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Pulmonary catabolism of interferons: alveolar absorption of ^{125}I-labeled human interferon alpha is accompanied by partial loss of biological activity

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Summary

The catabolism of interferon was examined in isolated rabbit lungs which were ventilated and perfused with homologous blood. Natural human interferon-α (HuIFN-α) from lymphoblastoid Namalwa cells or recombinant DNA-derived HuIFN-α_2 were labeled with ^{125}I, mixed with an excess of the respective cold interferons and added to the perfusion blood. Protein-bound and acid-soluble radioactivity, as well as antiviral activity, were measured at regular time intervals. During the first 3 h of perfusion, only very small fractions of the interferons disappeared from the perfusate, irrespective of whether lungs were inserted in the perfusion system. This indicated that catabolism of interferons in the pulmonary circulation was negligible. On the other hand, when the interferons were instilled into the bronchial-alveolar tree, absorption of antiviral activity differed from that of acid-precipitable protein-associated radioactivity. While most of the radioactivity was transferred into the perfusate, only 2% of antiviral activity of natural HuIFN-α and 30% of that of HuIFN-α_2 were recovered in the perfusate. In both cases acid-soluble radioactivity in the system reached about 10%. Since radioiodide, instilled in the bronchial-alveolar tree, was transported rapidly into the perfusate, this type of analysis did not help in locating the site(s) of degradation. Alveolar macrophages did not catabolize or inactivate interferons in vitro.

interferon; catabolism; respiratory system; pharmacokinetics

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Introduction

Preceding investigations [6,8] have indicated that the rapid disappearance of interferon-α from plasma is mostly due to renal filtration and tubular catabolism while, owing to the lack of carbohydrates in this interferon type, the role of the liver is minimal [5,9]. Recently it has been observed [1,3,33] that labeled interferon, once bound to its cellular receptor, may become internalized and broken down. This potentially important catabolic pathway [7] has been shown in isolated cells and it appeared of interest to evaluate its role in intact organs. The isolated and perfused lung, with its very vast endothelial surface, offered a unique possibility to assess this phenomenon in physiological condition. Moreover, in preliminary experiments, we had observed that, after instillation of interferon into the bronchi, it was hardly measurable in the perfusate and at the same time was recoverable neither in lung washes nor in the lung cytosol. This result suggested that either interferon is catabolized in the alveolar lumen or is transported through the epithelial lining with binding or inactivation in the interstitial fluid. It appeared to us that this finding was of such theoretical and potentially clinical importance as to request a more extensive investigation. As we have already shown that interferon labeled with ^{125}I can be reliably used for metabolic investigations [11], the study has been done with labeled interferon mixed with an excess of cold interferon so that radiolabel and antiviral activity could be followed simultaneously.

Material and Methods

Purification and iodination of interferons

Natural interferon-α, HuIFN-α (Ly), from Namalwa cells [31], was purified by immuno-affinity chromatography against specific antibody [22], to a specific biological activity of 2×10^8 IU/mg of protein. Labeling with ^{125}I was carried out by a modification of the original chloramine-T method of Hunter and Greenwood [19], as previously described [23]. Iodinated interferon was further purified on a column of Sephadex G-75 in 0.5% bovine serum albumin, to remove minor iodinated contaminants. Radioactivity incorporation was calculated from electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate [23]. A two-dimensional co-electrophoresis [25] of labeled and unlabeled interferon with subsequent extraction of the isolated spots, showed a complete coincidence of protein, radiolabel and antiviral activity. Estimations of recovery of biological activity after iodination showed no evidence of inactivation exceeding the error limits of our biological assay (95% confidence limits: $\pm 0.15 \log_{10}$ units/ml). Also, there was no decline in antiviral activity for a period of at least 2 months after iodination. Recombinant DNA-derived HuIFN-α_2 was obtained in pure form and was labeled with ^{125}I as described above. It was stable as documented by preservation of biological activity and minimal release of acid-soluble radioactivity.

Average specific radioactivities were 70 and 90 μCi/μg protein for HuIFN-α (Ly) and for HuIFN-α_2, respectively.

