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Subcutaneous Absorption Kinetics and Local Tissue Distribution of Interferon and Other Solutes

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Abstract—The subcutaneous absorption and consequent tissue distribution of interferon g was studied in an anaesthetized rat model. Interferon g showed a biphasic disappearance profile from a solution in a subcutaneous absorption cell. Both the initial rapid distribution phase and slower removal phase followed first order kinetics. The steady-state clearance of interferon g from the cell was $1{\cdot}41 \pm 0{\cdot}38 \times 10^{-3}$ mL min^{-1}, and the absorption half-life ($t_{\frac{1}{2}}$) was $3{\cdot}8 \pm 1{\cdot}1$ h (n = 4). Noradrenaline did not significantly alter either the clearance or absorption of interferon g ($1{\cdot}18 \pm 0{\cdot}44 \times 10^{-3}$ mL min^{-1}, $P > 0{\cdot}05$, absorption $t_{\frac{1}{2}}$ $4{\cdot}96 \pm 1{\cdot}9$ h, $P > 0{\cdot}05$). Given that the clearance of smaller solutes, such as tritiated water, is significantly reduced when noradrenaline is coadministered, it is suggested that interferon g is removed via the lymphatic system rather than by the local blood supply. The amount of interferon g recovered in the plasma, urine and muscle is minimal relative to other solutes where the recovery is almost complete.

The interferons comprise a group of inducible glycoproteins which possess antiviral, cytostatic and immunomodulatory actions (Isaacs & Lindenmann 1957; DeMaeyer & DeMaeyer-Guignard 1982; Gresser 1982). The recent availability of virtually unlimited quantities of these substances, through advances in recombinant DNA technology and the cloning of interferon genes into *Escherichia coli*, has allowed their potential as novel drug molecules to be investigated. One of the major problems associated with the use of interferons as drug molecules is in achieving successful administration and distribution of these large proteins, particularly with the production of reproducible effective therapeutic profiles, whilst minimizing side-effects. As paracrine hormones, interferons are endogenously produced and utilized in microenvironments, and it is their overspillage into the blood stream which can be correlated with their toxic side-effects (Scott 1982). This fact, together with plasma half-lives of under 2 h (Bocci 1987), precludes the routine use of intravenous administration. However, the bioavailability of interferon from both intramuscular and subcutaneous sites is reputedly high (Wills et al 1984a, b), and with the wider patient acceptance of the latter (Russo & Moore 1982), this route of administration is emerging as the most suitable for both interferons and many other biologically active proteins. Following subcutaneous administration, a drug can be transported to the general circulation by either the blood capillaries or the lymphatics. For macromolecules such as interferon, permeability through the capillary endothelium will be low and direct movement into the blood should be restricted. Supersaxo et al (1990) suggested that molecules with mol. wt > 16 kDa are absorbed mainly by the lymphatics which drain the application site. The interferons and other lymphokines are physiologically released and act upon immune cells at short range. It has been postulated that if immunoenhancers were absorbed preferentially into the lymphatics they would interact primarily with immunocompetent effector cells present in the lymph and lymph nodes (Bocci 1987). Targeted delivery to this body compartment would reduce the toxic side-effects of the interferons which are known to be dose- and plasma level-dependent (Scott 1982).

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The design of effective subcutaneous interferon dosing regimens may be assisted by knowledge of the mechanism and kinetics of drug uptake from the injection site. The present study was designed to determine the rate of diffusion of interferon g from the subcutaneous site and also to establish to what extent this absorption was dependent upon the blood supply. Tissue biopsies and plasma and urine concentrations were used to estimate the proportion of the absorbed dose which could be recovered in these particular body compartments, compared with other drugs whose kinetics are more established.

Materials and Methods

Materials

^{125}I-Interferon g was obtained from Amersham International (Sydney, Australia), [^{14}C]lignocaine HCl and [^{3}H]H_2O were obtained from New England Nuclear (Australia). Noradrenaline was obtained from Sigma (Sydney, Australia). Scintillation fluids and tissue solubilizer were supplied by Amersham (Australia), and all other reagents were of analytical grade.

