler *et al.* 1966), but to prove this point beyond question probably requires a radiolabelled pure interferon preparation. Whether or not the interferon is inactivated before or after inducing an antiviral state is of some importance in clinical practice.

Interferon could be inactivated directly in the circulation, urine or bile as suggested by Cesario *et al.* (1973*a*, *b*). However, the main organs of protein catabolism are liver and kidney. Bocci *et al.* (1977*a*, *b*) showed that the modification of rabbit serum interferon (induced by i.v. injection of Newcastle Disease virus) by the removal of sialic acid speeded its clearance from blood and increased its uptake in perfused rabbit liver. More recently human leucocyte and fibroblast interferons treated with sialidase were perfused through isolated rabbit liver. Whereas the former was significantly cleared, the latter was unaffected by this treatment, suggesting that human leucocyte interferons are probably not glycosylated and supporting the observations of Mögensen *et al.* (1974). We may surmise that fibroblast but not leucocyte interferon could be inactivated in the human liver (particularly if the fibroblast interferon molecule is modified in some way).

Could the interferon be cleared by the kidney? There is evidence that many low molecular mass proteins are filtered by the glomerulus, reabsorbed in the proximal tubule and probably inactivated in renal tubular cells (Strober & Waldmann 1974). Inconsistent, transitory or low levels of interferon in the urine could be explained by a tubular maximum reabsorption capacity. Bocci *et al.* (1981*a*, *b*) have shown extraction of rabbit interferon between renal arterial and venous circulations of normal rabbits and an increase in interferonuria when renal tubular resorption had been inhibited by maleate. Human leucocyte interferon was handled in a similar way in isolated perfused rabbit kidney. Bino *et al.* (1981) found most of an i.m. dose of human leucocyte interferon in rats and monkeys to be in kidney tissues, where it was concentrated in mitochondrial–lysosomal fractions. Progress could be made towards clarifying the fate of interferons in man by studying radiolabelled pure preparations and interferon kinetics in patients with disorders of glomerular function or tubular resorption.

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Toxicity

Introduction

When interferons were first given to volunteers (Scientific Committee on Interferon 1962, 1965), no toxic effects were reported. Somewhat larger doses of crude leucocyte interferon were administered by Falcoff *et al.* (1966) to patients with a variety of conditions. Although newborn babies with CMV and HSV tolerated daily doses of 5000 units without ill effect, most of the older leukaemic patients developed fever, shivering and malaise after 2000–6500 units i.v. There had been some indirect evidence that interferon production was associated with fever. For example, Petralli & Merigan (1965) indicated that protection against vaccinia virus induced by live measles immunization correlated with the development of fever in children. Despite consistent observations of febrile reactions to interferons through the 1970s, it was widely believed that fever and other toxic effects of interferon were due to unidentified contaminants, of which these interferon preparations were almost entirely composed. Very pure and single-clone interferon preparations are now available for testing in patients and volunteers.

(*a*) *Clinical reactions*

After a single i.m. injection of more than 10^6 units of human leucocyte interferon, a symptom complex develops characterized by fever, headache, malaise and myalgia, sometimes with rigors, nausea and vomiting (Scott *et al.* 1981). Symptoms begin 2–6 h after injection and last for 12–20 h. Reactions after i.v. interferon begin earlier but not immediately after injection (Strander *et al.* 1973). Systemic reactions may occur after subcutaneous or intradermal (Scott *et al.* 1981) and after intrathecal (Jacobs *et al.* 1981) injections. There is a clear relation between the dose of interferon given and the maximum fever that develops. The symptom complex is similar to that experienced by patients who are given pyrogen, endotoxin or have influenza or malaria. It has been reported in all clinical studies with leucocyte interferon (Jordan *et al.* 1974; Emödi *et al.* 1975*a*, *b*; Merigan *et al.* 1978; Greenberg *et al.* 1978; Pazin *et al.* 1979) except one (Cheeseman *et al.* 1979). It was reported with lymphoblastoid interferon in cancer patients (Priestman 1980) and with fibroblast interferon (DeSomer *et al.* 1977). Although there is some evidence that the purification of leucocyte interferon may cause a reduction in symptoms in chronic treatment (Ingimarrson *et al.* 1979; Merigan 1977), there have been no reports that reactivity may be abolished by any means of purification.