Isolation and perfusion of rabbit lungs

Male Californian rabbits weighing about 2.8 kg were used. Each animal was anesthetized by intravenous injection of Nembutal (25 mg/kg). A cannula was introduced in the trachea about 5 cm above the bronchial bifurcation. A heparinized (800 IU/ml) solution of saline was injected intravenously, and, after laparatomy and cannulation of the aorta, the animal was killed by exsanguination. The thorax was then opened, the pulmonary artery was cannulated, the lungs were removed and placed in the perfusion system previously described [5,13]. The organ was kept at 37°C and ventilated at 30 strokes/min with warm humidified air taking care to avoid inspiratory pressure above 8 cm H_2O. The perfusate was constituted by fresh homologous blood, deprived of platelets and leukocytes with a final hematocrit of about 20%. The blood, 230 ml for each perfusion, was delivered to the lungs at a mean perfusion pressure (Pp) of 20 mmHg and flow rate of 92 ± 14 ml/min. It was recirculated after being gassed with about 95% air – 5% CO_2 to achieve and maintain a pH of 7.4 and an oxygen saturation degree of about 98%. After an appropriate period of equilibration, 8–10 μCi of ^{125}I-labeled interferon, mixed with $3–5 \times 10^5$ IU of cold interferon, were either added to the perfusate or instilled into the left and right bronchi (a maximum of 0.25 ml each) A known volume of Evans Blue was added when the interferons were injected in the perfusion fluid. Direct instillation into the bronchi was preferred to aerosol administration [4] because preliminary experiments had indicated that the actual amount of interferon delivered could not be ascertained. Samples of blood were taken at predetermined intervals and both protein-bound radioactivity values and interferon titers were expressed as a percentage of the initial values, calculated on the basis of the plasma volume (determined by the Evans Blue dilution) and of the initial amount of interferon. Acid-soluble radioactivity was expressed as a percentage of total radioactivity after precipitation with trichloroacetic acid (TCA) (at a final concentration of 10%) in the presence of cold iodide and serum proteins. At the end of the perfusion, recoveries of radioactivity and biological activities were measured in fractions defined as follows. The residual plasma volume in the perfusion apparatus was defined as 'perfusate'. The residual pulmonary plasma volume was defined as 'circulatory wash' recovered after a 20 ml perfusion with plasma. The 'lung wash' was defined as the fluid obtained after rinsing the lungs with 40 ml saline via the trachea. After excising the trachea and large bronchi, the lungs were weighed and homogenized with 1 vol of saline. The homogenate was centrifuged at $105\,000 \times g$ for 1 h and the supernatant volume was defined as 'lung cytosol'. All samples were stored at –20°C until titration. Most of the cells present in the lung wash were macrophages.

Preparation of alveolar macrophage cultures

Alveolar macrophages were collected from normal rabbit lungs after instillation of 50 ml medium (RPMI-1640) containing 5 IU/ml of heparin. About 40 ml of medium was recovered, with an average of 2×10^7 cells, 93% of which, in separate tests, took up colloidal carbon and were identified as macrophages. The cell suspension was distributed in cell culture dishes and incubated at 37°C in an air/CO_2 (95/5, v/v) atmosphere for 2 h. Afterwards, the medium was collected and the dishes were thoroughly rinsed twice to remove nonadherent cells.

Antiviral activity assay

Antiviral activities of interferon were titrated in duplicate with a plaque reduction assay [20] using WISH cells and vesicular stomatitis virus as a challenge. The international reference preparation of HuIFN-α (G-023-901-527) with a defined potency of 4.3 $\log_{10}$IU/vial was included in all assays. When reconstituted in 1.0 ml of sterile distilled water, it had a geometric mean titer, in our assay, of 4.28 $\log_{10}$ IU/ml (SD = 0.069; n = 16). All titers reported here are in IU/ml.

Results

Fig. 1 shows the averaged results of several experiments in which mixtures of radiolabeled and cold, natural or recombinant DNA-derived interferons were introduced in the blood compartment of the lung perfusion system.