Preparation of absorption sites

The diffusion of each solute was studied using glass cells fixed to the exposed abdominal subcutaneous tissue of rats, following the method described by Levy & Rowland (1972). Female Sprague-Dawley rats, 225–260 g, were used throughout. Briefly, anaesthesia was induced with intraperitoneal sodium pentobarbitone (30 mg kg^{-1}), followed by 4 mg kg^{-1} at 2·5 and 4 h where necessary. The abdominal hair was clipped with electric clippers and a circle approximately 2 cm in diameter marked in a standard position on the lower abdomen. A section of the skin was lifted with forceps and an incision made to the level of the subcutaneous tissue; the whole area was then excised by cutting along the marked

circle, drawing the skin upwards and separating it from the subcutaneous layer. The subcutaneous absorption cell (8 cm high × 1·8 cm internal diam.) was fixed to the moist tissue with cyanoacrylic adhesive. The cell was clamped in position, warmed with a heating wire wound in a standard fashion around the cell, and maintained at 37°C throughout the experiment.

Absorption studies

Two millilitres of isotonic phosphate-buffered saline (PBS) interferon g (0·5 μCi) solution at 37°C (n = 4), or 3 mL of other specified radiolabelled drug PBS solutions, was introduced into the cell at time 0, a 10 μL sample being taken from the solution before it was introduced to the cell and further 10 μL samples taken from the cell at each of the time points specified, to a maximum of 6 h. The solution was stirred throughout by a fixed glass rod with paddles at approximately 45 rev min^{-1}. At the end of each absorption period the remaining solution in the cell was drawn out with a pipette, the cell removed, the subcutaneous tissue blotted with filter paper, and a 1–2 mL blood sample taken into a heparinized tube from the tail vein. The rat was killed with an overdose of anaesthetic and tissues sequentially removed from below the position of the cell, and then from an untreated area high on the abdomen, for differential concentration determinations. Dissection instruments were carefully cleaned with filter paper between sampling layers to reduce to a minimum any cross-contamination of radioactivity between tissues. Where specified, urine samples were taken by aspirating the entire contents of the bladder into a syringe and into a preweighed vial.

Assays

The analysis of aqueous and tissue samples containing interferon g was by gamma-counting. Aqueous and tissue samples were collected into preweighed capped plastic Eppendorff tubes and the sample weights determined before the radioactivity was measured. Beta-counting was used to estimate the concentrations of [^{14}C]lignocaine and [^{3}H]H_2O in tissue and aqueous samples. The sample weights were measured in preweighed scintillation vials and the other tissue samples treated with the tissue solubilizer NCS (1 mL tube for 5 h at 45°C) before the addition of the appropriate scintillation fluid, and counting in a liquid scintillation counter.

Data analysis

Clearances (CL) were estimated from the slopes (k) of the regression lines fitted to the log % remaining vs time plot for the terminal phase for each solute, and the volume (V) of the solute applied to the cell, i.e. $CL = k \cdot V$.

Tissue concentration of recovered solutes are expressed as a % of the total dose lost from the cell at the time of biopsy. Plasma and contralateral tissue concentrations are expressed as % mL^{-1} and % g^{-1}, respectively. Statistical analyses were performed using a non-paired Student's *t*-test.

Results and Discussion

Absorption of interferon g

To assess the reproducibility of our kinetic studies with those of Levy & Rowland (1972), who first introduced the model, we determined the clearance of the local anaesthetic lignocaine from the cell as in their original studies. Our estimated value for lignocaine of $6{\cdot}2 \pm 0{\cdot}2 \times 10^{-3}$ mL min^{-1} (Fig. 1C) is comparable with their reported clearance of $5{\cdot}6 \times 10^{-3}$ mL min^{-1}.

