

Effect of Induced Interferon in Experimental Rhinovirus Infections in Volunteers

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Nasal administration of an interferon inducer, CP-20,961 (*N,N*-dioctadecyl-*N',N'*-bis(2-hydroxyethyl)propanediamine), was evaluated in a double-blind, placebo-controlled study of experimental rhinovirus infection in 29 volunteers. Detectable nasal interferon developed in 10 of 15 subjects treated with CP-20,961, and 2 of 14 in the group receiving placebo ($P < 0.006$). Titers of CP-20,961-induced interferon ranged from 10 to 250 international units, concentrations similar to those observed in rhinovirus infections. Ten in the CP-20,961 group and eight in the placebo group became ill ($P > 0.05$). However, mean total sign scores were significantly diminished among CP-20,961-treated subjects as compared with placebo-treated subjects ($P < 0.025$). No significant effect was noted on quantitative virus shedding patterns or neutralizing antibody responses. These findings suggest that such concentrations of induced interferon do not protect against rhinovirus infection, and that factors other than interferon may be important in recovery from rhinovirus infection.

Viral respiratory illnesses account for more time lost from school and from work than any other cause (6). Rhinovirus infections, which are a major cause of such illnesses, present a formidable problem to control because of the multiplicity of serotypes and reported failures of candidate vaccines (4, 10). Thus, chemotherapeutic agents should be evaluated in regard to prophylaxis and treatment of such infections. Topical use of interferon or an inducer of interferon is especially attractive because previous studies have suggested a role for nasal interferon in recovery from rhinovirus infections (1, 5, 7) and because of the possible broad-spectrum effect of interferon.

Merigan et al. (9) have shown an effect of topically applied interferon in experimental rhinovirus infections. Hill et al. (7) showed reduced incidence of colds in subjects receiving poly I:C, a toxic interferon inducer, intranasally, and Gatmaitan et al. (5) showed decreased severity of illness in subjects receiving CP-20,961 [*N,N*-dioctadecyl-*N',N'*-bis(2-hydroxyethyl)propanediamine], a novel low-molecular-weight interferon inducer. Since the presently available interferon preparations for humans are both expensive and difficult to obtain, nontoxic interferon inducers now available need more investigation. In the present study, nasal administration of CP-20,961 was evaluated in a double-blind, placebo-controlled

study of experimental rhinovirus type 13 infection in volunteers.

MATERIALS AND METHODS

Volunteers. Subjects were healthy adult male university students between the ages of 18 and 25 who were shown to possess low or undetectable titers of serum-neutralizing antibody to rhinovirus type 13. Procedures used to recruit the volunteers insured their freedom of choice to participate, and volunteers gave written informed consent to participate in the study (8). Before and at the end of the study, volunteers underwent complete history, physical examination, and laboratory evaluation of hematological, renal, and hepatic function.

Drugs. CP-20,961 was supplied by Central Research, Pfizer, Inc., in individual dose pipettes containing 25 mg/0.5 ml in a vehicle consisting of equal parts glycerin and polysorbate 80. Identical-appearing placebo containing only the vehicle was also supplied.

Study design. For the first 10 days of the study, volunteers were confined to a 30-bed unit of a university dormitory which contained 10 one-bed rooms, 10 two-bed rooms, and common bathroom and dining facilities. Volunteers were randomly assigned to CP-20,961 or placebo treatment in a double-blind fashion. Treatments, 0.25 ml by coarse spray per nostril, given at 8:00 AM, 12:00 noon, 4:00 PM and 8:00 PM began on the first day of confinement. Forty-nine hours after starting treatment, and 1 h after a drug dose, the virus inoculum was administered to the volunteers. Drug administration was

continued for 5 full days after virus inoculation, or 7 days in all.

Clinical evaluations. Volunteers were examined by a physician, who was uninformed about treatment group, twice daily, at 8:00 AM and 5:00 PM, for signs and symptoms of respiratory tract illness. Severity scores (1 to 3+) for specific signs and symptoms referable to the respiratory tract (e.g., nasal obstruction, nasal discharge, etc.) were assigned, and mean total scores for each group were calculated. In addition, overall evaluations for presence of illness and site of involvement (e.g., rhinitis, pharyngitis, tracheobronchitis, systemic illness) were made at the time of each evaluation. Oral temperatures were recorded at 8:00 AM, 12:00 noon, 4:00 PM, and 8:00 PM.

Virus inoculum. The rhinovirus type 13 stock used in the present study was prepared, safety tested, and administered by described methods (2, 3). Simultaneous titration in WI-38 cells revealed that the inoculum contained eight 50% tissue culture infectious doses (TCID₅₀). Previous studies with this inoculum have shown the 50% human infectious dose (HID₅₀) to be 0.10 TCID₅₀ (2).

