JOURNAL OF CLINICAL MICROBIOLOGY, July 1976, p. 106-107
Vol. 4, No. 1

Printed in U.S.A.

Recovery of Applied Human Leukocyte Interferon from the Nasal Mucosa of Chimpanzees and Humans

PAUL E. JOHNSON,* STEPHEN B. GREENBERG, MAURICE W. HARMON, BOBBY R. ALFORD, AND ROBERT B. COUCH

Department of Otorhinolaryngology and Communicative Sciences, and Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030*

Received for publication 9 February 1976

Intranasally applied human leukocyte interferon was recovered locally in 9 of 10 chimpanzees and 3 of 3 human volunteers tested. A 5- to 50-fold reduction in interferon was observed over 60 min.

A recent study in man demonstrated a prophylactic effect of intranasally applied interferon (IF) on infection and disease induced by a rhinovirus (4). A very large quantity of human leukocyte IF (14,000,000 units) was used and complete protection did not ensue, indicating a need for improved efficacy. One possible explanation for the apparently high in vivo dose requirement could be the clearance of IF before it can gain access to the nasal epithelial cells (5). This note reports on the recovery of locally applied leukocyte IF in both chimpanzees and normal volunteers.

Ten chimpanzees were anesthetized, and 10,000 units of human leukocyte IF (3) (kindly provided by the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases) were applied onto the right inferior turbinate by a Pasteur pipette calibrated to deliver 0.01 ml. The area of application (approximately 1 cm^2) was immediately scraped with a Freimuth curette (2) to obtain a zero-hour or base-line sample. The same dose of human leukocyte IF was then applied to the left inferior turbinate of each chimpanzee and scraped after an interval varying from 5 to 60 min. The samples were placed in 1.0 ml of medium 199 containing HEPES (*N*-2-hydroxyethyl - piperazine - N' - 2 - ethanesulfonic acid) buffer (25 mM), 0.3% bovine serum albumin, 2% fetal bovine serum, L-glutamine (10 mM), and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/ml; gentamicin, 50 μg/ml) and stored at −70°C.

To assay for IF activity, the samples were thawed, thoroughly mixed in a Vortex-Genie (Scientific Ind., Springfield, Mass.), and diluted in Eagle minimal essential medium containing 5% fetal bovine serum. Samples of 1.0 ml were applied in duplicate to monolayers of human foreskin fibroblasts (cell strain HR-202 from HEM Research Inc.). A yield reduction assay using 4.5×10^6 plaque-forming units per 0.1 ml of vesicular stomatitis virus as the challenge virus was used to measure IF activity. One unit of IF was that dilution of the sample that reduced the vesicular stomatitis virus yield by 50% compared with controls. In this assay system, 5 units of IF were equivalent to 1 unit of human IF research standard 69/19 from the Medical Research Council (Mill Hill, London, England).

Preliminary studies using 0.01 ml of a 2% methylene blue solution revealed the rapid spread of the dye immediately upon application to the nasal mucosa. The area scraped for the base line or zero-hour sample covered approximately one-tenth of the area in which the methylene blue or IF spread.

IF was recovered in the base-line sample in 9 of 10 chimpanzees tested and ranged from 200 to 1,000 units (Table 1). This low and variable recovery probably represents the immediate spread of the applied IF beyond the scraped area. An immediate loss of activity of leukocyte IF when mixed with human nasal secretions has not been found in other experiments performed in our laboratory (Harmon et al., submitted for publication).

A considerable loss of recoverable IF appears to have occurred within the first 5 min. The continued spread of the applied IF to the posterior nasopharynx during the first few minutes might account for the rapid decline of IF from the application site.

The amount of IF recovered 5 to 60 min after application was 5- to 50-fold less than the base-line sample. Although the loss of IF did not appear to be linear over the 60 min, there was a definite reduction in all samples tested. Three human volunteers also exhibited a similar 5- to 50-fold reduction in IF recovered 30 and 60 min after application (Table 2).

The method of sampling requires that some

TABLE 1. *Recovery of human leukocyte interferon[a] applied intranasally to chimpanzees*

Chimpanzee	Interferon recovered (units)[b]					
	0[c]	5	10	15	30	60
1	<10	10				
2	500	100				
3	500		10			
4	300		<10			
5	500			50		
6	500			50		
7	500				100	
8	200				10	
9	500					10
10	1,000					100

[a] 10,000 units of human leukocyte interferon in 0.01 ml applied to inferior turbinate.

[b] Determined by a vesicular stomatitis virus yield reduction assay.

[c] Time after application (min).

TABLE 2. *Recovery of human leukocyte interferon[a] applied intranasally to human volunteers*

Volunteer	Interferon recovered (units)[b]		
	0[c]	30	60
1	1000	100	
2	500		10
3	500		100

[a] 10,000 units of human leukocyte interferon in 0.01 ml applied to inferior turbinate.

[b] Determined by a vesicular stomatitis virus yield reduction assay.

[c] Time after application (min).

variability occur in the amount of mucus and cells removed with each scraping. The relatively constant recovery of IF at zero hour in most of the chimpanzees and human volunteers suggests that this should not be a major source of error. Because of the limited number of test animals used at each time period, statistical comparisons could not be made.

The recovery of IF 60 min after application suggests that not all of the applied IF was removed from the nasal mucosa. This IF was either attached to nasal epithelial cells or bound to mucus. Since the entire mucus content of the nasal passages is removed every 30 min as a result of mucociliary clearance, the recovered IF at 60 min may be cell associated (1, 6). The marked individual differences in clearance rates that have been demonstrated in volunteers may contribute to the variability in rate of loss of IF (5).

Our data in chimpanzees and man indicate that a significant removal of IF from the nose occurs within 1 h and confirm the low recovery of IF by Merigan et al. in nasal rinses obtained 2 h after the last dose of IF (4). We are presently evaluating alternative dosage schedules and methods of application so that human leukocyte IF can be used more economically and successfully for prophylaxis of respiratory viral infections.

We would like to thank Dr. Peter Gerone of the Delta Regional Primate Laboratory, Covington, La., for his cooperation in performance of the primate studies. We would also like to thank Shellie Scott and Christopher Thomas for their technical assistance.

This work was supported by Public Health Service Contract AI-42530 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Abramson, M., and L. A. Harker.** 1973. Physiology of the nose. Otolaryngol. Clin. North Am. 6:623-635.
2. **Alford, B. R., R. G. Douglas, Jr., and R. B. Couch.** 1969. Atraumatic biopsy of nasal mucosa. Arch. Otolaryngol. 90:180-184.
3. **Cantell, K., S. Hirnonen, K. E. Mogensen, and L. Pyhälä.** 1974. The production and use of interferon for the treatment and prevention of human virus infections, p. 3. *In* C. Waymouth (ed.), In vitro monograph no. 3. The Tissue Culture Association, Rockville, Md.
4. **Merigan, T. C., S. Reed, T. S. Hall, and D. A. Tyrrell.** 1973. Inhibition of respiratory virus infection by locally applied interferon. Lancet 1:563-567.
5. **Proctor, D. F., I. Andersen, and G. Lundquist.** 1973. Clearance of inhaled particles from the human nose. Arch. Intern. Med. 131:132-139.
6. **Proetz, A. W.** 1941. Essays on the applied physiology of the nose, p. 192-221, 239-266. Annals Publishing Co., St. Louis.