The disappearance of both natural HuIFN-α (Ly) and HuIFN-α_2 was mono-exponential and their fractional turnover rates were similar: 0.09 ($t^1/_2$ of 13 h) and 0.05 ($t^1/_2$ of 23.5 h) for the antiviral activities and protein-bound radioactivities, respectively. The increase of acid-soluble radioactivity was as little as 0.5% during 4 h. Thus, the biological behavior of both radioiodinated interferons was similar to that of the cold interferons. No disappearance occurred when the interferons were examined in the same conditions, without the lungs being inserted in the perfusion system. Table 1 (upper part) shows recoveries of antiviral activity and of total, protein-bound and acid-soluble radioactivities. It can be seen that recoveries were complete. The results were strikingly different when the interferons were instilled in the bronchial alveolar tree. Fig. 2 shows results obtained with natural HuIFN-α (Ly), when instilled into the bronchi at the beginning of perfusion. After a short lag period, protein-bound radioactivity rose rapidly and progressively in the perfusate. However, a concomitant

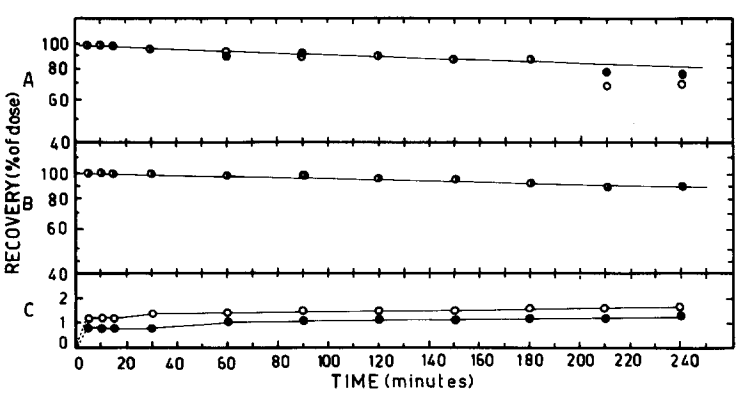

Fig. 1. Kinetics of disappearance of natural HuIFN-α (Ly) (o) and recombinant DNA-derived HuIFN-α_2 (•) from blood perfusing isolated, ventilated rabbit lungs. Mixtures of ^{125}I-labeled and cold interferons were added at time 0 h. Each point is the average of at least 2 experiments. A, antiviral activity; B, protein-bound radioactivity; C, acid-soluble radioactivity.

TABLE 1

Evaluation of interferon catabolism by the isolated rabbit lung

Introduction of interferon in perfusion system		Activity tested	% Recovery of interferon (4 h perfusion) in			
Site of administration	Type of interferon		Perfusate samples + circulatory washes	Alveolar washes	Lung tissue cytosol	Total
Intravascular	HuIFN-α(Ly)	Antiviral activity	86.6[a]	0.9	0.2	87.7
		Total radioactivity	106	0.55	1.1	107.6
		Protein-bound radioactivity	104	0.5	0.9	105.4
		TCA-soluble radioactivity	1.8	5.7	8.0	-
	HuIFN-α_2	Antiviral activity	96	3.2	3.0	102.2
		Total radioactivity	106	4.2	2.3	112.5
		Protein-bound activity	105	4.1	2.2	111.3
		TCA-soluble radioactivity	1.1	2.4	5.1	-
Intrabronchial	HuIFN-α(Ly)	Antiviral activity	1.9	0.4	0	2.3
		Total radioactivity	92	14.4	8.6	115
		Protein-bound radioactivity	82	14.0	7.8	103.8
		TCA-soluble radioactivity	12.5	2.5	9.1	-
	HuIFN-α_2	Antiviral activity	30.7	6.7	1.1	38.5
		Total radioactivity	81	16.2	5.3	102.5
		Protein-bound radioactivity	73	15.3	4.8	93.1
		TCA-soluble radioactivity	10	5.6	9.4	-

[a] Averages of at least 2 values obtained in separate experiments.