Fig. 1 shows the time course for the disappearance of solutes from the absorption cell. The profile obtained for interferon g (Fig. 1A) indicates that after an initial distribution phase, the loss of interferon g from the cell is monoexponential, indicative of a constant clearance with time. An initial rapid loss of 22·1 ± 0·1% of the initial dose applied corresponds to an apparent partition coefficient, or tissue distribution ratio, of 0·22 between the underlying subcutaneous tissue and the cell test solution. The clearance of interferon g estimated from the rate of loss from the applied

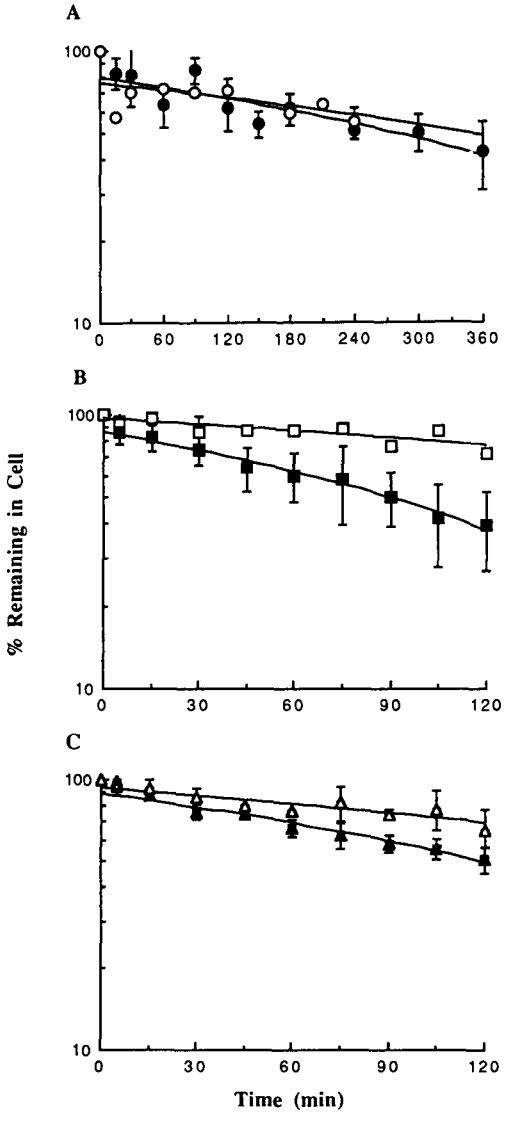

FIG. 1. Effect of noradrenaline on the absorption of (A) interferon g, (B) water and (C) lignocaine. Open symbols are experiments in the absence of noradrenaline, closed symbols are experiments in the presence of 1 μM noradrenaline.

solution was $1{\cdot}41 \pm 0{\cdot}38 \times 10^{-3}$ mL min^{-1}. The absorption half-life ($t_{\frac{1}{2}}$) from the solution was $3{\cdot}8 \pm 1{\cdot}1$ h.

Effect of noradrenaline

Noticeable blanching of the area under the absorption cell was observed at a concentration of 10^{-6} M noradrenaline. The clearance of $[^3H]H_2O$ from the cell was found to be significantly reduced when coadministered with this concentration of noradrenaline ($P<0{\cdot}05$) (Fig. 1B), and the absorption $t_{\frac{1}{2}}$ increased from $1{\cdot}7 \pm 0{\cdot}6$ to $4{\cdot}2 \pm 0{\cdot}2$ h ($P<0{\cdot}01$).

