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The lymphatic route. IV. Pharmacokinetics of human recombinant interferon α_2 and natural interferon β administered intradermally in rabbits

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Summary

We have evaluated the feasibility of administering human recombinant interferon α_2 and human natural interferon β via the intradermal route with an air-pressure injector in the rabbit model. This is the first report showing pharmacokinetic parameters after intradermal administration of interferon. The prolonged permanence of circulating interferon α_2 and its excellent bioavailability make this route an attractive one to be tested in patients because it may increase the therapeutic index of IFN. On the other hand, this route seems less practical for IFN β for reasons probably connected with inactivation and/or scarce absorption of this drug from the skin.

Introduction

Interferons (IFN) are new drugs which can be used as antiviral, cytostatic and immunomodulatory agents (Oldham and Smalley, 1983). Mainly based on empirical approaches several routes and schedules of administration have been used with more than often conflicting results (Bocci, 1984; Bonnem and Spiegel, 1984). This is true particularly when IFN are used as immunomodulatory agents because the type of response is influenced by a number of parameters such as age, sex, metabolic conditions, nutritional status and preceding therapies that may have severely depressed the immune system. Thus, it is felt that studies aiming to evaluate novel routes of administration are warranted, particularly now when other immunomodulators, normally acting at short range, such as interleukins and thymic hormones, are becoming available (Bocci 1985b). So far we have examined in some detail the subcutaneous (s.c.) route with the innovation of administering IFN together with a high albumin (ALB) concentration (13%) in order to enhance the absorption of IFN via lymphatics (Bocci et al., 1986a and b).

We would now like to report results obtained testing the same drugs administered via the intradermal (i.d.) route. The reasons for choosing this route are three-fold: firstly, the skin is an important site of the immune system (Edelson and

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Fink, 1985; Friedmann, 1981) and the amount of the skin-associated lymphoid tissue (SALT) is one of the largest of the body (Streilein, 1978). Secondly, as the skin contains "continuous"-type blood capillaries and is rich in lymphatic vessels (Bennett et al., 1959), IFN and other proteinaceous immunomodulators should be preferentially absorbed and transferred via lymphatics and lymph nodes, hence increasing the interaction of the drug with the effector cells. Thirdly, because of the large cutaneous surface the drug could be administered in multiple sites thus involving a good deal of the peripheral immune system.

Materials and Methods

Animal experiments and collection of samples

Five New Zealand male rabbits (2.5 kg) were used for evaluating the plasma disappearance rates of human recombinant IFN α_2 (Schering, U.S.A.) and human natural IFNβ (Toray Industries, Japan) after intravenous (i.v.) administration as a bolus to three and two rabbits, respectively.

The recombinant IFN α_2 had a potency of 42.8×10^8 IU/ml and it was at least 98% pure and the natural IFNβ had a specific activity higher than 1×10^7 IU/mg proteins. Doses of 20 and 9 mega U of IFN α_2 and β, respectively, were used for each animal. Small samples of blood were withdrawn at various times thereafter from the left auricularis vein into tubes containing dry heparin (15 IU/ml of blood).

Fourteen New Zealand male rabbits (2.4-2.6 kg) were used for the main investigation as follows: four served as controls receiving IFN α_2 in saline and four received IFN α_2 in 13% human ALB solution in saline; three served as controls receiving IFN β in saline and three received IFN β in 13% ALB solution in saline.

The skin of the abdomen of each animal was shaved with an electric clipper and depilatory cream (Klorane, P.F. Cosmetique, Paris) applied for about 5 min the day before IFN administration. IFN α_2 (16 mega-U) and IFNβ (10 mega-U) diluted up to 3 ml either with saline, or with 13% ALB solution was administered intradermally in 60 sites (0.05 ml per site) for each animal in about 2 min. Administration was carried out with an injector (Mesoflash M 40, B.P. 41; Lons, Billere, France) without needle by simply taking advantage of the high hydrostatic pressure built in the volume (0.05 ml) during delivery. The "pistol" does not need any attachment to a high pressure reservoir and is commonly used in patients for mesotherapeutic treatment.

Human ALB for therapeutical use was a gift from Sclavo (Siena, Italy). During the experiment the animal had free access to pellets and water and were free in their cage. Samples of blood were withdrawn from the ear veins in dry heparin at predetermined intervals (1, 3, 6, 9, 12, 14, 24, 30, 33, 36, 48, 51, 58, 72 h) after IFN administration and all plasma samples were kept at $-70\,^{\circ}$C until IFN titration.