Virological procedures. Ten milliliters of veal infusion broth containing 0.5% bovine albumin was instilled into the nasopharynx, and the effluent (nasal wash) was collected daily during the confinement period and tested in WI-38 cell cultures by described methods (3). The first and last virus isolates from each volunteer were identified by neutralization tests with hyperimmune guinea pig sera (3). Quantitation of virus in specimens was performed by 10-fold tube dilution titrations in WI-38 cultures (3). Before inoculation, throat and anal swabs were also collected and tested for the presence of extraneous viruses in rhesus monkey kidney cell cultures.

Antibody determinations. Serum and nasal secretion (NS) specimens were obtained before and 4 to 6 weeks after inoculation. NS were obtained by instilling 10 ml of lactated Ringer solution into the nasopharynx and collecting the effluent fluid plus secretions discharged by blowing. This material was homogenized in glass tissue grinders, clarified by centrifugation, and concentrated 10-fold by air drying in dialysis tubing. In previous tests, the mean immunoglobulin A level in concentrated specimens was 0.340 mg/ml, and the range was 0.125 to 1.78.

Interferon assay. NS were collected each morning 1 h after treatment. Before testing, they were concentrated 10-fold and treated with ultraviolet irradiation. Tests for interferon were performed in human neonatal foreskin fibroblast cell cultures challenged with vesicular stomatitis virus (11). To insure sensitivity of the assay, each assay incorporated a standard interferon preparation containing 600 international units as compared with the human interferon standard A (10,000 units/ml) obtained from Medical Research Council, England. Titers are expressed as reciprocals of dilutions inhibiting 50% plaque-forming units. Previous studies have shown that the vesicular stomatitis virus inhibitor detected in nasal secretions is heat and acid resistant but sensitive to trypsin (1).

RESULTS

Illness. Clinical responses are shown in Table 1. As indicated, 15 subjects were treated with CP-20,961, and 14 subjects received placebo. Ten of the 15 in the former group and 8 of 14 in the placebo group became ill ($P > 0.10$; Fisher exact test). In addition to the lack of differences in frequency of illness, the type of illness (not shown) in both groups was similar. The most prominent manifestation was rhinitis (1 or 2+ severity), and many volunteers also exhibited pharyngitis (1+ severity). Only one volunteer had fever (102 F; 38.9 C), and he was in the CP-20,961 group. The mean duration of illness was 4.2 days for the CP-20,961 group and 4.75 days for the placebo group ($P > 0.05$), although two illnesses in the CP-20,961 group lasted only 24 h.

Mean total symptom and total sign scores were calculated from the twice-daily observations. The mean total sign score for the CP-20,961 group was significantly less on the evening of the first day ($P < 0.05$; two-sample *t*-test) and morning of the second day after virus inoculation ($P < 0.025$) (Fig. 1). At the same time, mean total symptom scores also tended to diverge, 2.2145 versus 4.3000, but the difference was not significant ($P = 0.10$). No other significant differences were noted between total sign or symptom scores at other intervals.

In addition to total symptoms and sign scores, scores for each sign and symptom were analyzed separately. The total area under the curve for each sign and each symptom was not significantly different between the two groups either before or after viral inoculation.

Infection. Fourteen of 15 in the CP-20,961 group and 10 of 14 in placebo group shed rhinovirus type 13 in nasal wash specimens. ($P > 0.20$; chi-square test). The one volunteer in the CP-20,961 group who did not shed rhinovi-

TABLE 1. Clinical responses to intranasal inoculations with rhinovirus type 13^a

Group	No. of volunteers	No. of volunteers with indicated response			
		Illness	Virus shedding	Four-fold serum antibody response	Interferon in nasal secretions ^b
CP-20,961	15	10	14	14	10
Placebo	14	8	10	11	2

^a Dose = 8 TCID₅₀.

^b $P < 0.006$; Fisher exact test.

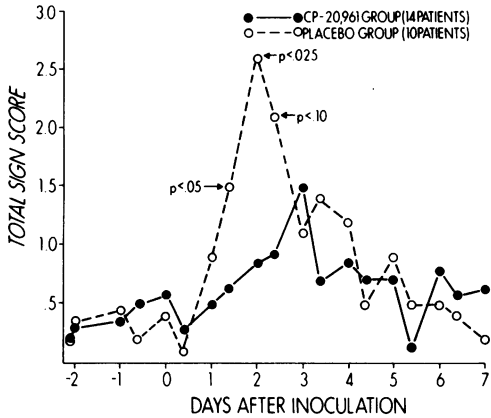


Fig. 1. Comparison of mean total sign scores of CP-20,961-treated and placebo-treated subjects. *P* value calculated from paired sample *t*-tests.

rus type 13 was shedding another rhinovirus type at the time of inoculation. In addition, two volunteers shed herpes simplex virus. These were the only extraneous viruses isolated.

