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Intranasal Interferon-α_2 Treatment of Experimental Rhinoviral Colds

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The therapeutic efficacy of recombinant interferon-α_2 (HuIFN-α_2) in experimental infection with rhinovirus type 39 was assessed in two randomized, double-blind, placebo-controlled studies. Adult volunteers (serum neutralizing antibody titer, ≤1:2) were given 9×10^6 international units of HuIFN-α_2 three times a day for five days by intranasal spray (study 1) or drops (study 2) beginning 28 hr after rhinovirus inoculation. HuIFN-α_2 did not prevent rhinovirus infection or colds in either study. Treatment by nasal drops and to a lesser extent by spray was associated with significant reductions in duration and quantity of viral shedding. Treatment by drops was associated with significant but modest effects on nasal symptom scores and trends toward reduced quantities of production of nasal mucus. Despite lower nasal wash concentrations of interferon, HuIFN-α_2 drops appeared to have greater antiviral activity and therapeutic efficacy than did HuIFN-α_2 spray. These findings suggest that HuIFN-α_2 may not be therapeutically useful in treating naturally occurring rhinoviral colds.

Several studies have documented that intranasal administration of either leukocyte-derived human interferon (HuIFN-α) [1, 2] or recombinant DNA-produced interferon-α_2 (HuIFN-α_2) [3–5] is effective in preventing experimentally induced rhinoviral colds. The extent of protection depends on the dosage administered; high dosages ($2.25–4.56 \times 10^7$ IU per day) can prevent both infection and illness [2, 3, 5], whereas lower dosages (10^7 IU per day) reduce illness rates but do not prevent infection [4]. Two recent field trials have confirmed that intranasal administration of HuIFN-α_2 at a dosage of 10^7 IU per day is effective in preventing naturally occurring rhinovirus colds [6, 6a] (B. Farr, J. M. Gwaltney, Jr., K. F. Adams, and F. G. Hayden, unpublished observation). However, both tolerance studies [4, 7] and field trials [6] (B. Farr, J. M. Gwaltney, Jr., K. F. Adams, and F. G. Hayden, unpublished observation) have found unacceptable rates of nasal side effects after several weeks of interferon (IFN) administration at a dosage of 10^7 IU per day. Nasal irritation (blood-tinged nasal mucus, nasal stuffiness, and mucosal erosions or ulceration) has developed in about one-quarter of volunteers within three weeks of initiation of administration, and pronounced mucosal histopathologic abnormalities have occurred in more than one-half of HuIFN-α_2 recipients after administration for four weeks [7].

One alternative to long-term or seasonal prophylaxis is therapeutic administration. This communication describes two placebo-controlled, double-blind trials to assess the therapeutic efficacy of intranasal HuIFN-α_2 in a volunteer model of experimentally induced rhinovirus infection.

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Written informed consent in a form approved by the Human Investigation Committee of the University of Virginia was obtained from all participants. The guidelines for human experimentation of the U.S. Department of Health and Human Services and the University of Virginia were followed in the conduct of these studies.

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Materials and Methods

Subjects. Fifty-two healthy adult volunteers with titers of serum neutralizing antibody to rhinovirus type 39 of ≤1:2 were randomly assigned to receive HuIFN-α_2 or placebo in two separate studies (23 in the first and 29 in the second). Individ-

uals who had had upper respiratory tract illness or fever within two weeks or who were concurrently taking intranasal or oral medications (except oral contraceptives) were excluded from participation. The ratios of male to female subjects (mean age) were 7:5 (22.2 years) in the IFN group and 7:4 (20.6 years) in the placebo group in the first study and were 7:8 (20.5 years) in the IFN group and 9:5 (20.0 years) in the placebo group in the second study. Volunteers were housed individually in separate motel rooms from the day of viral challenge until five days afterward.

IFN. HuIFN-α_2 (Schering Corp., Bloomfield, NJ) was provided as a lyophilized powder (specific activity, $10^{8.0}$ IU/mg of protein) containing 2 mg of human serum albumin/ml and phosphate buffers. Lyophilized albumin identical in appearance and protein content served as placebo. The methods used for IFN reconstitution, viral challenge, and assays for IFN and antibody to HuIFN-α_2 have been detailed previously [5, 7].

Viral challenge. In both studies rhinovirus type 39 was administered by intranasal drops (0.25 ml per nostril) on two separate occasions 4–6 hr apart on the first day of the study. The total inoculum administered was 56 $TCID_{50}$ in the first study and 44 $TCID_{50}$ in the second.