Determinations

The IFN assay was carried out in microtiter plates (Costar, Scotland) as described by Langford et al. (1981) using human amnionic cells (WISH) and vesicular stomatitis (VSV, Indiana strain) as a challenge virus. All samples were assayed at least twice in triplicate. The assays were always made employing the international reference preparations for human IFN α and β (obtained from National Institute for Biological Standards and Control, London and from NIAID, NIH, Bethesda, MD, respectively). The standard of human IFN α with a defined potency of 3.69897 $\log_{10}$/vial, when reconstituted in 1.0 ml of sterile distilled water, yielded in our assay system a geometric mean titer of 3.98001 $\log_{10}$ IU/ml (S.D. = 0.044; n = 10). The standard of human IFN β (G-023-902-527 with a defined potency of 4.0 $\log_{10}$ IU/vial) when reconstituted in 1.0 ml of sterile distilled water, yielded in our assay system a geometric mean titer of 4.01 $\log_{10}$ IU/ml (S.D. = 0.106; n = 16).

All titers are reported in IU/ml.

Data analysis

Inspection of the IFN α_2 and β concentration –time data after i.v. administration plotted on semilogarithmic paper revealed a three- and bi-exponential decay pattern (Fig. 1). The half-lives, and the slopes of the slow and fast components

were determined according to standard formulae on the basis of the process known as curve stripping (Gibaldi and Perrier, 1982).

The IFN concentration–time data after i.d. administration were also plotted on semilogarithmic paper. The total areas under the curve (AUC) were estimated using the expression:

$$AUC_T = AUC_0^{nh} + C_{nh}/\beta$$

where AUC_0^{nh} was the area under the curve between 0 and nh calculated by the trapezoidal rule, C_{nh} was the plasma concentration at nh and β was the terminal elimination rate constant estimated by least-squares linear regression. The elimination half-life ($t_{1/2(\beta)}$), IFN volume of distribution (V_d) and plasma clearance (Cl) were calculated from the equations:

$$t_{1/2(\beta)} = \frac{0.693}{\beta}; \quad V_d = \frac{\text{Dose}}{\beta \times \text{AUC}}; \quad Cl = \beta \times V_d$$

V_d and plasma clearance were normalized to per kg body weight. Extended bioavailability (EBA) was measured by calculating the $AUC_{i.d.}/AUC_{i.v.}$ ratio.

Statistical evaluation of the experimental plasma curves versus controls was performed using Student's *t*-test with $P \leqslant 0.01$ (two-tailed) as the minimal level of significance.

Results and Discussion

Typical disappearance curves after i.v. injection of either human recombinant IFN α_2 or natural IFN β in rabbits are shown in Fig. 1. A plot of the logarithm of plasma concentration versus time yields curves with three (left panel) and two (right panel) distinct linear components. Only 29 ± 3% and 36 ± 14% of the dose were present in the circulation 10 min after injection of IFN α_2 and

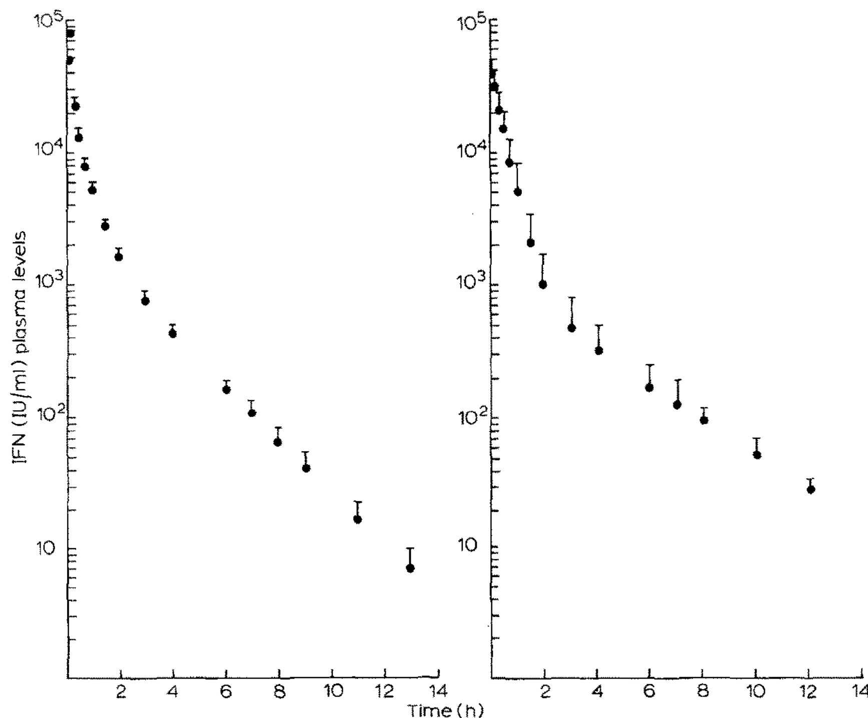

Fig. 1. Plasma pharmacokinetic for human recombinant IFN α_2 (left panel) and human natural IFN β (right panel) after i.v. administration in rabbits. Average ± S.D. of 3 and 2 rabbits, respectively.