Recently we examined this symptom complex in normal volunteers given about 2×10^6 units of partly purified (PIF) and monoclonal antibody (NK2-) purified leucocyte interferons and placebo on separate occasions (Scott *et al.* 1981). There were no differences between the active preparations in terms of the degree of reactions seen. However, the second active injection given after 4 or 7 days caused significantly less severe subjective symptoms.

Gutterman *et al.* (1982) have recently reported systemic reactions in cancer patients given Hoffman–LaRoche cloned IFN–α2 (IFL-rA). We have examined an increasing dose schedule of Schering-Plough IFN-α2 in normal volunteers. A single intramuscular injection was given each week and one dose in the ascending schedule was randomly allocated to placebo. Slight fever but no symptoms were seen at 10^6 units i.m., but the complete symptom complex as described above was seen at 3×10^6 units i.m. (G. Scott, unpublished).

In two volunteers given NK2-interferon, we observed glycosuria in urine samples collected 8–12 h after injections. For this reason, we examined serum total 11-hydroxycorticosteroids

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(G. Scott & D. Wright, unpublished observations). In one volunteer given a 'toxic' dose of NK2-interferon, a significant rise was observed with a peak 8 h after injection. Serum cortisol levels were low 8 h after a placebo injection (17h00). Similar increases were also seen in volunteers given cloned IFN-α2.

When patients are given aspirin, reactions may be suppressed but not abolished (Priestman 1980). We have determined the effects of indomethacin on reactions to pyrogenic doses of NK2-interferon and cloned IFN-α2 in volunteers. Significant reductions in fever, tachycardia and symptom scores were seen but increases in serum cortisol levels induced by interferon were not altered by indomethacin.

Patients on steroids may or may not get fever. The group of renal transplant recipients studied by Cheeseman *et al.* (1979) had no fever after large doses of interferon, possibly because they were also receiving prednisone and azathioprine. Nevertheless, patients treated for *tic doloureux* by surgery to the trigeminal ganglia were given very large doses of dexamethasone around the time of surgery, and this was not sufficient to abolish interferon fever (Pazin *et al.* 1979). It is of interest that this group was able to perform a double-blind placebo-controlled trial despite interferon reactions.

When leucocyte interferon is given as an intravenous bolus injection, dramatic reactions may occur: 90 min after the injection of 4.5×10^5 units, one patient became nauseated with fever and underwent a dramatic rise in both systolic and diastolic blood pressure and pulse rate. He vomited and had rigors (Strander *et al.* 1973). Another patient became severely hypotensive after 30×10^6 units i.v. (Emödi *et al.* 1975*a*). Recent studies of lymphoblastoid interferon have shown that at an upper dose level of 2×10^8 u ml^{-1} d^{-1}, severe metabolic changes with hyperkalaemia and hypocalcaemia and drowsiness may occur (Rohatiner & Lister, personal communication).

There seems no doubt that febrile reactions also occur to crude fibroblast interferons (DeSomer *et al.* 1977; Billiau *et al.* 1979; Scott, unpublished observation). However, purification does reduce its pyrogenicity in cancer patients (Horoszewicz *et al.* 1978). Borg *et al.* (1981) have shown that crude fibroblast interferons are contaminated by material pyrogenic to rabbits, which can be removed by a simple purification method. Because patients with certain diseases may not react to crude fibroblast interferons that are clearly pyrogenic (Billiau *et al.* 1979; Kingham *et al.* 1978), or even to crude leucocyte interferon (Cheeseman *et al.* 1979), it is necessary to demonstrate the absence of pyrogenicity in healthy volunteers before being certain that a preparation is not pyrogenic. Even with leucocyte interferons, animals may be insensitive to the pyrogenic effects seen in man (Schellekens *et al.* 1981). Studies with cloned fibroblast interferons are awaited with interest.