Surveillance and sampling. The frequency and severity of clinical illness were determined by monitoring clinical symptoms (days 1–9 after challenge) and weighing expelled nasal secretion (days 1–5 after challenge) by previously described methods [8, 9]. Complete blood and differential counts were performed before viral challenge and one day after the end of treatments. In the second study nasal examinations were performed one day after the end of treatments and again two days later.

The rates of infection were determined by viral isolation and by measurements of titers of homotypic serum neutralizing antibody in paired specimens obtained on the day of viral challenge and three weeks later. Nasal wash specimens were collected before viral inoculation, on the first day after challenge before initiation of treatments, and for seven subsequent days; they were used for viral isolation [10] and for assay of IFN concentrations [11]. Viral collection broth containing sheep antibody to HuIFN-α_2 (final concentration, ~2,500 neutralizing units/ml) and repetitive washing of cell monolayers with PBS after an adsorption period of 1 hr were used to reverse the potential inhibitory effects of residual IFN in nasal wash specimens [10].

Experimental plan. In both studies IFN treatments were initiated at 28 hr after the first viral inoculation and continued three times a day for five days (total, 15 doses). Treatments were given at 4 PM, 8 PM, and midnight on the first day and at 9 AM, 3 PM, and 9 PM on the subsequent four days. The HuIFN-α_2 dosages per treatment, per day, and per volunteer were 9×10^6, 2.7×10^7, and 1.35×10^8 IU, respectively. In the first study, treatments were self-administered by the subjects by metered pump spray. Two sprays (0.10 ml) were given per nostril per treatment. In the second study, treatments were administered by the study staff in the same method used for administering the viral inocula [5, 12]. These treatments were given by nasal

Table 1. Infection rates and viral shedding in rhinovirus type 39–inoculated volunteers.

Study (delivery method)	Treatment (*n*)	Infection (%)	Seroconversion (%)	Virus-positive days (% of total days of observation)	Median duration of shedding (days)
1 (spray)	IFN (12)	83	17	46*	5.0
	Placebo (11)	100	55	71*	7.6
2 (drops)	IFN (15)	80	27	31*	3.8†
	Placebo (14)	93	36	68*	8.2†

NOTE. Infection was determined by viral isolation and/or seroconversion (a fourfold or greater rise in titer of homotypic serum neutralizing antibody). All infected volunteers, except one IFN recipient in study 2, shed rhinovirus type 39. The mean ± SD titers of neutralizing antibody in convalescent-phase serum were 1.6 ± 1.4 $\log_2$ in the IFN group and 2.9 ± 2.0 $\log_2$ in the placebo group in the first study and 1.9 ± 1.5 $\log_2$ for both groups in the second study. The total numbers of days of observation after the initiation of treatments were 83 and 77 in the IFN and placebo groups, respectively, in the first study and 105 and 97, respectively, in the second study.

* $P < .01$, IFN vs. placebo group, χ^2 test.

† $P < .03$, IFN vs. placebo group, Lee-Desu statistic.

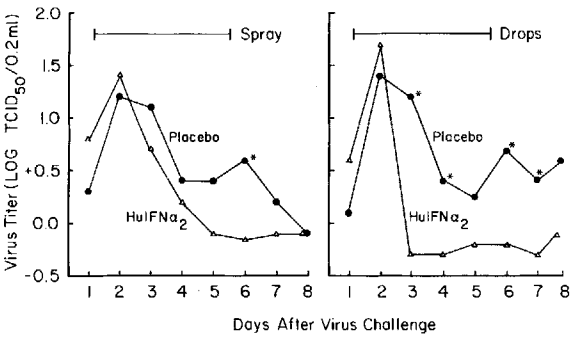

Figure 1. Quantitative shedding of virus in rhinovirus type 39–infected volunteers. Specimens that were positive for rhinovirus on initial isolation were serially diluted and titrated in quadruplicate monolayers of MRC-5 fibroblasts after one freeze (−70 C)-thaw cycle. The treatment period is indicated by the horizontal bar above each panel. The numbers of volunteers with laboratory-documented infection were 11 placebo and 10 IFN recipients in the first study and 13 placebo and 12 IFN recipients in the second study. Specimens that were negative for virus on initial isolation were assigned a value of -0.5 $\log_{10}$ $TCID_{50}$/0.2 ml for the purposes of calculation. The asterisk indicates a statistically significant difference ($P < .05$) between the IFN and placebo groups by Student's t test.

drops (0.25 ml per nostril) while the subjects were in a supine position. All treatments, clinical evaluations, symptom analyses, and virological studies were conducted under double-blind conditions.

Data analysis. The significance of differences in proportions was calculated by Fisher's exact test, of differences in symptom scores and IFN concentrations by Mann-Whitney U test, and of differences in other measures by Student's t test. In each instance P values were those for two-tailed testing.