TABLE 1

PHARMACOKINETIC PARAMETERS AFTER i.v. (BOLUS) ADMINISTRATION OF HUMAN RECOMBINANT IFN α_2 AND β IN 5 RABBITS

	α	β	γ	$t_{1/2(\alpha)}$ (h)	$t_{1/2(\beta)}$ (h)	$t_{1/2(\gamma)}$ (h)	AUC (IU/ml/h)	V_d (1/kg)	Cl (ml/min/kg)
IFN α_2 *	8.03 ± 0.2.86	1.73 ± 0.34	0.45 ± 0.04	0.09 ± 0.03	0.41 ± 0.08	1.53 ± 0.14	32,876 ± 1,041	0.5 ± 0.02	3.8 ± 0.2
IFN β **	–	2.45 ± 0.47	0.29 ± 0.04	–	0.29 ± 0.05	2.45 ± 0.35	23,098 ± 9,011	0.6 ± 0.3	2.8 ± 1

* Mean ± S.D. of 3 experiments
** Mean ± S.D. of 2 experiments

β, respectively. The pharmacokinetic parameters obtained after i.v. injection of IFN α_2 and β are reported in Table 1.

As was mentioned in the Methods section, IFN was administered via the i.d. route in the skin of the abdomen. To our knowledge this is the first report showing that IFNs administered intradermally with a mesotherapeutic device can reach the plasma pool thereby allowing the measurement of pharmacokinetic parameters. As it is shown in Fig. 2 (upper panel), administration is practically painless and extremely rapid. Occasionally there is a little abrasion followed by a very limited bleeding. The small blebs visible in the middle panel appear immediately after the administration and indicate that the solution has collected underneath the epidermis. Neither wheal- nor erythema-type reactions were noted for both IFNs around the blebs at any time: their size decreased progressively in a few hours so that one day later the skin had re-acquired normal appearance (lower panel). Fig. 3 displays graphically the disappearance of IFN α_2 from plasma in two groups of rabbits when the drug was injected intradermally in 60 sites either in saline (left panel) or in ALB solution at a final concentration of 13% (right panel). It is interesting to note that at variance with the s.c. route (Bocci et al., 1986a and b) the pattern of the plasma curve is hardly in fluenced by the addition of ALB. This is probably due to the fact that i.d. absorption of proteins, namely IFN, occurs via dermis lymphatics and therefore the ALB in itself may be unable to enhance further lymphatic absorption. The pharmacokinetic parameters are reported in Table 2: it is remarkable that, by using the i.d. route, the half-time of the elimination phase is over 6-fold longer than that measured after i.v. injection (Table 1). The apparent volume of distribution is also 6-fold larger suggesting a better distribution and a more extensive interaction of IFN with cell receptors. The IFN bioavailability by i.d. route was quantitative.

On the contrary the administration of natural IFN β by i.d. route presents some problems and appears rather inefficient. Fig. 4 displays graphically the disappearance of IFN β from plasma in two groups of rabbits after i.d. administration in 60 sites either in saline (left panel) or in ALB solution at a final concentration of 13% (right panel). The pharmacokinetic parameters are reported in Table 3. Also in this case the addition of ALB to IFN does not significantly affect either the distribution, or the catabolism. Although we administered very high doses of IFN β per rabbit (4 mega-U/kg), IFN plasma levels were disappointingly low. IFN β is a glycoprotein (Morser et al., 1978) and is much more hydrophobic (Sulkosky and Goeddel, 1982) than IFN α_2; while it is notoriously difficult to achieve the theoretically expected IFN plasma level after intramuscular (i.m.) and s.c. administrations (Billiau et al., 1979; Hanley et al., 1979; Hilfenhaus et al., 1981; Lucero et al., 1982; Quesada et al., 1982; Satoh et al., 1984; Treuner et al., 1981; Vilcek et al., 1980), it was unknown whether i.d. administration would have yielded better results. It appears now that also i.d. administration of IFN β results in poor bioavailability and actually this remains very low even if ALB is present. By contrast when we evaluated (Bocci et al., 1986b) administration of IFN β via s.c. route with the addition of ALB, we