Repeated injections of interferon cause less fever and reduced symptoms compared with the first (Merigan 1977; Priestman 1980). However, Ingimarrson *et al.* (1979) showed that some features of the acute reaction (particularly fatigue and malaise) continued in some patients throughout prolonged treatment courses.

(*b*) *Local inflammation*

When interferons are injected intradermally, inflammatory reactions are seen (DeSomer *et al.* 1977; Scott *et al.* 1977*a*; Billiau *et al.* 1979). Purification reduced the skin reactivity of fibroblast interferon (Billiau *et al.* 1979; Carter & Horoszewicz 1979), and chemical purification of leucocyte interferon also reduced its skin reactivity (Scott *et al.* 1980*b*). The first phase of the

skin reaction is an immediate flare. Contaminants in partly purified leucocyte interferon may cause larger flare reactions, which can be suppressed by histamine antagonists. However, the flare reactions to purified interferons are similar to those after saline and other control solutions, suggesting that they may be due to the trauma of injection.

The flare fades towards the end of 1 h and is replaced by discrete dense erythema maximal in size and intensity between 4 and 24 h after inoculation, and which fades gradually over several days. The intensity of the late reaction may be suppressed by indomethacin. Histological appearances at skin sites biopsied after interferon injections show immediate polymorphonuclear responses and a later mononuclear infiltration (Scott *et al.* 1977*a*; Billiau *et al.* 1979). There is no deposition of IgG, IgM or complement components at these sites.

The skin reactions occur in volunteers not previously exposed to exogenous interferons and do not increase if they are repeated. However, repeated injections of crude fibroblast interferon may stimulate type I (Billiau *et al.* 1979) and type IV (Scott *et al.* 1980*b*) hypersensitivity reactions (see below).

TABLE 1. TOLERANCE OF INTRANASAL INTERFERONS

(Total clinical scores and nasal secretion masses (n.s.m.) (g) in individual volunteers given repeated intranasal sprays of leucocyte interferon (IFN) preparations (5×10^6 u ml^{-1}, 9×10^7 u over 4 days) or placebo (human albumin, 2 mg ml^{-1} in buffered saline). Interferons tested were monoclonal-antibody (NK2-) purified leucocyte-derived IFN (Cantell–Secher) and *Escherichia coli*-derived IFN-α2 (Weissmann, Schering-Plough).)

trial 1				trial 2			
placebo		NK2-IFNα		placebo		IFNα2	
score	n.s.m.	score	n.s.m.	score	n.s.m.	score	n.s.m.
1	3.5	2.5	17.7	1.5	2.0	11.0†	12.2
1	3.2	2.5	0.4	1.0	0.8	2.0	10.2
0	2.2	2.0	13.5	1.0	0	1.5	2.8
0	1.6	1.5	3.5	0	5.8	1.0	11.8
0	1.4	1.5	0.9	0	2.5	1.0	2.7
0	1.4	0	12.3	0	1.1	1.0	1.5
0	0.3	0	2.3	0	0	0§	8.9
0	0	0	1.8	0‡	(35.2)	0	1.2
—	—	—	—	0‡	(35.9)	0	0.5
—	—	—	—	—	—	0	1.8

† Assessed as a 'very mild' cold. ‡ Assessed as having perennial rhinitis. § Wild rhinovirus in pre-trial nasal wash.