Results

Infection rates and viral shedding. Of volunteers in the IFN and placebo groups, respectively, 83% and 100% developed infection in the first study and 80% and 93% in the second study (table 1). All infected subjects, except one IFN recipient in the second trial who only seroconverted, shed rhinovirus type 39. Slightly lower rates of infection were observed in the IFN groups in both studies. Overall, five (19%) of 27 IFN recipients did not have laboratory evidence of infection, as compared with one (4%) of 25 placebo recipients ($P = .23$ by Fisher's exact test). In the first study the proportion of IFN recipients who seroconverted (17%) was lower than in the placebo (55%) group ($P = .14$ by Fisher's exact test), but comparable seroconversion rates were observed in the two groups in the second study.

In both studies the proportion of virus-positive days, expressed as a percentage of the total days of observation after initiation of treatments, was significantly reduced in the IFN recipients compared with the placebo recipients (table 1). Relative to the corresponding placebo group, the magnitude of the reduction in the IFN group was 35% in the first study and 54% in the second. The median duration of viral shedding in IFN recipients was also reduced to a greater extent than in placebo recipients in the second study (4.4 days less) as compared with the first study (2.6 days less). The proportions of virus-positive specimens on days 7 and 8 after challenge, when nasal wash concentrations of IFN were low or absent, were 18% in IFN recipients vs. 50% in placebo recipients ($P = .028$) in the first study and 17% in IFN recipients, vs. 59% in placebo recipients ($P = .002$) in the second study.

Table 2. Illness, symptom scores, and production of nasal mucus in rhinovirus type 39–inoculated volunteers.

Study (delivery method)	Treatment (n)	Colds (% of all subjects)	Colds (% of infected subjects)	Nasal symptom score	Nasal mucus weight (g/4.5 days)	Nasal tissue count (no./4.5 days)
1 (spray)	IFN (12)	83	80	18 ± 7	17 ± 18	38 ± 46
	Placebo (11)	73	73	19 ± 16	26 ± 39	46 ± 53
2 (drops)	IFN (15)	73	75	16 ± 11	8 ± 8*	19 ± 13*
	Placebo (14)	79	77	20 ± 9	17 ± 16*	40 ± 44*

NOTE. Symptom scoring and the determination of colds were performed by using a modification [8] of the method of Jackson et al. [9]. Nasal symptom scores represent days 1–9 after challenge for all subjects; nasal mucus weights and tissue counts represent days 1–5. Where indicated, data are mean ± SD values.

* $.05 < P < .1$, IFN vs. placebo, Student's t test.

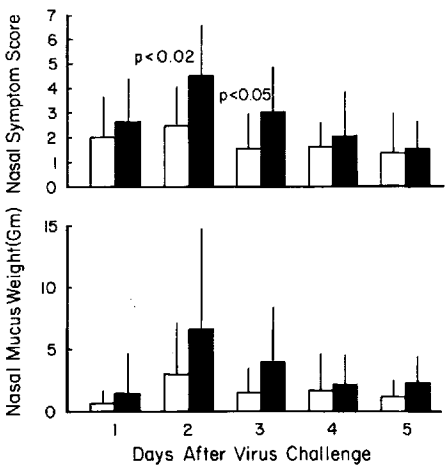

Figure 2. Nasal symptom scores and production of mucus in rhinovirus type 39–inoculated volunteers treated with HuIFN-α_2 or placebo by nasal drops. Treatments were initiated on the first day after viral challenge at 4 P.M. The nasal mucus weights on the first day after virus challenge represent 12-hr collections beginning at 8 P.M.

Figure 1 gives results of quantitative viral shedding studies on nasal wash samples from infected volunteers. Both placebo and IFN groups had rapid increases in mean viral titers, which peaked on the second day after viral challenge. In the first study (spray), the IFN recipients tended to have lower titers than the placebo recipients, but no significant differences were observed except for day 6 after challenge ($P < .01$). In the second study (drops), the IFN group had a prompt decrease in mean viral titers from day 2 to 3 after challenge and continued to shed low quantities of virus subsequently. Compared with the placebo group, the IFN group had significantly lower titers on days 3 ($P < .001$), 4 ($P < .05$), 6 ($P < .02$), and 7 ($P < .05$) after challenge.