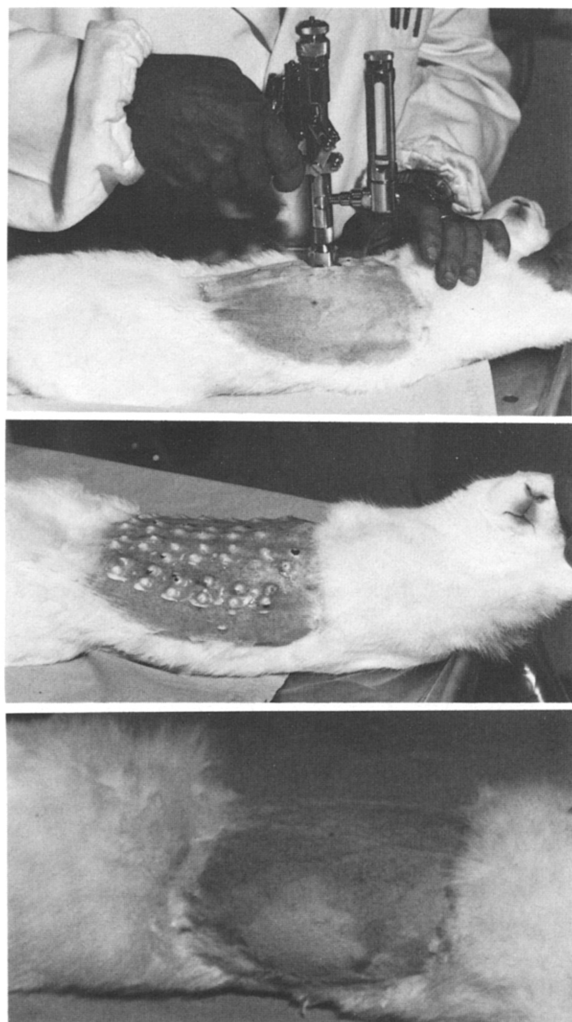

Fig. 2. Administration of human recombinant IFN α_2 in 13% albumin solution in rabbits. Top panel: the drug is applied in multiple sites of the abdomen with an air-pressure injector (0.05 ml per site). The arrow indicates the reservoir with a capacity of 13 ml. Mid and lower panels show the appearance of the skin immediately and one day after administration, respectively.

could raise the bioavailability from 12 up to 40%. The reasons for the scarce bioavailability of IFN β remain obscure and one of us (Bocci, 1983) has discussed at length this issue elsewhere. Administration of IFN β via i.d. route with the device used in the present experiment seems to have a pejorative effect and reasonable explanations are the possible denaturation of IFN β during the administration due to shear forces (Stewart, 1979) and/or inactivation of IFN in the dermis accompanied by scarce absorption or increased fixation at the injection site. We hope to clarify in the future some of these aspects as the problem of the low IFN β bioavailability after i.m. and s.c. administration is an ongoing endeavour of this laboratory.

In conclusion, we have found that i.d. administration of human recombinant IFN α_2 using an air-pressure injector could be a suitable route susceptible to be applied in patients. As far as it can be judged from the present pharmacokinetic

TABLE 2

PHARMACOKINETIC PARAMETERS FOR HUMAN RECOMBINANT IFN α_2 ADMINISTERED VIA i.d. ROUTE IN 8 RABBITS

Parameters	IFN in saline (control)	IFN in saline +13% albumin
β	0.072±0.032	0.056±0.015
$t_{1/2(\beta)}$ (h)	12.4±7.9	13.2±3.5
AUC (IU/ml/h)	32,871±4269	32,699±3400
$V_{d(\beta)}$ (l/kg)	3.2±1.9	3.3±0.9
Cl (ml/min/kg)	3.0±0.4	2.9±0.2
EBA (%)	100±10	100±13

Values are means±S.D. Experimental versus control: not significant.

TABLE 3

PHARMACOKINETIC PARAMETERS FOR HUMAN NATURAL IFN β ADMINISTERED VIA i.d. ROUTE IN 6 RABBITS

Parameters	IFN in saline (control)	IFN in saline +13% albumin
β	0.056± 0.027	0.035± 0.014
$t_{1/2(\beta)}$ (h)	14.2 ± 5.6	22.7 ± 11.5
AUC (IU/ml/h)	3521 ±474	2274 ±390
$V_{d(\beta)}$ (l/kg)	24.1 ± 13.6	58.1 ± 26.2
Cl (ml/min/kg)	18.7 ± 3.9	30.0 ± 6.9
EBA (%)	17 ± 2	11 ± 2

Values are means±S.D. Experimental versus control: not significant.