Topical interferon has not previously been reported to cause inflammation. However, at the Common Cold Unit (Scott *et al.* 1982*c*), we have seen mild reactions to repeated nasal sprays with high titre interferon. A total dose of about 10^8 units was given over 4 days. These reactions were seen both to NK2-interferon (Cantell–Secher) and cloned IFNα-2 interferon (Schering–Plough). The reactions consisted of nasal obstruction, rhinorrhoea, mild epistaxis and sneezing, and also occurred, but to only a lesser extent, on placebo (table 1) (Scott *et al.* 1982*c*). They were not severe enough to interfere with the evaluation of these interferons in experimental rhinovirus infections.

Intrathecal injections also cause inflammatory reactions characterized by an increase in c.s.f. protein with a c.s.f. pleocytosis. A delayed symptom complex with fever and headache follows injection (DeClercq *et al.* 1975; Jacobs *et al.* 1981).

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(*c*) *Changes in circulating cell counts and cell growth*

Single intramuscular injections of leucocyte, lymphoblastoid or fibroblast interferon cause reversible lymphopenia. The trough appears after fibroblast interferon at 1–3 h (Billiau *et al.* 1979), after lymphoblastoid interferon at 6 h (Priestman 1980) and after purified leucocyte interferon at 8 h (Scott *et al.* 1981). Whereas the last preparation causes a polymorphonuclear leucocytosis at this time (also seen by Billiau *et al.* (1979) with fibroblast interferon), Priestman saw a decrease in polymorphonuclear counts in cancer patients. Changes in white cell counts due to interferons still occur when volunteers take indomethacin despite the suppression of the clinical reactions (Scott *et al.* 1981). On the other hand, dexamethasone caused changes in white cell counts in placebo recipients similar to those seen in interferon recipients (Pazin *et al.* 1979). This suggests that the redistribution of white cells caused by interferon may be mediated by corticosteroid production.

Repeated injections of leucocyte interferon cause reversible lymphopenia, thrombocytopenia and anaemia, which sometimes require a reduction in dose frequency (Cheeseman *et al.* 1979). This may be a manifestation of the cytostatic effects of interferon, which could also account for hair loss (Ingimarrson *et al.* 1979) and inhibition of weight gain in children (Arvin *et al.* 1976).

(*d*) *Liver toxicity*

In some patients, a modest increase in serum hepatocellular enzymes is seen to accompany interferon treatment (Arvin *et al.* 1976; Kingham *et al.* 1978), but this has never been seen in normal volunteers. Higher doses of leucocyte, cloned leucocyte and lymphoblastoid interferons appear to be hepatotoxic in cancer patients (Gutterman *et al.* 1982; Rohatiner & Lister, personal communication). This suggests that interferon in moderate doses only causes an increase in liver enzymes if there is pre-existing liver disease.

(*e*) *Allergy*

Billiau *et al.* (1979) showed that type I cutaneous hypersensitivity to fibroblast interferon preparations could be induced by repeated intramuscular injections. One volunteer, who had not received interferon previously, had a type I hypersensitivity reaction to crude fibroblast interferon given intradermally. He did not react to highly purified fibroblast interferon or to foetal calf serum (Scott *et al.* 1977*a*). Ingimarrson *et al.* (1979) reported itchy erythema of the trunk and arms immediately after injections of leucocyte interferons, but this side-effect disappeared when a purer preparation was introduced. Occasionally, urticarial rashes are seen (Jordan *et al.* 1974), even after intranasal inoculation of very pure leucocyte interferon (Scott, unpublished observation). Merigan *et al.* (1973) also reported a transient erythematous rash after intranasal application of crude leucocyte interferon.

Mouse interferon has been shown to accelerate autoimmune haemolytic anaemia in susceptible inbred mice (Heremans *et al.* 1978) but a similar effect has not been reported in man. However, neutralizing antibodies to exogenous interferons have been seen in one cancer patient after repeated injections of fibroblast interferon (Vallbracht *et al.* 1981), in one normal patient given moderate doses of leucocyte interferon (Mögensen *et al.* 1981) and in patients treated with milligram amounts of cloned IFN-α2 (Gutterman *et al.* 1982). This unwanted effect of treatment could be disastrous, as experiments using a potent anti-interferon serum in experimental infections have indicated (Gresser *et al.* 1976*a*, *b*), and should give cause for serious consideration before interferons are given repeatedly in trivial infections.

