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Intranasal Interferon-α2 for Prevention of Natural Rhinovirus Colds

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The prophylactic activity of intranasal human interferon-α2 (HuIFN-α2) against natural rhinovirus colds was determined in a randomized, double-blind, placebo-controlled trial. A total of 304 working adults self-administered sprays of HuIFN-α2 (10^7 IU/day) or a placebo once daily. During 22 days of treatment, 13 (8.5%) placebo recipients but no HuIFN-α2 recipients had respiratory illnesses documented secondary to rhinovirus infection ($P = 0.0002$). The occurrence of illness with symptoms of tracheobronchitis was lower in HuIFN-α2 recipients (one eposide) than in placebo recipients (eight episodes, $P = 0.04$). In contrast, the frequency of nasal symptoms and the overall rate of respiratory illness were significantly higher in HuIFN-α2 recipients during weeks 2 and 3 of treatment. Symptoms (obstruction, discomfort, blood-tinged nasal mucus) or signs (punctate bleeding sites, erosions, superficial ulcerations) of nasal irritation occurred in 40 HuIFN-α2 recipients during week 3 ($P < 0.0001$ versus placebo recipients). Although the results of the current study were partially confounded by the nasal side effects of prolonged administration, they showed that intranasal HuIFN-α2 was efficacious in preventing rhinovirus colds under natural conditions.

The common cold is a major cause of morbidity and industrial absenteeism in the United States, afflicting adults an average of 2 to 4 and children an average of 6 to 8 times annually (2, 6). The antigenic heterogeneity of the causative viruses has prevented the development of a cold vaccine. For example, the most frequently isolated agent, the rhinovirus, has 89 identified serotypes and more that are recognized but presently unnumbered. Specific antiviral compounds have not yet been shown to have clinically useful prophylactic or therapeutic activity.

Prevention of natural colds has not been achieved previously except by geographical isolation. However, experimental rhinovirus infections in susceptible volunteers have been prevented by intranasal administration of multiple daily doses of either leukocyte-derived human interferon (HuIFN) (3, 13, 14) or recombinant DNA-produced HuIFN-α2 (9, 15). A recent study has also shown that single daily intranasal doses of HuIFN-α2 gave protection against experimental rhinovirus colds in volunteers (9).

The current randomized, placebo-controlled, double-blind study determined the prophylactic efficacy and tolerance of daily intranasal spraying with HuIFN-α2 in the prevention of natural rhinovirus colds.

MATERIALS AND METHODS

Population. The volunteer population consisted of 304 adult employees of the Eastern Regional Office of the State Farm Mutual Insurance Co. in Charlottesville, Va. Of the volunteers, 63% were women and 63% were younger than 35 years. The two treatment groups were similar in sex distribution, number of smokers, and mean age (HuIFN-α2 group [n = 151]: 94 females, 43 smokers, mean age 34.6 years; placebo group [n = 153]: 98 females, 33 smokers, mean age 34.9 years). The volunteers were randomly distributed throughout the workplace, an air-conditioned, 5-acre, partitioned, single-story building. Pregnant employees and those having serious medical conditions or using inadequate contraceptive methods were excluded from the study. This trial was conducted in September and October 1982 because previous epidemiological studies showed that the rate of rhinovirus-specific upper respiratory illness in this population peaks during this period (6).

Drug administration. Lyophilized HuIFN-α2 (Schering Corp., Bloomfield, N.J.), with a specific activity of $10^{8.0}$ IU/mg of protein, and a placebo identical in appearance (human albumin with protein content, pH, and tonicity identical to those of HuIFN-α2) were reconstituted in a phosphate-buffered solution containing the preservative thimerosal (0.002%). These solutions were stored at room temperature (22 to 24°C) in individual nebulizer units for use during 7 days of spraying and were replaced weekly.

Volunteers were randomly assigned (computer-generated randomization) to receive HuIFN-α2 (0.05 ml per spray, 10^7 IU/day) or placebo, two sprays per nostril once each day for 4 weeks. The sprays were self-administered under the supervision of a study nurse on weekdays; volunteers administered their weekend doses unsupervised. The treatment was originally intended to continue for 4 weeks, but drug administration was stopped after 22 days because of the frequent occurrence of symptoms and signs of nasal irritation.