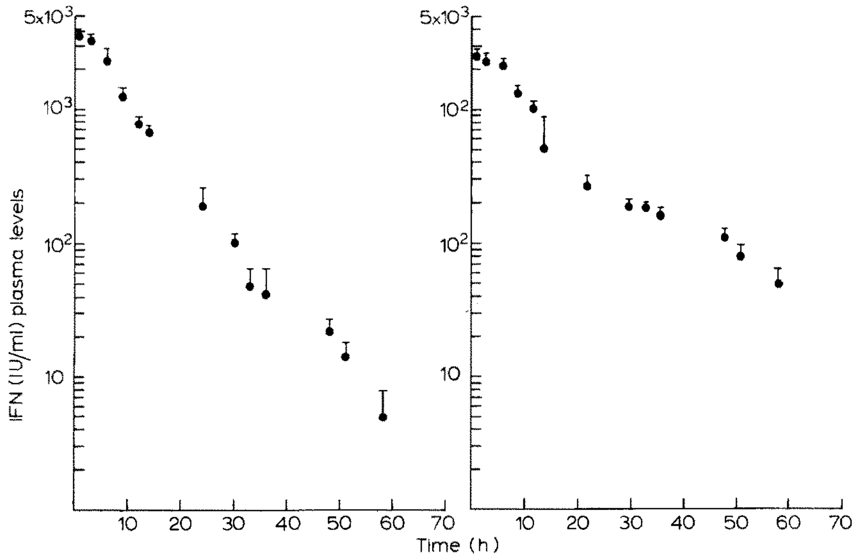

Fig. 3. Plasma pharmacokinetics of human recombinant IFN α_2 in 8 rabbits after multisite i.d. administration of IFN in saline (left panel) and in 13% ALB solution (right panel). Each point is the average ± S.D. of 4 experiments.

parameters, multisite application of IFN α_2 in the skin may increase the therapeutic effectiveness of IFN and other immunomodulators and reduce their toxicity. One important point that remains to be clarified is the wheal and flare reactions observed in patients after i.d. injection of more or less purified leukocyte IFN in order to test local reactions induced by IFN (Scott et al., 1981). Interestingly, as reported by Scott (1983) i.d. injection of human recombinant IFN α_2 caused no visible inflammation at 8–24 h and this was confirmed by histological examination of skin biopsies. Scott's finding raises hopes of good cutaneous tolerability of IFN α_2 after i.d. administration.

On the other hand, on the basis of the present

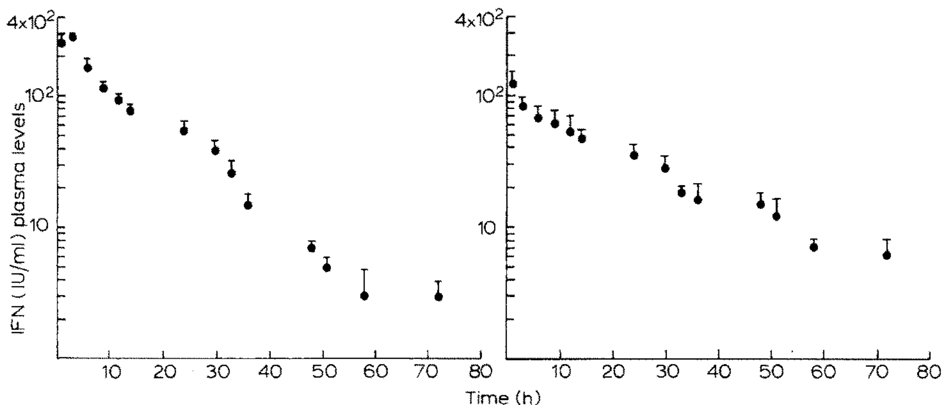

Fig. 4. Plasma pharmacokinetics of human natural IFN β in 6 rabbits after multisite i.d. administration of IFN in saline (left panel) and in 13% ALB solution (right panel). Each point is the average ± S.D. of 3 experiments.

results, i.d. administration of human natural IFN β seems to be an impracticable route of administration and this may be due either to the device used and/or to scarce diffusion of IFN β into the body fluids. Moreover, skin tests of IFN β in man used to detect hypersensitivity to this particular type of IFN have evidentiated wheal and flare reactions when 3 mega-U of IFN β are injected per site (Muranaka and Suzuki, 1984). Although, usually one would inject no more than 0.5 mega-U per site, the possibility of these cutaneous reactions should be borne in mind and, for the time being, it seems more practical to inject IFN β added with 13% ALB via the s.c. route as previously described (Bocci et al., 1986b).

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