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Antiviral Activity of Intranasally Applied Human Leukocyte Interferon

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Previous studies in our laboratory have demonstrated that the development of antiviral activity of human leukocyte interferon (IF) in nasal epithelial cells is time and concentration dependent and that the loss of intranasally applied human leukocyte IF is rapid. The present studies compared the activity of IF applied intranasally either by nasal drops or by a saturated cotton pledget. Adult volunteers had IF applied to an area of nasal mucosa (2 by 2 cm^2) either by repeated nose drops or by a saturated cotton pledget that was applied to the nasal mucosa and left in place for 1 h. Nasal epithelial cells scraped from the area of application, as well as the control, untreated side of the same volunteers, were challenged with vesicular stomatitis virus. No significant reduction in mean virus yield was found in volunteers who received 80,000 U by nose drops. Significant reduction ($P < 0.025$) in mean virus yield was found in cells obtained 4 h after 80,000, 50,000, or 20,000 U was applied by cotton pledget or in volunteers pretreated with oral antihistamines prior to receiving 80,000 U by nose drops. These experiments indicate that nasal epithelial cells can be made antiviral in vivo by application of human leukocyte IF. However, practical usefulness of human leukocyte IF for prophylaxis against respiratory viral infections may depend on the method of local application.

Because of the antigenic diversity of respiratory pathogens, viral infections of the human respiratory tract are an appropriate target for the clinical application of interferon (IF) by virtue of its broad spectrum of antiviral activity. Several studies have shown that a variety of respiratory viruses are susceptible to small doses of IF in vitro, whereas human trials with locally applied IF have been followed thus far by variable effectiveness. Russian investigators (14) reported that human leukocyte IF preparations protected volunteers or naturally exposed individuals against an influenza virus infection; however, Merigan et al. found no significant effect of large doses of intranasally applied human leukocyte IF on an influenza type B virus infection in volunteers (11). In this latter study, a prophylactic effect on the clinical disease induced by a rhinovirus infection was demonstrated.

The large quantity of human leukocyte IF (approximately 14×10^6 U) used by Merigan et al. (11) for prophylaxis of the rhinovirus infection in volunteers indicates the need for improved efficacy of IF. Our results obtained in an in vitro nasal epithelial cell culture system and in our nasal clearance studies in chimpanzees and volunteers suggest that the development of significant in vivo antiviral activity may depend on the method of IF application employed (6-9). Since cells from the human nasal mucosa can be removed and studied in vitro (1, 8), the antiviral activity of locally applied human leukocyte IF on human nasal epithelium can be evaluated. The present study reports on the antiviral activity resulting from intranasally applied human leukocyte IF in volunteers.

MATERIALS AND METHODS

IF preparation. Human leukocyte IF (lot no. 73-2-12) was provided by the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases, Bethesda, Md. Leukocyte IF was prepared from buffy coat leukocytes stimulated with Sendai virus and purified by the technique of Cantell et al. (4).

Virus. Vesicular stomatitis virus (VSV), Indiana strain, was obtained from W. Rawls. The preparation of the virus pool used in this study has been described previously (7).

Cell cultures. Human foreskin fibroblasts (cell strain HR202 and HR207) from HEM Research, Inc.

(Rockville, Md.) were used from passage levels 7 through 12 to assay IF. Mouse L-cells were used for plaque assays of VSV. Both cell lines were maintained in Eagle minimal essential medium with Earles salts (MEM) purchased from Grand Island Biological Co. (Grand Island, N.Y.). MEM was supplemented with 10% heat-inactivated fetal bovine serum (Grand Island Biological Co.), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

IF assay. A description of the yield reduction assay used to measure IF activity has been previously published (8). Virus yields were determined by plaque assay in L cells. One unit of IF was defined as the dilution of the original sample that reduced the VSV yield by 50% when compared with controls. One unit of IF was equivalent to 0.4 U of human IF research standard B (69/19) from the Medical Research Council, Mill Hill, London, England. All values for IF are expressed in human IF research standard B (69/19) units.

