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The lymphatic route. 1) Albumin and hyaluronidase modify the normal distribution of interferon in lymph and plasma[1]

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Summary. When human recombinant interferon-α_2 diluted in saline was injected s.c. into rabbits, the total amount recovered in thoracic lymph was less than 0.4%. Recoveries increased from 2- to 8-fold if interferon was injected in 4% albumin or with hyaluronidase, respectively. Albumin added to interferon acts as an interstitial fluid expander, thus favoring interferon absorption through lymphatics rather than blood capillaries. This strategy may increase the therapeutic index of interferon.
Key words. Interferon; immunomodulator; catabolism; pharmacokinetics; administration routes.

The problem of distribution of interferon (IFN) in lymph and plasma has attracted little attention so far[4], but there is no doubt about the occurrence of transcapillary passage of IFN. After i.v. administration of IFN into rats, its concentration in lymph was similar to that in the plasma[5]. However, because of renal filtration[6,7] and hepatic catabolism[8] IFN has a very short half-life in plasma so that, when IFN is administered via i.m. and s.c. routes that favor blood capillary rather than lymphatic absorption, it is likely that lymphatic organs may exchange little IFN with the plasma pool[9]. This uneven distribution may represent an important drawback as it remains uncertain whether in cancer therapy IFN acts more as a cytostatic drug or as an immunomodulatory agent. Thus, in order to reproduce the physiological distribution of IFN[10], one of us has proposed[11] that lymphatic absorption should be facilitated and expanded as far as possible, in order to minimize IFN plasma levels and to improve the interaction of IFN with effector cells. In this report we investigated the distribution of human recombinant interferon-α_2 (rec. IFN-α_2) in plasma and thoracic lymph in the rabbit after s.c. injection of IFN, diluted either in saline, or in 4% human albumin (ALB) solution, or in saline with the addition of 75 U hyaluronidase (HYAL).

Materials and methods. Human rec. IFN-α_2 was obtained through the courtesy of Dr I. I. A. Tabachnich (Schering Corp. Bloomfied, N. J.). It had a potency of 4.2×10^9 IU/ml and it was at least 98% pure. 20 adult male rabbits (3.488±0.285 kg) were randomly assigned to one of the 4 groups necessary for the investigation.

The animals were anesthetized with Nembutal throughout the experiment: after cannulation of the thoracic duct and a femoral artery, lymph and blood samples were collected at predetermined times. A constant flow of lymph was ensured by a slow infusion of saline into a marginal vein of the ear and by passively moving the extremities of the animals every hour. Rabbits of group A received a single s.c. injection (0.5 ml) in the hind leg of 11 mega units (MU) human rec. IFN-α_2 (from E. coli) in saline. Groups B, C, D were injected at 5 sites (0.1 ml/site) of the hind legs with the same amount of IFN diluted in saline (B), in saline-4% ALB. solution (C) and in saline containing 75 U (0.5 ml) of HYAL (D).

Lymph and blood were collected in heparinized vials and, after centrifugation, the volumes of the cell-free lymph and plasma were measured and stored at −20 °C until IFN determination. IFN titration was carried out by virus plaque-assay using HEp 2 cells and VSV as challenge virus[12]. All samples were assayed at least twice in duplicate, and the titres were referred to an international standard of IFN-α. The results presented here are means ± standard error of the means. Statistical evaluation presented in the table was made by using the t-test.

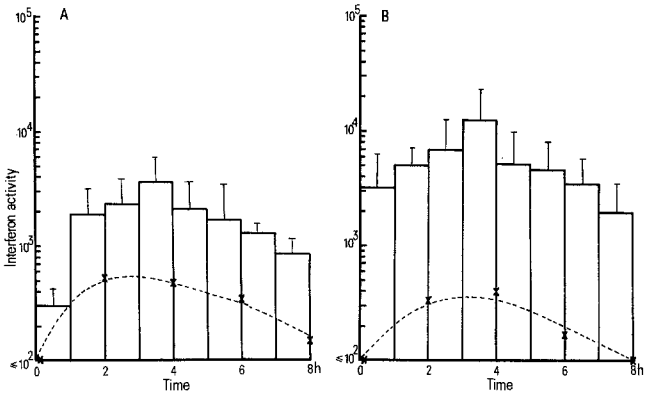
Results and discussion. The figure shows amounts of IFN recovered hourly in thoracic lymph and profiles of the IFN plasma levels during 8 h after s.c. injection of human rec. IFN-α_2. The peak of IFN concentration in the lymph was reached within 4 h irrespective whether IFN was injected at 1 or 5 sites. However, in the latter case, IFN concentration was almost 1 log unit higher than after a single administration. The amount of IFN recovered hourly in lymph increased when IFN was injected with either 4% ALB or HYAL. This enzyme was used as a well-known means of favoring lymphatic absorption of proteins, so that its effects could be compared with those of albumin. Clearly, HYAL cannot be injected in cancer patients because it can favor cancer cell metastatization. In all cases, lymph-to-plasma IFN concentration ratios were above 1 and were increased when IFN was injected with ALB and particularly with HYAL.