Surveillance. The presence or absence of symptoms of respiratory (runny nose, stopped-up nose, sneezing, sore or scratchy throat, hoarseness, cough) and constitutional (sick feeling, fever, chills, headache, muscle ache, nausea, diarrhea) illness were recorded daily by the volunteers (6). Volunteers complaining of unusual, severe, or protracted symptoms were examined by a study physician. Complete blood counts, differential leukocyte counts, and platelet counts were done before the study and 1 day after spraying was discontinued.

Virology. Nose and throat specimens were collected from subjects reporting upper respiratory illness, and the swabs were transported on wet ice in beef heart infusion broth containing 1% bovine serum albumin, vancomycin (20 μg/ml), gentamicin (50 μg/ml), amphotericin B (1 μg/ml) and sheep anti-HuIFN-α2 antibody (2,500 neutralizing units per ml) (8). The broth was inoculated in 0.2-ml portions onto duplicate monolayers of MRC-5 fibroblast, HEp-2, primary rhesus monkey kidney, and human embryonic kidney cells

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TABLE 1. Rhinovirus infections in subjects with upper respiratory illness during treatment with HuIFN-α2 or placebo

Group	No. of subjects	No. of illness episodes	No. (%) of episodes cultured	No. (%) of episodes positive for rhinovirus
Placebo	153	64	41 (64)	13 (32)
HuIFN-α2	151	94	49 (52)	0 (0)
P		<0.001	>0.20	<0.001[a]

[a] $P < 0.0001$, placebo versus HuIFN-α2 group (number of positive episodes divided by the number of episodes cultured).

in 16- by 125-mm screw-capped tubes. After incubation for 1 h, the monolayers were washed three times with phosphate-buffered saline and then fed with a maintenance medium appropriate to the cell type. The use of anti-HuIFN-α2 antibody and monolayer washing has previously been shown to reverse the inhibitory effect of residual HuIFN-α2 on the recovery of rhinoviruses from respiratory specimens (8).

Acid-sensitive (pH 3) virus isolates from MRC-5 fibroblast monolayers that showed typical rhinovirus cytopathic effect were characterized as rhinovirus. Fibroblast monolayers showing rhinovirus-like cytopathic effect on initial culture to uninfected monolayers which failed to pass were characterized as yielding possible picornavirus isolates (five from the placebo and three from the HuIFN-α2 group).

Neutralizing activity of serum against HuIFN-α2. Sera were obtained from all subjects before and 2 weeks after the completion of drug administration to determine their neutralizing activity against HuIFN-α2. Blind samples were initially screened by a competitive radioimmunoassay (W. Protzman, unpublished data) in which samples are considered positive if a 1:5 dilution neutralized 10 IU of HuIFN-α2. Positive samples were retested by a microtiter bioassay (10, 16) in the laboratory of the Pediatric Infectious Disease Unit, University of Utah School of Medicine, Salt Lake City. The results of this assay are expressed as the serum dilution which neutralized the antiviral activity of 4 to 10 IU of HuIFN-α2. Four control sera, to which sheep anti-HuIFN-α2 antibody had been added to a final concentration of ca. 250 neutralizing units per ml, had neutralizing titers of 74 to 216 (mean, 160) in the bioassay.

Data analysis. An episode of upper respiratory illness was defined by the criteria used in previous studies of this population as one respiratory symptom (except sneezing) on 2 or more consecutive days or at least two respiratory symptoms on the same day (6). Other criteria for defining colds (rhinorrhea on at least 3 consecutive days) or tracheobronchitis (cough on at least 3 consecutive days) were also used for episode analysis. Separate illnesses were defined as occurring at least 3 days apart.

Significance of differences in proportions was calculated by Fisher's exact test. The occurrence rate of upper respiratory illness was analyzed for each week of the study after subtracting volunteers who were no longer at risk of becoming ill (e.g., because of illness continuing from the previous week), and the rates of the two treatment groups were compared by the chi square test. In each instance, *P* values were determined by two-tailed testing.

RESULTS

Rhinovirus infection. The number of rhinoviruses isolated from volunteers with upper respiratory illness was significantly reduced by HuIFN-α2 prophylaxis (Table 1, Fig. 1). Among the placebo recipients, rhinoviruses were isolated from 13 (8.5%) volunteers with symptoms, whereas none were isolated from the HuIFN-α2 recipients during treatment ($P = 0.0002$, Fisher's exact test). If the enterovirus and possible picornavirus isolates are included, 19 (12.4%) placebo recipients had positive picornavirus cultures, compared with 3 (2.0%) HuIFN-α2 recipients ($P = 0.0006$). The calculated efficacy of intranasal HuIFN-α2 in preventing rhinovirus-specific illness was therefore 100%; its efficacy in preventing illness related to proven and possible picornavirus infection was 84%.