In contrast, the total amount of interferon g cleared from the cell after 4 h remained relatively unchanged by the presence of 10^{-6} M noradrenaline ($P>0{\cdot}05$) (Fig. 1A). The clearance of interferon g in the presence of noradrenaline was $1{\cdot}18 \pm 0{\cdot}4 \times 10^{-3}$ mL min^{-1} and similar to that obtained when no noradrenaline was present ($1{\cdot}41 \pm 0{\cdot}38 \times 10^{-3}$ mL min^{-1}). The absorption $t_{\frac{1}{2}}$ increased slightly from $3{\cdot}8 \pm 1{\cdot}1$ to $4{\cdot}96 \pm 1{\cdot}9$ h. However, the change was not significant. The loss of $21{\cdot}7 \pm 0{\cdot}02\%$ of the dose in the initial distribution phase is similar to that reported earlier in the absence of noradrenaline, suggesting that interferon g distribution into local tissues is not affected by blood supply. Given that the clearance of $[^3H]H_2O$ is significantly reduced when noradrenaline is coadministered, it is suggested that interferon g is not removed by the local blood supply, and that it is cleared from the site by the lymphatic system. The clearance of $[^{14}C]$lignocaine (Fig. 1C) was also not significantly attenuated by noradrenaline. However, the amount of lignocaine in the subcutaneous tissues and adjacent tissues increased and is consistent with tissue diffusion being a major determinant of lignocaine tissue penetration after topical application (Singh & Roberts unpublished results).

Tissue distribution of interferon g compared with other solutes

Fig. 2 shows the percentage recovery of different solutes in the plasma, tissues underlying the cell and contralateral muscles. Following uptake from the cell it can be expected that smaller solutes would enter the underlying tissues, diffuse into the bloodstream, enter the general circulation, and consequently be distributed to contralateral tissues. It would therefore follow that the majority of the radioactivity lost from the cell would be able to be recovered by determining the concentration in the blood plasma, the tissues beneath the cell and in the contralateral tissues. The hypothesis that interferon g is taken up by the lymphatics is further supported by the finding that the majority of the doses of water and lignocaine could be recovered in these body compartments, but not interferon g.

Over 60% of the initial dose of water applied was lost from the cell. Uptake into the tissues directly beneath the cell accounted for $21{\cdot}8 \pm 10{\cdot}8\%$ of this dose, with a large proportion of the remainder being associated with the plasma and contralateral tissues (Fig. 2B). The presence of noradrenaline significantly reduced the amount of $[^3H]H_2O$ taken into the blood ($P<0{\cdot}01$), and distributed to the contralateral tissues, and increased the proportion of the dose associated with the tissues underlying the cell. Water is a small solute (mol. wt 18) which both readily diffuses in tissues and crosses capillary walls (Paaske & Sejrsen 1989). A rapid

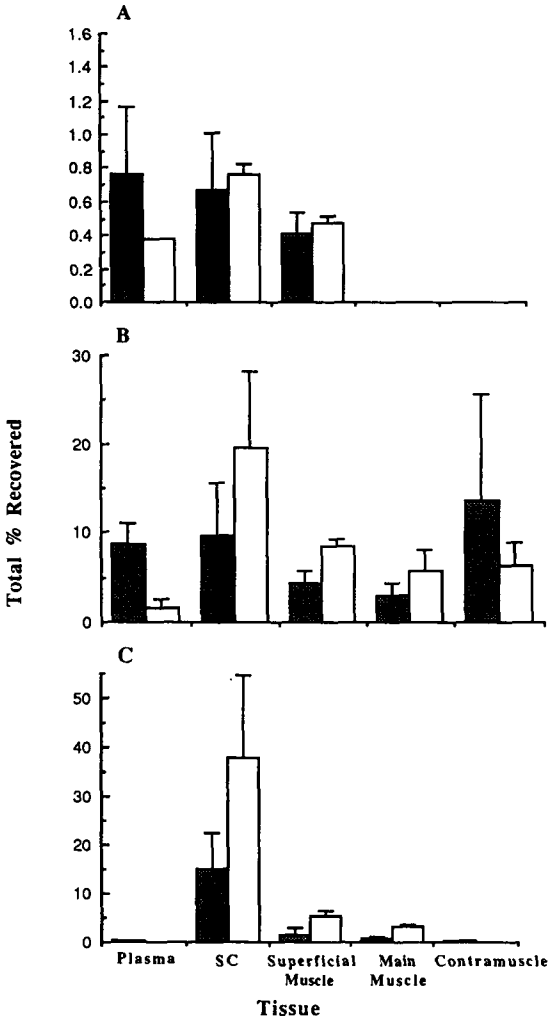

FIG. 2. Percentage recovery of the absorbed doses of (A) interferon g, (B) water and (C) lignocaine, in various body compartments (shaded columns) and in the presence of 1 μM noradrenaline (open columns).

loss from the site to the blood stream is consistent with these characteristics.