Volunteers. Volunteers were healthy adults between the ages of 20 and 35. Written informed consent was obtained for all studies. Throat swabs were processed for routine virus isolation on the day of intranasal application of IF. Laboratory tests which included serum glucose, creatinine, electrolytes, liver function studies, and routine urinalysis were performed before and 1 month after the study. In addition, chest and sinus X rays were performed prior to administration of IF. Tests for hepatitis B surface antigen (HB_sAg) and anti-HBs antibody were kindly performed by F. Blaine Hollinger (Baylor College of Medicine). Parainfluenza type 1 (Sendai virus) antibody was measured by standard neutralization tests (5) in pre- and 1-month follow-up serum samples.

Intranasal application of IF. Volunteers received various doses of IF (8,000 to 80,000 U) which were applied onto the inferior turbinate of one nasal passage either by drops or by a saturated cotton pledget. The other inferior turbinate of each volunteer served as a control. Nose drops were applied once every 5 min for 1 h by a Pasteur pipette calibrated to deliver 0.01 ml. In other volunteers, a sterilized cotton pledget (2 by 2 cm^2, 75 mg; Johnson & Johnson absorbent cotton) saturated with 0.5 ml of phosphate buffered saline (PBS) containing IF was placed on one inferior turbinate; a similar cotton pledget saturated with PBS was placed on the opposite (control) inferior turbinate. After 1 h, both pledgets were removed. In several experiments, volunteers received antihistamine, 4 mg of chlorpheniramine maleate (Schering Corp., Kenilworth, N. J.), 12 and 1 h before IF was applied intranasally.

Nasal epithelial cells in the area of IF contact were removed with a Freimuth curette at various times after IF application and placed in a petri dish. Nasal epithelial cells removed from the untreated, control sides of the same volunteers were added to a second petri dish.

The nasal epithelial cell samples were placed in medium 199 which contained 0.3% bovine serum albumin (North American Biologicals, Inc., Miami, Fla.), l-glutamine (10 mM), and antibiotics (penicillin, 100 u/ml; streptomycin, 100 μg/ml; amphotericin B, 2.5 μg/ml). Medium 199 was buffered with 0.15% $NaHCO_3$ and 25 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (Grand Island Biological Co.)

Human nasal epithelial cell cultures. In each experiment, cells removed from volunteers were pooled into two groups, those removed from the IF-treated side of the nose and those removed from the control side of the nose. Both groups of cells were processed in parallel. The pooled cells were incubated at 37°C for 30 min. After incubation, the medium was removed, the cells were transferred into 15-ml plastic conical centrifuge tubes (Falcon Plastics, Oxnard, Calif.) and centrifuged at 200 × *g* for 10 min. Medium was removed, and the cells were dispersed by incubation at 37°C for 10 to 20 min in medium 199 containing 2.5% *N*-acetyl-l-cysteine and 0.05% ethylenediaminetetraacetic acid. For this treatment, the pH of medium 199 was adjusted to 8.1 (13).

After two washes in fresh medium 199, cells were counted and viability was determined by using 0.04% Trypan blue exclusion; viability averaged 50%. A total of 3×10^5 cells (1.5×10^5 viable cells) was aliquoted into each tube and challenged with 0.1 ml of VSV (average inoculum, 5×10^6 plaque-forming units). After virus adsorption, cell cultures were washed and assayed for VSV as described previously (8). The results are expressed as the sum of the 24-, 48-, and 72-h virus yields minus the 0-h virus titer. The difference in total virus yield between the cells removed from the IF-treated side and those obtained from the control side was used as a measure of antiviral activity. Statistical analysis employed Student's *t* test.

RESULTS

Antiviral activity of human leukocyte IF applied intranasally by drops. When 50,000 or 80,000 U of IF was applied by drops over 1 h, the cells removed from the area of application demonstrated no significant reduction in VSV yield compared with cells removed from the control (untreated side) of the same volunteers (Table 1). When 80,000 U of IF were applied by drops over 1 h to volunteers who were pretreated with antihistamines, the reduction in VSV yield (0.54 log) was found to be significant ($P < 0.025$ by Student's *t* test).