During the 4-week followup period rhinovirus was isolated from five members of the placebo group and three members of the HuIFN-α2 group (Fig. 1). The first posttreatment rhinovirus isolation from a HuIFN-α2 recipient occurred on day 11 after cessation of therapy, whereas the first posttreatment isolation from placebo recipient occurred on day 6. Herpes simplex virus was isolated from two HuIFN-α2 recipients with herpes labialis (one during and the other 3 days after spraying).

Upper respiratory illness. The overall number of upper respiratory illness episodes was not reduced by HuIFN-α2 during the treatment period (Table 1). In fact, episodes were more frequent during weeks 2 and 3 of spraying in the HuIFN-α2 group than in the placebo group (Fig. 2) ($P < 0.02$, chi square test). During treatment, however, the number of episodes with at least 3 days of rhinorrhea was lower in the HuIFN-α2 recipients (15 episodes) than in the placebo recipients (20 episodes). Of 14 placebo recipients who had cultures taken, 6 had proven rhinovirus infection with such illness episodes compared with 0 of 10 HuIFN-α2 recipients ($P = 0.04$, Fisher's exact test). The number of episodes with at least 3 days of cough was lower in the HuIFN-α2 group (one episode) than in the placebo group (eight episodes) during the treatment period ($P = 0.04$). The number of volunteers who reported missing work because of illness of any type (13 HuIFN-α2, 10 placebo) and the total of number of work days missed (17 days for HuIFN-α2 and 14 days for placebo recipients) were similar for the two groups during the treatment period.

Weekly analysis of individual symptoms showed that the increased overall illness rate in the HuIFN-α2 group was due to an increased frequency of nasal symptoms, particularly nasal obstruction (stopped-up nose), in this group during

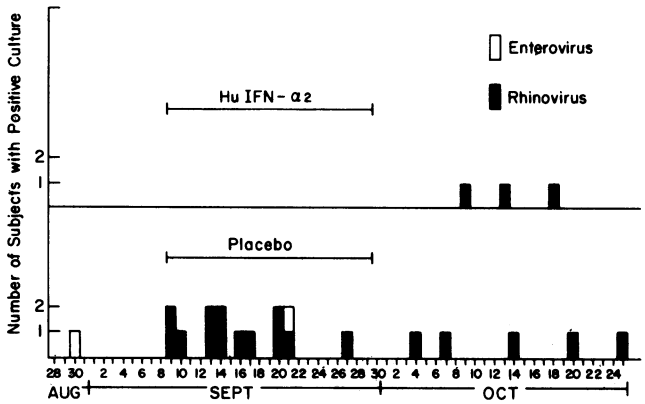

FIG. 1. Number of ill subjects from whom rhinovirus or enterovirus was isolated, shown by date of isolation. The 22-day treatment period is indicated by the horizontal bar above each graph. The enterovirus isolated during the treatment period was identified as echovirus type 24 by neutralization testing.

weeks 2 and 3 of therapy. During week 3, 41 (27%) HuIFN-α2 and 14 (9%) placebo recipients reported this symptom ($P < 0.0001$). In contrast, the frequency of cough was lower for the HuIFN-α2 recipients (3 of 151) than for the placebo recipients (11 of 153) during week 3 ($P = 0.055$).

During week 3 of treatment, 48 subjects complained of persistent nasal obstruction, nasal discomfort, or blood-tinged nasal mucus or a combination of these symptoms; this group included significantly more HuIFN-α2 recipients (40 of 151, 26.5%) than placebo recipients (8 of 153, 5%) ($P < 0.0001$). HuIFN-α2 recipients with persistent complaints also had abnormalities of the nasal mucosa more frequently (39 of 40, 97.5%) than did placebo recipients with similar complaints (4 of 8, 50%) ($P = 0.003$). Among the HuIFN-α2 recipients, 25 had mucosal erythema, 28 had erosions or superficial ulcerations of the mucosa, and 24 had punctate bleeding sites. Among the placebo recipients, the numbers were 1, 1, and 2, respectively. These symptoms usually resolved spontaneously during the first (66% of subjects) or second (90%) week after cessation of HuIFN-α2 therapy. Objective mucosal abnormalities usually healed within 2 weeks, but one-third of those with ulcerations required more than 2 weeks for complete healing. All subjects had normal nasal mucosa when reexamined 4 months after cessation of spraying.