Lignocaine is a larger solute (mol. wt 234) which exists predominantly in the ionized form. In this work, a total of $49{\cdot}3 \pm 5{\cdot}75\%$ of the initial dose administered was lost from the cell during a 2 h period. The tissues directly beneath the absorption cell contained the largest single proportion of this dose, $19{\cdot}0 \pm 9{\cdot}1\%$ (Fig. 2C). The relatively low concentrations of the lignocaine in the blood but high concentrations in the tissue in the presence of noradrenaline is consistent with a poor removal of lignocaine by the blood and the subcutaneous and other underlying tissues acting as an effective 'sink' for the lignocaine. The attenuated uptake of lignocaine from the cell failed to show a significant change on the addition of noradrenaline due to the dominant effect of tissue diffusion in the removal process.

A high loss ($38{\cdot}9 \pm 10{\cdot}3\%$) of the large mol. wt solute inteferon g is also evident from the absorption cell after 2 h of application, although the solute did not appear to be undergoing 'steady-state' absorption at this point. Clearances were therefore calculated over a 6 h absorption period.

After 6 h, 57·5 ± 11·9% of the interferon g was lost from the cell. However, only around 1% of this dose is recovered in the tissues directly beneath the cell, and 0·77% mL^{-1} was associated with the circulating blood volume (Fig. 2A). Interferon g was not detectable in the contralateral tissues (Fig. 2A). Interferon g is therefore not associated with extensive tissue distribution into the tissues below the absorption site, and is cleared to some other body compartment by a mechanism other than blood transport. Less than 1% of the interferon g radioactivity was recovered in the urine upon complete aspiration of the bladder contents. The catabolic role of the kidney with respect to interferons has been investigated in a series of papers by Bocci (for review see Bocci 1987). Up to 45% of interferon molecules carried through the kidney are passed from the glomerular capillaries into the ultrafiltrate, and are then taken up by the tubular cells and degraded. The lack of interferon g radioactivity in the urine in the present study suggests that little of the drug has been excreted in the urine as either intact interferon g or its metabolites in the 6 h period. A possible site for the unaccounted radioactivity is the lymphatic system.

Poor absorption of interferon g would be consistent with its protein nature and molecular size (around 17 kDa). Large molecular weight solutes are usually more efficiently cleared from body tissues by the lymphatic system. Absorption of interferon g through the large pores of the lymphatic capillaries and movement into the labyrinth of the lymphatic system would explain why significant amounts of the radioactivity lost could not be found in the tissues and fluids biopsied. Interferons undergo extensive binding in the lymph, which together with the slow rate of lymph flow (0·04% that of blood flow (Courtice 1981)), means that only very low levels of free interferon g should emerge in the plasma.

The lymphatic clearance hypothesis is supported by the studies of Supersaxo et al (1990) on the role of mol. wt in the lymphatic absorption of water-soluble compounds following subcutaneous administration in sheep. They suggested that molecules with a mol. wt > 16 kDa will be absorbed mainly by the lymphatics that drain the application site. From the mol. wt of interferon, it would therefore be expected to be cleared primarily by the lymphatics. The results also correlate with the earlier findings of Bocci et al (1988), who showed that interferon $\alpha 2$ could be preferentially absorbed by the lymphatics following intramuscular or subcutaneous administration compared with intravenous dosing.