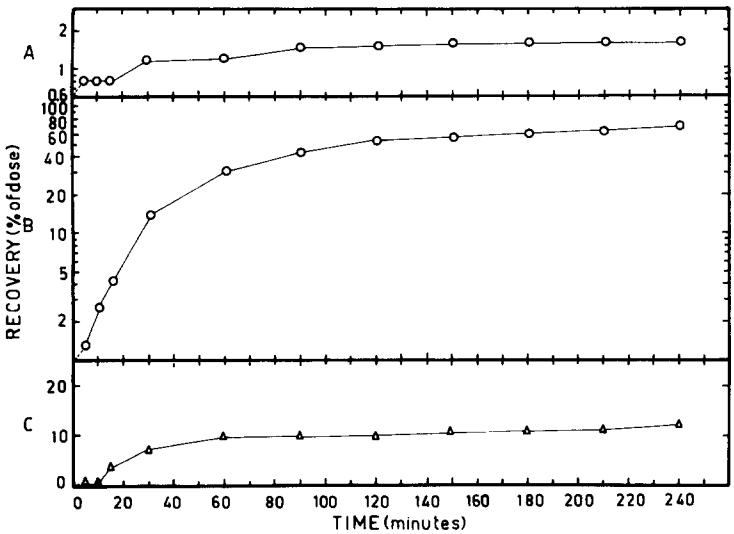

Fig. 2. Kinetics of appearance of natural HuIFN-α (Ly) in blood perfusing isolated, ventilated rabbit lungs. A mixture of ^{125}I-labeled and cold interferon was instilled in both bronchi at time 0 h. Each point is the average of 2 experiments. A, antiviral activity; B, protein-bound radioactivity; C, acid-soluble radioactivity.

increase of antiviral activity failed to occur. As shown in Table 1 (lower part) after 4 h, up to 82% of protein-bound radioactivity, but only 1.9% of antiviral activity had been transferred from the bronchial lumen to the perfusate and acid-soluble radioactivity had only increased up to 12.5%. The final protein-bound radioactivity/antiviral activity ratio was about 44. This result indicated that labeled and cold natural HuIFN-α (Ly) were absorbed by the bronchial-alveolar epithelia, with a limited breakdown, but that biological activity was lost while most of the protein-bound radioactivity could emerge in the circulating blood. In accordance with this only 14% of labeled and 0.4% of cold interferons were recovered in the lung washes.

Alveolar macrophages possess a potent proteolytic enzyme system which might participate in the breakdown of interferon. To test this possibility macrophage cultures (10^6 cells/ml) were incubated for 24 h with medium containing 0.01 μCi/ml of ^{125}I-labeled and 2000 – 10000 IU/ml of cold natural HuIFN-α (Ly). As a control, the interferons were also incubated with aliquots of the clarified alveolar lavage medium. Neither a significant increase of acid-soluble radioactivity (from 2.4% to 3.2%), nor any decrease in antiviral titer were seen. Very similar results were obtained when the interferons were incubated with the alveolar lavage medium, deprived of cells.

Because HuIFN-α (Ly) is a complex mixture of interferon subtypes [2], it was of interest to investigate the metabolic behavior of a single subtype. Fig. 3 shows results of such an analysis on the HuIFN-α_2 from *E. coli*, administered by bronchial instillation. In much the same way as it was the case with natural HuIFN-α (Ly), protein-bound radioactivity rapidly rose to an asymptote maximum level of about 60% of the injected dose. However, in contrast to the situation with HuIFN-α (Ly), significant

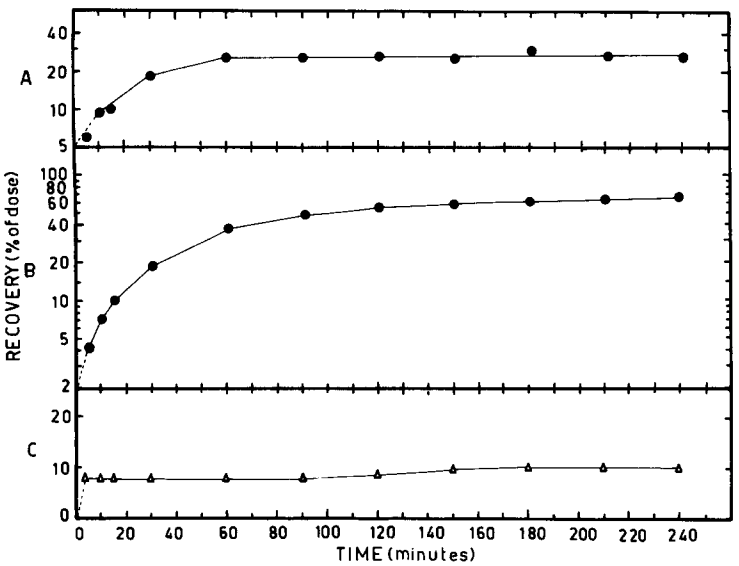

Fig. 3. Kinetics of appearance of human recombinant DNA-derived HuIFN-α_2 in blood perfusing isolated, ventilated rabbit lungs. A mixture of ^{125}I-labeled and cold interferon was instilled in both bronchi at time 0 h. Each point is the average of 2 experiments. A, antiviral activity; B, protein-bound radioactivity; C, acid-soluble radioactivity.

levels of antiviral activity became detectable in the perfusate. Thus, the final ratio between protein-bound radioactivity and antiviral activity levels was 2.5, against 44 in the case of natural HuIFN-α (Ly). Increments in levels of acid-soluble radioactivity were minimal. These data indicate that inactivation of HuIFN-α_2 during transepithelial transport was limited.