Hematology. Compared with pretreatment values, hematology studies revealed a mean 10.3% decrease in leukocytes in the HuIFN-α2 recipients (−740 cells per mm^3), compared with a mean 5.8% increase in placebo recipients ($P < 0.01$, Student t test). The decline in the mean leukocyte count was principally accounted for by a decrease in the mean granulocyte count (−733 cells per mm^3), in the HuIFN-α2 group. Sixteen HuIFN-α2 recipients developed leukopenia (<4,000 leukocytes per mm^3) during therapy, compared with two placebo recipients ($P = 0.0009$). The count was ≥3,000/mm^3 in each instance and had returned to base-line values when retested 2 months after the trial. Of 16 HuIFN-α2 recipients with leukopenia, 10 (62.5%) had symptoms and signs of nasal irritation during week 3 of treatment, compared with 30 to 135 (22%) without leukopenia ($P = 0.003$).

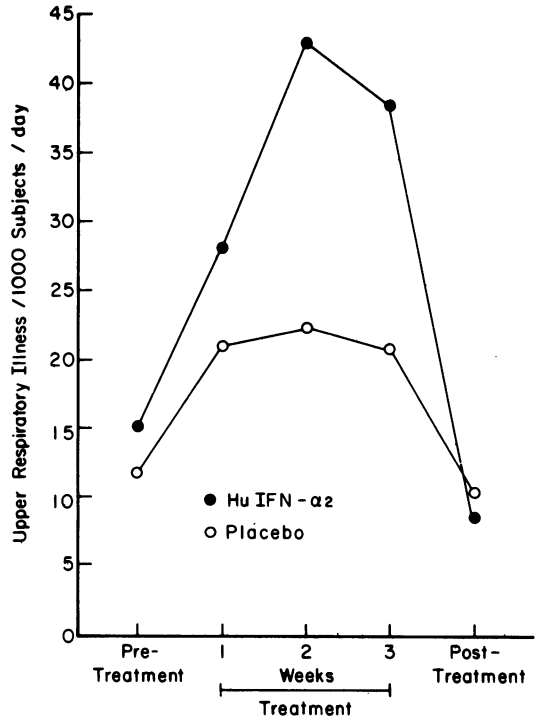

FIG. 2. Rate of upper respiratory illness during a 10-day pretreatment period, treatment weeks 1, 2, and 3, and a 10-day posttreatment period. The illness rates were significantly ($P < 0.02$) higher in the HuIFN-α2 group than in the placebo group during weeks 2 and 3.

HuIFN-α2-neutralizing activity of serum. Neutralizing activity against HuIFN-α2 was found by radioimmunoassay in the posttreatment serum sample from 1 of 151 HuIFN-α2 recipients and 0 of 153 placebo recipients. In the bioassay, the titer in the serum of this one subject was 1:15 in the pretreatment sample, 1:81 at 2 weeks after the completion of treatment, and 1:16 when tested 7 months later. This volunteer was then reexposed to intranasal HuIFN-α2 (10^6 U twice daily for 12 days). At 2 weeks after the end of exposure, the titer was <1:3 in the bioassay and negative in the radioimmunoassay.

HuIFN-α2 concentrations in serum. Of 37 randomly selected HuIFN-α2 recipients who had pretreatment IFN concentrations in serum of ≤9 IU/ml, as determined by microtiter bioassay, 3 had equivocal (19 IU/ml) and 1 had detectable (75 IU/ml) IFN activity in serum samples collected approximately 24 h after the last HuIFN-α2 dose. Two of these four volunteers had abnormal nasal mucosa, but none developed leukopenia. When the samples were tested again after an additional freeze-thaw cycle, they were negative for IFN activity as determined by microtiter bioassay in two separate laboratories (16).