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(*f*) *Other toxicity in animals*

Daily injections of mouse L-cell interferon (8×10^5 u d^{-1}), but not human leucocyte or mock interferon preparations, given to newborn mice result in death from hepatocellular necrosis between days 8 and 14. The $l.d._{50}$ of the preparation was estimated to be 10^5–2.5×10^5 u d^{-1} (*ca.* 88 ng d^{-1}) (Gresser *et al.* 1975). Glomerulonephritis associated with lymphocytic choriomeningitis virus infection in suckling mice has been shown to be due to endogenous interferon induction (Rivière *et al* 1977); it can be inhibited by anti-interferon serum and mimicked with exogenous interferon. Pyhälä *et al.* (1978) did not see any such toxicity with human or rabbit interferons in newborn rabbits, and the relevance of the observation in mice to the use of interferons in man is not known.

Discussion

Interferon treatment is not innocuous. The febrile reactions described could be due to interferons themselves (but perhaps only to certain species within the interferon families) or to contaminants. If interferons are pyrogenic, then they act either by stimulating the production of intermediate endogenous pyrogens or directly on the thermoregulatory centre of the hypothalamus. The systemic reaction complex seen with leucocyte interferon cannot be abolished by purification but may be suppressed by prostaglandin synthetase inhibitors or corticosteroids. It is possible that interferon preparations are contaminated with traces of highly pyrogenic material, and this was suggested by Fumarola (1981). However, we and others (Scott *et al.* 1981; Priestman *et al.* 1981) have seen reactions with interferons containing less than 1.25 ng endotoxin and our placebo preparations contain similar levels of endotoxin. An alternative contaminant would be endogenous leucocyte pyrogen, but this could not be present in cloned preparations. If the reaction complex is due to interferon, it would be reasonable to suppose that systemic reactions with local virus infections such as influenza could in part be mediated by endogenous interferon production. The mechanism for the reaction is not known. That it may be suppressed by indomethacin, aspirin or steroids suggests that it is mediated by inflammatory prostaglandin (PG) synthesis and the reduction in reaction after repeated doses could occur because arachidonic acid precursors are consumed. Indeed, peculiar changes in lipid metabolism have been observed with leucocyte interferon in normal individuals (Cantell *et al.* 1980). Although fibroblast interferon has been shown to induce PGE production in synovial fibroblasts (Yaron *et al.* 1977), this has not been reported for leucocyte interferon. As far as the corticosteroid response is concerned, it may be that interferon given to man behaves like ACTH, as proposed by Blalock & Smith (1981), but there are differences in the kinetics of serum corticosteroid production after interferon and synthetic ACTH administration. Alternatively, this may be a pituitary–adrenal axis response to 'stress'.

Febrile and inflammatory responses to interferons may be important features of non-specific host defence: interferon production is one of the earliest features of the innate immune response to a new virus infection. Influenza virus secretion in nasal mucus resolves rapidly, coincides with interferon production (and symptoms) and precedes the detection of specific antibody (Baron & Dianzani 1977). Local inflammation caused by interferon could be important in eradicating virus-infected cells, and enhanced body temperature may suppress the replication of some viruses. It may therefore be unwise to interfere with the febrile reactions to exogenous or endogenous interferons.

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Discussion

Dr Scott was asked whether the local skin reactions upon intradermal injection of interferon purified by chromatography on the monoclonal antibody, NK2, could have been due to other proteins leached off the column. He had no explanation to offer for the tiredness and lethargy seen in patients who had been injected with interferon, but he pointed out that the only side-effects seen in volunteers who had received interferon intranasally were mild local symptoms such as nasal stuffiness.

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