Discussion

Our previous work aiming at defining catabolic sites for circulating interferon has emphasized the major role of the kidney [6,8] and the liver [9,10]. The present results show that the lung, although possessing one of the largest endothelial surfaces of the body, has a minimal catabolic role. Clearance of both HuIFN-α (Ly) and HuIFN-α_2 from the perfusate was very slow, either with or without the lungs inserted in the perfusion system. Contrastingly, in vivo half-life values for interferon are of the order of magnitude of minutes [7,29]. Furthermore, the almost total absence of breakdown products is another indication of negligible cellular uptake and catabolism. Although minimal endocytosis (of the interferon–receptor complex) and catabolism may occur in all cells [1,3,7,33], only a few cell types, namely proximal tubular cells [6,8] and hepatocytes [9], can extensively internalize and break down circulating interferon. We have now shown that pulmonary endothelial cells and alveolar macrophages do not share this function.

In planning the present work we considered it theoretically and practically relevant to explore the fate of interferon administered in the bronchial lumen. It has already been demonstrated that inhaled albumin [15] and antigens [12] can be readily absorbed. Furthermore, studies done by Merigan et al. [21], Greenberg et al. [16] and Scott et al. [30], attempting to prevent rhinovirus infections by topical treatment with interferon indicated that a good degree of protection could be achieved only when applications were given frequently and when potent interferon preparations were used. These important observations, although only relevant to the nasal mucosa, suggested that several factors, such as the presence of the mucous layer [14] and proteases [26], possible absence of interferon receptors on the luminal pole of some cells and the efficiency of the mucociliary escalator [14] would, to variable extents, reduce the efficacy of interferon treatment in higher and lower tract respiratory infections. From our present work, it appears that HuIFN-α instilled into the bronchi is rapidly absorbed. However, considerable amounts of both interferons appeared to be inactivated during transport. The slightly different metabolic behaviour of these interferons is in line with the findings of Sarkar [28], that in mice, leukocyte-derived natural HuIFN-α and *E. coli*-derived HuIFN-α_1 have a different disappearance from plasma.

Actual protein breakdown appeared limited because increments in acid-soluble radioactivity in the perfusate were very modest. Inactivation could occur in the alveolar fluid due to proteases released from macrophages [17,18] and mast cells [32]. However, protease inhibitors, usually present in excess, ought to inhibit such proteolysis. In our in vitro experiments with alveolar macrophages no breakdown of interferon was noticed. Other putative sites of breakdown are the several cell types of the bronchial mucosa [27], the interstitial fluid and the endothelial cells during cellular transit. At this time there are no observations favoring one or the other site. The fact that the highest acid-soluble radioactivity was detected in the lung cell extract does not imply that this is the site of interferon inactivation. Indeed, lung homogenization is likely to increase proteolysis and acid-soluble radioactivity moves continuously towards the vascular pool.

Finally, on the basis of the present results, we would like to consider which route of interferon administration (parenteral and/or aerosol) during viral diseases of the lower respiratory tract is likely to be therapeutically advantageous. Because several physiological mechanisms operating in the bronchial-alveolar tract hinder the contact of interferon with the mucosa, it appears useful to administer frequent doses of potent interferon preparations by means of inhalation aerosol [24]. Although wastage of interferon due to partial deposition in the upper respiratory tract, exhalation, inactivation and absorption may be considerable, some interferon may reach mucosal cells and limit the spread of infection. Inhalation aerosol of interferon seems advantageous also because of the rapidity in achieving a local effect particularly important as interferon is mainly a prophylactic drug. Topical application should reduce also general side effects [10] mostly because the absorbed interferon becomes inactive. It will however be important to evaluate carefully the possible noxious effects at the bronchial level, although these appear fairly trivial after nasal spray [30]. On the other hand, parenteral administration of interferon may be less useful than inhalation

because circulating interferon is eliminated rapidly in vivo [7,29] and displays adverse side effects.

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