DISCUSSION

In this study, HuIFN-α2 spray was highly effective in preventing natural rhinovirus colds. This is the first study to show the prophylactic efficacy of any IFN preparation against natural colds. We believe that the results indicate prevention of infection rather than suppression of viral growth in cell culture, because previous work has shown the effectiveness of the isolation techniques used for this study in recovering rhinoviruses from respiratory secretions containing HuIFN-α2 (8). This conclusion is further supported by the lower frequency of illness episodes with symptoms characteristic of tracheobronchitis in the HuIFN-α2 recipients during the treatment period. In addition, Betts and coworkers recently reported that a comparable dose of intranasal HuIFN-α2 given in divided doses was also effective in preventing rhinovirus infections (R. F. Betts, S. Erb, F. Roth, et al., Proc. Int. Congress Chemother. 13th, abstr. no. SE 4.7/1-5, p. 60/13–60/15, 1983).

One particularly appealing feature of IFN antiviral activity is its prolonged time of effectiveness after interaction with host cells. Earlier in vitro studies with human leukocyte- or fibroblast-derived IFN showed that a concentration-dependent antiviral effect is achieved within minutes after human fibroblast or nasal epithelial cells are exposed to IFN (1, 7), and studies with nasal epithelial cells showed that this effect persists for at least 72 h after the IFN is removed. Greenberg et al. (4) found that significant in vitro antiviral activity persists in nasal epithelial cell scrapings obtained up to 18 h after in vivo exposure to leukocyte-derived HuIFN. The results of the current study confirm our observations in experimentally induced rhinovirus infections in volunteers (9) that single daily applications of HuIFN-α2 give protection against rhinovirus colds.

The HuIFN-α2 recipients in the current study had significantly more episodes that met our criteria for upper respiratory illness than did the placebo recipients. These episodes were principally caused by the nasal side effects of the treatment and occurred during weeks 2 and 3 of treatment. The HuIFN-α2 group in this study also showed abnormalities of the nasal mucosa more frequently than did the placebo group. These side effects are believed to be primarily due to the HuIFN-α2 itself (10), but the preservative (thimerosal) or the mechanical effect of repeated spraying could have contributed to them. Earlier studies that used similar doses of HuIFN-α2 without preservatives found only mild signs or symptoms of nasal mucosal irritation in 23% of the recipients after 28 days of exposure (10). However, sequential nasal biopsies documented the occurrence of reversible mononuclear cell infiltrates in 58% and epithelial microulcerations in 15% of the recipients after 4 weeks of intranasal administration (10). In the current study, both the placebo and the HuIFN-α2 groups reported substantially higher rates of respiratory illness during the treatment period than were observed in previous epidemiological studies of this population (6). Although the placebo recipients did not experience increased rates of respiratory illness during successive weeks of exposure (Fig. 2), we cannot exclude the possibility of a toxic interaction between HuIFN-α2 and the thimerosal preservative.

Neutropenia has previously been observed after parenteral therapy with various IFN preparations (5). The results of this and of previous volunteer studies (9) indicate that reversible leukopenia and neutropenia can occur when HuIFN-α2 is administered intranasally. The higher occurrence rate of signs and symptoms of nasal irritation in the leukopenic volunteers compared with that in the nonleukopenic volunteers suggests that abnormalities in the nasal mucosa induced by HuIFN-α2 were related to the development of leukopenia. One explanation for this association could be a greater systemic absorption of intranasal HuIFN-α2 in subjects with altered mucosal integrity. However, this study did not document the presence of HuIFN-α2 in serum samples collected 24 h after the last treatment, and we cannot be certain that HuIFN-α2 was the cause of the neutropenia.

The occurrence of the local side effects documented in this study suggest that long-term prophylaxis with daily, intranasal administration of 10^7 IU of HuIFN-α2 is not feasible. However, this does not exclude the possibility of short-term use immediately after exposure to a common cold. The most appropriate site for this use would be in the home, since most colds are acquired there and the time of exposure would be known. Other approaches to reducing the nasal side effects may include altering the dosage or dosage intervals, using different preservatives or methods of administration (i.e., as drops rather than as spray), and adding topical anti-inflammatory compounds (e.g., beclomethasone) to the HuIFN preparation. Two controlled trials that used intranasal drops with lower dosages of leukocyte-derived HuIFN (5×10^3 to 10×10^3 IU/day) have been performed; partial protection against febrile respiratory illness was found, and no intolerance over the 2- to 3-month exposure period was reported (11, 12),

Although the results of the current study were partially confounded by the nasal side effects of prolonged topical administration, they did show that intranasal HuIFN-α2 was effective in preventing rhinovirus colds under natural conditions. Further investigations into the practical application of HuIFN in controlling the spread of colds are warranted.

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