Antiviral activity of human leukocyte IF applied intranasally by saturated cotton pledget. Cotton pledgets saturated with varying amounts of IF in PBS were placed on the inferior turbinate for 1 h and then removed. Similarly, cotton pledgets saturated with an equal volume of PBS were placed on the opposite inferior turbinate and also removed after 1 h. Cells were removed from both sides of the nose at 4, 18, or 24 h after IF application and challenged with VSV, and the virus yields were measured as described. The results of these experiments are presented in Table 2. A significant reduction in

the VSV yield was found when 20,000 or more U of IF was applied by the saturated cotton pledget and the cells were removed by nasal scraping 4 h later. When 8,000 U was applied for 1 h and the cells were removed 4 h later, no significant reduction in VSV yield was found. Cells removed 18 h after application of 80,000 U demonstrated significant reduction in VSV yield, but cells removed 24 h after IF application did not.

There was no significant difference in results when volunteers were pretreated with antihistamines before IF treatment by cotton pledget. Those who received 80,000 U of IF demonstrated significant antiviral activity and those who received 8,000 U after pretreatment with antihistamines did not. However, no augmentation of antiviral activity was apparent in those volunteers pretreated with antihistamines who had received 80,000 U of IF by cotton pledget. In addition, IF-treated cotton pledgets removed from the nasal cavity after the 1-h application retained 50 to 100% of the IF originally employed.

Clinical observations in volunteers receiving intranasal IF. No virus isolates were found in the throat swabs taken on the day of the study in any volunteer. No volunteer experienced an untoward reaction after IF was administered. Although no temperatures were recorded, no subjective febrile reactions were reported by any of the volunteers. Tests of HBsAg and anti-HBs antibody on serum obtained before and 1 month after administration of IF showed no change in titers. No seroconversions to parainfluenza type 1 (Sendai virus) were demonstrated in three volunteers who were tested. In three volunteers tested, no IF was detected in the peripheral blood 10, 30, or 60 min after 80,000 U of IF was applied to the nasal mucosa by drops or saturated cotton pledget.

TABLE 1. *Antiviral activity of human leukocyte IF applied intranasally by drops*[a]

IF application (U/0.1 ml)[b]	VSV yield[c]		Reduction in VSV yield (log_{10})
	Untreated cells	IF-treated cells	
50,000	6.05 ± 0.03 (3)	5.88 ± 0.02 (2)	0.17
80,000	5.24 ± 0.11 (8)	5.34 ± 0.09 (7)	+0.10
80,000 + AntiH[d]	5.07 ± 0.16 (8)	4.53 ± 0.13 (7)	0.54[e]

[a] A 0.01-ml portion of IF was applied every 5 min for 1 h by a calibrated Pasteur pipette. Nasal epithelial cells from the IF-treated and untreated inferior turbinate were scraped 4 h later.

[b] Expressed in human IF reference standard 69/19 units.

[c] Geometric mean virus yield of 24-, 48-, and 72-h samples minus the 0-h sample ± standard error of the mean. Numbers in parentheses indicate the number of test samples containing 3×10^5 cells per tube. Each experiment used pooled cells obtained from at least three volunteers.

[d] Four-milligram antihistamine (AntiH) tablets given 12 and 1 h before application of IF.

[e] $P < 0.025$ by Student's t test.

TABLE 2. *Antiviral activity of human leukocyte IF applied intranasally by saturated cotton pledget*[a]

IF application (U/0.5 ml)[b]	Interval to nasal scraping (h)	VSV yield[c]		Reduction in VSV yield (log_{10})
		Untreated cells	IF-treated cells	
8,000	4	5.99 (1)	5.82 (1)	0.17
8,000 + AntiH[d]	4	6.23 (1)	6.18 (2)	0.05
20,000	4	6.65 ± 0.01 (2)	6.11 ± 0.05 (6)	0.54[e]
50,000	4	5.55 ± 0.23 (7)	4.81 ± 0.13 (9)	0.74[e]
80,000	4	6.26 ± 0.16 (7)	4.97 ± 0.05 (10)	1.29[e]
80,000 + AntiH	4	5.22 ± 0.01 (2)	4.13 ± 0.17 (3)	1.09[e]
80,000	18	5.18 ± 0.03 (7)	4.70 ± 0.18 (5)	0.48[e]
80,000	24	5.91 ± 0.17 (2)	5.55 ± 0.16 (2)	0.36

[a] A cotton pledget (2 by 2 cm^2) was saturated with IF or PBS and placed on each inferior turbinate for 1 h and then removed. Nasal epithelial cells from each inferior turbinate were scraped 4, 18, or 24 h later.