Illness rates. About three-quarters of the subjects in each of the treatment groups met the criteria for colds (table 2). No differences in the proportion of subjects with colds were noted between the groups when all inoculated subjects or only those with laboratory-documented infection were considered. In the first study no important differences existed between the IFN and placebo groups with regard to nasal symptom scores, production of nasal mucus, or use of nasal tissues during or after treatments (table 2). In the second study total nasal symptom scores tended to be lower in IFN recipients compared with placebo recipients and were significantly lower on days 2 and 3 after challenge (figure 2). HuIFN-α_2 administration did not delay the time of illness onset but was associated with reductions in peak nasal symptom scores and production of mucus (figure 2). Production of nasal mucus and use of nasal tissues averaged ~50% lower in IFN recipients than in placebo recipients (table 2). In neither study were differences noted between the IFN and placebo groups in the frequencies of or scores for respiratory tract (cough, sore throat, and hoarseness) or systemic symptoms when analyzed separately (data not shown).

IFN concentrations. The results of bioassay of nasal wash samples for IFN activity are listed in table 3. In both IFN groups (spray or drops) considerable variability existed between individuals and to a lesser extent in the same individual on successive days. The IFN concentrations observed later in the treatment period (days 4, 5, and 6 after challenge) did not indicate substantial intranasal accumulation of HuIFN-α_2 after administration

Table 3. Nasal wash concentrations of IFN in HuIFN-α_2-treated volunteers.

Study (delivery method)	No. of subjects	Geometric mean (range) IU/ml of nasal wash on day after challenge.						
		1	2	3	4	5	6	7
1 (spray)	12	<19 (<19)	2,538 (75–38,400)	2,536 (300–38,400)*	5,061 (300–76,800)†	3,385 (75–19,200)	3,389 (150–38,400)	64 (<19–1,200)
2 (drops)	15	<19 (<19)	1,199 (300–9,600)	909 (19–19,200)*	1,376 (300–19,200)†	1,138 (75–19,200)	1,316 (75–38,400)	32 (<19–300)

NOTE. IFN concentrations were determined by bioassay [11]. Samples on day 2 after challenge were collected ~9 hr after the preceding IFN dose, those on days 3–6 at 12 hr after the preceding dose, and those on day 7 at ~36 hr after the last dose. Only 14 subjects were sampled on posttreatment day 5 in study 2.

* $P < .05$, spray vs. drops, Mann-Whitney U test.

† $P < .002$, spray vs. drops, Mann-Whitney U test.

Table 4. Effect of HuIFN-α_2 or placebo administered intranasally on blood counts in rhinovirus type 39–inoculated volunteers.

Cell type	Study 1		Study 2	
	IFN (n = 12)	Placebo (n = 12)	IFN (n = 15)	Placebo (n = 14)
Leukocytes ($\times 10^3$)	−0.51 (−7)*	1.15 (19)	−0.93 (−9)*	1.76 (32)
Granulocytes	−866 (−17)†	115 (5)	−1,079 (−18)*	848 (28)
Lymphocytes	281 (23)†	861 (61)	160 (15)†	870 (53)
Platelets ($\times 10^3$)	−28.3 (−9)*	5.3 (3)	−13.5 (−5)	−3.9 (−1)
Erythrocytes ($\times 10^6$)	0.11 (3)	0.08 (2)	0.08 (2)	0.22 (4)

NOTE. Data are mean absolute (%) change in no. of cells/mm³ from the baseline value. Baseline specimens were collected on the day of viral challenge and posttreatment specimens at ~12 hr after the last treatment.

* $P < .01$, IFN vs. placebo, Student's t test.

† $P < .05$, IFN vs. placebo, Student's t test.

by either method. Only low residual IFN activity was detected in nasal wash samples collected 36 hr after the last treatment (day 7 after challenge). The geometric mean concentrations observed in the group given IFN by spray were consistently two-to-four times higher than those of the group given IFN by drops on all treatment days and differed significantly on days 4 and 5 after challenge.

None of the volunteers had neutralizing activity against HuIFN-α_2 detected in convalescent-phase sera or nasal wash samples.

Side effects. In the first study, three IFN recipients but no placebo recipients reported the occurrence of blood-tinged mucus on one or two days during the postchallenge period. Because of these complaints all volunteers in the second study were asked daily about blood-tinged nasal mucus and examined at the end of the treatment period. In this study blood-tinged mucus was reported by 10 (67%) of 15 IFN recipients and by seven (50%) of 14 placebo recipients during or after the treatment period ($P > .5$). In those reporting blood-tinged mucus the mean and range of duration of symptoms were 2.7 and one to nine days in IFN recipients and 2.6 and one to five days in placebo recipients. On the last day of treatments nasoscopic examinations detected mucosal abnormalities of erythema, crusting, and punctate bleeding sites in two, four, and four placebo recipients, respectively, and in four, six, and five IFN recipients, respectively. Two days later another examination revealed fewer abnormalities in both groups, and bleeding sites were detected in only two placebo and two IFN recipients.