The present data on noradrenaline's differential effects on solute clearance provides a simple method of comparing the importance of the blood vessels and lymphatic system in solute clearance from the site. There was no change in the amount of interferon g absorbed from the cell with or without 10^{-6} M noradrenaline, although this concentration of noradrenaline has been reported to cause a significant vasoconstriction of blood vessels underlying tissue (Mahe et al 1989). Such a vasoactive effect was confirmed by the significant reduction in the clearance of $[^3H]H_2O$ and observed blanching when noradrenaline was added to the diffusion cell. Although both blood and lymphatic vessels are responsive to the effects of circulating catecholamines, noradrenaline has been shown to have no depressive effect on the activity of lymphatic vessels at concentrations of 10^{-6} M and below (Mahe et al 1989). The lack of effect of noradrenaline is thus supportive of clearance by the lymphatics.

Although the current approved use of interferons is limited, being listed only for malignancies such as hairy-cell leukaemia, Kaposi's sarcoma in AIDS, and a small number of infections due to human papilloma virus, chronic active hepatitis C virus infection and chronic granulomatous disease, their therapeutic promise continues to broaden (Volz & Kirkpatrick 1992). This work has defined the absorption kinetics of interferon g given subcutaneously as a two-phase process involving a distribution from solution into local tissues and clearance by the lymphatic system.

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References

Bocci, V. (1987) The metabolism of anticancer agents. Pharmacol. Ther. 34: 1–49

Bocci, V., Pessina, G. P., Paulesu, L., Nicoletti, C. (1988) The lymphatic route. VI. Distribution of recombinant interferon alpha-2 in rabbit and pig plasma and lymph. J. Biol. Resp. Mod. 7: 390–400

Courtice, F. C. (1981) The lymphatic circulation. In: Schwartz, C. J., Werthessen, N. T., Wolf, S. (eds) Structure and Function of the Circulation. Vol. 2, Plenum Press, New York, pp 487–602

DeMaeyer, E., DeMaeyer-Guignard, J. (1982) Immuno-modulating properties of interferons. Phil. Trans. R. Soc. Lond. B299: 77–90

Gresser, I. (1982) How does interferon inhibit tumour growth? Phil. Trans. R. Soc. Lond. B299: 69–76

Isaacs, A., Lindenmann, J. (1957) Virus interference. 1. The interferon. Proc. R. Soc. Lond. B147: 258–267

Levy, R. H., Rowland, M. J. (1972) Development of a system for studying the subcutaneous absorption of local anaesthetic amines. Pharm. Sci. 61: 1263–1267

Mahe, L., Chapelain, B., Neliat, G., Gargouil, Y.-M. (1989) The role of alpha- and beta-adrenoceptors in the response to noradrenaline of lymphatic vessels isolated from the bovine mesentery. Eur. J. Pharmacol. 167: 31–39

Paaske, W. P., Sejrsen, P. (1989) Permeability of continuous capillaries. Dan. Med. Bull. 36: 570–590

Russo, G., Moore, W. V. (1982) A comparison of subcutaneous and intramuscular administration of human growth hormone in the therapy of growth hormone deficiency. J. Clin. Endocrinol. Metab. 55: 1003–1006

Scott, G. M. (1982) Interferon: pharmacokinetics and toxicity. Phil. Trans. R. Soc. Lond. B299: 91–107

Supersaxo, A., Hein, W. R., Steffen, H. (1990) Recombinant interferon alpha 2a: delivery to lymphoid tissue by selected modes of application. Pharm. Res. 7: 167–169

Wills, R. J., Spiegel, H. E., Soike, K. F. (1984a) Pharmacokinetics of recombinant alpha A interferon following IV infusion and bolus, IM and PO administration to Green African monkeys. J. Interferon Res. 4: 399–409

Wills, R. J., Dennis, S., Spiegel, H. E., Gibson, D. M., Nadler, P. I. (1984b) Interferon kinetics and adverse reactions after IV, IM and SC injection. Pharmacol. Ther. 35: 722–727

Volz, M. A., Kirkpatrick, C. H. (1992) Interferons 1992. How much of the promise has been realised? Drugs 43: 285–294