The table summarizes cumulative recoveries of IFN in lymph expressed as percentages of the administered dose. Simultaneous administration of either IFN and ALB, or IFN and HYAL, increased IFN yields in lymph between 2- and 8-fold, respectively. This result indicates that an increase of the oncotic pressure of the interstitial fluid, like the one obtained here with 4% albumin, is hardly comparable to the HYAL effect and there-

Recoveries of human rec. IFN-α_2 in thoracic lymph of rabbits after s.c. injection

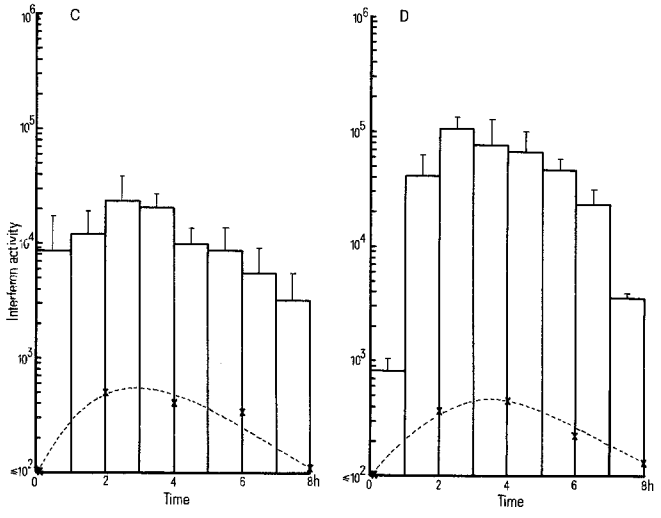
Group	Total units recovered in 8 h (means ± SE)	% of dose (means ± SE)
A (saline, 1 site)	11,266 ± 6,519	0.10 ± 0.06
B (saline, 5 sites)	41,406 ± 34,405	0.38 ± 0.32
C (4% ALB, 5 sites)	91,397 ± 19,829	0.83 ± 0.18
D (75 U HYAL, 5 sites)	360,386 ± 58,747	3.28 ± 0.53

B vs C: NS, B vs D: $p < 0.05$.



fore, for optimizing IFN absorption via lymphatics, the albumin concentration needs to be increased. The consistency of the edema induced at the site of injection is clearly an important driving force facilitating IFN absorption through the lymphatics.

A striking finding is the very low recovery of IFN in lymph; although IFN entering into the plasma pool is very rapidly eliminated[6–8], we were expecting a somewhat higher IFN recovery in lymph. At present we can only speculate that the low yield is due not so much to dilution in the lymph pool as to extensive cell-binding of IFN. In fact the concentration of lymphoid cells is normally from 15- to 1000-fold higher in lymph and nodes, respectively, than in plasma[9] so that little IFN emerges free into the thoracic lymph. Further studies with radioactively labeled IFN are necessary for quantitating this phenomenon, but the possibility of increasing the IFN concentration in the lymph and lymph nodes, where most of the effector cells with important anti-tumor activities are concentrated, is well worth studying.



Mean plasma levels, IU/ml (x—x) and recoveries in thoracic lymph (mean ± SE, total units/h) after s.c. injection of rec. IFN-α_2 in saline: *A* one site, and *B* five sites; *C* in 4% ALB, and *D* with HYAL.

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Cellular and secreted tumor plasminogen activator: the effects of NaCl

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Summary. Plasminogen activator, secreted by metastatic tumor cells, was strongly inhibited in buffer or tissue culture medium containing physiological concentrations of NaCl. Intact cells, however, expressed strong activity under similar conditions. Thus, if plasminogen activator is involved in invasion and metastasis, the cellular activity, acting as an ectoenzyme, may be more important than secreted enzyme under physiological conditions.
Key words. Plasminogen activator; NaCl; metastasis; MAT 13762 cells.