None of the volunteers developed leukopenia (white blood cell counts, <4,000/mm³). In both studies the posttreatment leukocyte and, in particular, granulocyte counts decreased in IFN recipients relative to their baseline values (table 4). These net decreases were significantly different from the net increases in leukocyte and granulocyte counts observed in the corresponding placebo groups (table 4). Furthermore, in both studies the placebo groups manifested increases in lymphocyte counts compared with baseline values that were significantly greater than the changes noted in the corresponding IFN groups.

Discussion

These studies are the first to assess systematically the therapeutic efficacy of intranasal IFN for infections with rhinovirus or other respiratory viruses. Intranasal administration of HuIFN-α_2 at a relatively high dosage (2.7×10^7 IU per day) beginning late in the incubation period did not prevent the development of experimental rhinoviral colds in susceptible volunteers. However, treatment with HuIFN-α_2 by nasal drops and to a lesser extent by nasal spray modified the virological course of infection and was associated with significant reductions in the duration and quantity of viral shedding. Treatment by nasal drops but not by spray was associated with significant but modest effects on the clinical course of the illness, as reflected in nasal symptom scores and trends toward lesser production of nasal mucus.

The incubation period of experimental rhino-

viral colds is variable and may be as long as seven days in a minority of cases [13]. In both the present (figure 2) and previous [5, 8] studies of experimental infection with rhinovirus type 39, increases in production of nasal mucus and nasal symptom scores have been observed on the first day after challenge. We chose to initiate IFN treatment 28 hr after viral inoculation to maximize the likelihood of detecting therapeutic efficacy. The dosage of IFN used in these studies was selected to fall within the range of dosages that had been previously shown to provide significant protection against both infection and illness when administered prophylactically and to be well tolerated during short-term (four to five days) administration [2, 3, 5]. The finding that IFN recipients tended to have lower infection rates than did placebo recipients suggests that IFN administration during the incubation period may have abrogated some infections, perhaps those in the subgroup of individuals who have longer incubation periods than the two- to three-day average [13].

HuIFN-α_2 administration by nasal drops appeared to have greater antiviral activity and therapeutic efficacy than administration by spray. These differences occurred in the presence of higher IFN concentrations in nasal wash samples from spray-treated volunteers (table 3). An explanation for this apparent paradox may lie in the observations of Aoki and Crawley [12] that the distribution of radiolabeled human serum albumin was wider when the solution was dropped in the nose of supine individuals than when administered by intranasal spray to seated persons. Nasal drops were used to administer HuIFN-α_2 in the second study, and it is possible that wider distribution and retention of IFN occurred with this method of administration. Because the viral inocula were also administered by nasal drops, it is possible that HuIFN-α_2 administered by drops was more likely to be distributed to the initial sites of viral replication in the nose. High concentrations of IFN recoverable in nasal wash samples were not predictive of a positive response in this model and may have simply reflected residual HuIFN-α_2 in the vestibule or other sites, where it was unlikely to exert an antiviral effect.

HuIFN-α_2 administration by spray or drops was generally well tolerated. In the second study (drops) the proportion of volunteers complaining of irritative symptoms or having mucosal abnormalities was relatively high in both the placebo and the IFN group. Other factors, such as the mechanical trauma associated with nasal washing and the treatments or the occurrence of rhinovirus-induced illness, may have contributed to this relatively high frequency of local abnormalities. As has been previously observed in volunteers treated with relatively high dosages of HuIFN-α_2 [5], significantly lower total leukocyte and granulocyte counts occurred in IFN recipients than in placebo recipients at the end of the five-day treatment period (table 4), although none developed leukopenia. The mechanism(s) accounting for HuIFN-α_2 related hematologic changes remain uncertain. In vitro studies have found that HuIFN-α_2 does not affect chemotaxis, adherence, or other functions of mature human polymorphonuclear leukocytes [14]. We did not collect blood samples during treatment in the current studies to look for evidence of systemic absorption of HuIFN-α_2 from the nasal mucosa.

The failure of HuIFN-α_2 administered intranasally to modify more substantially the clinical course of experimental rhinoviral infection despite administration of high dosages during the incubation period suggests that HuIFN-α_2 as a single agent may not be therapeutically useful in treating naturally occurring rhinoviral colds. Although correlations between the effects of therapeutic interventions in experimental rhinoviral infection and those in naturally acquired colds have not been clearly established, the results of the present studies suggest that HuIFN-α_2 administered intranasally as currently used would not significantly alter the clinical manifestations of an established illness but could possibly reduce the transmissibility of infection.

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