[b] Expressed in human IF reference standard 69/19 units.

[c] Geometric mean virus yield of 24-, 48-, 72-h samples minus the 0-h sample ± standard error of the mean. Numbers in parentheses indicate the number of test samples containing 3×10^5 cells per tube. Each experiment used pooled cells obtained from at least two volunteers.

[d] Four-milligram antihistamine (AntiH) tablets given 12 and 1 h before application of IF.

[e] $P < 0.025$ by Student's t test.

DISCUSSION

Previous studies in our laboratory had defined the time and dose requirements of in vitro antiviral activity in human nasal epithelial cells (6, 8). From these results, nasal epithelial cells appeared to have reduced IF sensitivity when compared with human foreskin fibroblasts. The development of full antiviral activity, however, appeared to follow a similar course in both types of cells. Equivalent antiviral activity could be demonstrated in both types of cells if the concentration of IF was substantially increased in the nasal epithelial cells (M. W. Harmon and S. B. Greenberg, unpublished data).

Even though nasal secretions do not inactivate large doses of IF, mucus could hinder the access of IF to the surface of the nasal epithelial cell, thereby contributing to the apparent disparity in the IF susceptibility between human nasal epithelial cells and the human fibroblast cells. In addition to the mucus barrier, mucociliary clearance mechanisms could alter the IF dose requirements needed in vivo (11). Recovery of intranasally applied IF in chimpanzees and humans 5 to 60 min after application was found to be 5- to 50-fold less than that in the base-line sample (9). These studies, therefore, suggested that significant antiviral activity in vivo would depend on the method of delivery of IF to the nasal mucosa and the extent of IF contact with the nasal epithelial cells.

The experiments presented in this report demonstrate the development of significant in vivo antiviral activity after intranasal application of human leukocyte IF. This antiviral activity was found when IF was applied by a saturated cotton pledget for 1 h or by nasal drops applied to volunteers who had been pretreated with antihistamines. Cells removed 4 or 18 but not 24 h after IF had been applied were resistant to virus challenge. Reduced VSV yields were obtained from nasal epithelial cells from volunteers who received either 80,000, 50,000, or 20,000 U of IF. However, cells exposed to 8,000 U in a saturated cotton pledget did not show evidence of reduced VSV yield. Therefore, the development of in vivo antiviral activity is dose dependent and the persistence of in vivo antiviral activity is of relatively short duration.

Although the IF concentration delivered by nose drops or cotton pledget was different, the total dose was similar. Indeed, the IF dose delivered by drops was actually fivefold more concentrated than the dose delivered by cotton pledget. In addition, the cotton pledgets were tested for residual IF activity, and they demonstrated almost complete recovery of the administered dose. This result suggests that only the amount of IF which was in direct contact with the cell was needed for the development of antiviral activity.

Pretreatment with antihistamines did not enhance the antiviral activity already demonstrated after application of 80,000 U of IF in a saturated cotton pledget; however, significant reduction in virus yield was found in volunteers pretreated with antihistamines before the application of 80,000 U of IF by nasal drops. These results suggest that the mucus layer overlying the nasal epithelial cells may be a barrier to IF. Because of the atropine-like activity of antihistamines (10), a decrease in mucus production may expedite access of IF to the surface of the nasal epithelial cells before normal mucociliary clearance mechanisms remove it from the nasal cavity. Alternatively, antihistamines or cotton pledget may be slowing mucociliary clearance through the presence of the cotton or reduced production of mucus.

Although the physiology of the nose has been described in great detail, we know little about the optimal method for intranasal administration of chemotherapeutic agents, such as IF (12). Indeed, several studies have described marked differences in distribution and effectiveness of intranasally applied substances (2, 3). Since the cotton pledget would not be a practical method of administering IF and because of the limited antiviral activity demonstrated with nasal drops, other methods of IF application need to be attempted. Nevertheless, the results of these experiments indicate that the nasal epithelial cells lining the nasal cavity can be made antiviral in vivo and that new methods can be found that will allow IF to be used in doses that are effective and economical for the prevention of respiratory viral infections.

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