The most frequent day of onset of shedding of rhinovirus type 13 was day 2, and all volunteers in the placebo group began to shed virus by day 4 (Table 2). In contrast, three volunteers in the CP-20,961 group began to shed virus after day 4. This tendency for mean onset of virus shedding to occur earlier for the placebo group is not statistically significant ($P = 0.08$; Wilcoxon test). During the period of drug administration, 11 of 15 in the CP-20,961 group and 10 of 14 in the placebo group shed virus.

Quantitative shedding patterns are shown in Fig. 2, omitting the volunteers with late onsets. As indicated, the patterns were similar for both groups, and peak virus titers were observed on the 4th day after inoculation. Geometric mean quantities were similar for the two groups at each time tested ($P > 0.05$; Wilcoxon two-sample rank test).

Mean duration of virus shedding was similar for the two groups, 4.92 and 6.0 days, respectively ($P > 0.05$; Wilcoxon test). If the three individuals with late onsets who were tested for only 1 or 3 days for virus shedding are deleted, the mean duration was 5.72 and 6.0 days, respectively.

Antibody responses. All individuals who shed virus in both groups developed fourfold or greater antibody responses, and one individual in the placebo group who did not shed virus, developed such a response (Table 1). Mean serum neutralizing antibody titers, \log_2 , were <1 and <1 before virus inoculation and 7.00 and 6.44 4 weeks after inoculation for the CP-20,961

and placebo groups, respectively ($P > 0.05$; *t*-test).

Interferon in nasal secretions. Ten volunteers in the CP-20,961 group and two in the placebo group had detectable levels of interferon in NS ($P < 0.006$; Fisher exact test) (Table 1). The cumulative number of volunteers in each group with detectable interferon in NS is shown in Fig. 3. Interferon was detected in secretions of three volunteers in the CP-20,961 group before virus inoculation and in none of the placebo group. After virus inoculation, interferon was detected in NS of seven additional subjects in the CP-20,961 group and in two subjects in the placebo group.

A total of 26 specimens were found to contain detectable quantities of interferon. Duration of

TABLE 2. Distribution of onsets of virus shedding

Group	Day after inoculation								Mean ^a
	1	2	3	4	5	6	7	8	
CP-20,961	1	7	1	2	1	0	0	2	3.36
Placebo	3	6	0	1	0	0	0	0	1.90

^a $P = 0.08$; Wilcoxon test.

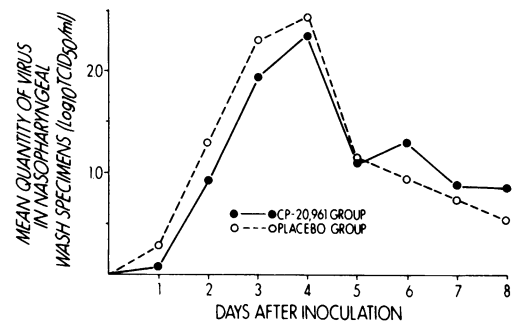


Fig. 2. Quantitative rhinovirus shedding patterns.

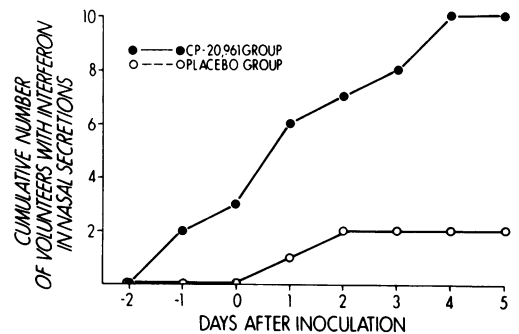


Fig. 3. Comparison of cumulative incidence of detection of interferon in nasal secretions of CP-20,961-treated and placebo-treated subjects.

detection ranged between 1 and 6 days, with a mean of 2.2 days. Titers varied from 10 to 250, and the geometric mean was 87.

The relationship of CP-20,961 administration, presence of interferon, and virus shedding is shown in Fig. 4 for 10 volunteers. As indicated, interferon titers detected in NS prior to virus inoculations were low. Interferon was detected in NS from 8 of 10 subjects before detection of rhinovirus type 13. Higher titers were detected after virus inoculations, and the highest titers were obtained on the 4th day after inoculation at the time of peak virus shedding.

Duration of shedding and peak quantity of virus shed were not different among individuals who shed interferon and those who did not in the CP-20,961 group. In addition, in this group ill subjects did not have interferon in NS less frequently than did subjects who were not ill (7/10 versus 3/4).

Of the three subjects with interferon detected in nasal secretions before virus inoculation, three shed virus (one late) and two became ill. Of the three subjects who demonstrated late onset of virus shedding, two had interferon in NS before virus shedding.

DISCUSSION

By administering CP-20,961 beginning 2 days before virus inoculation and continuing for 7 days, the present study was designed to evaluate possible prophylactic or therapeutic effects of a nasally administered interferon inducer in experimental rhinovirus infections in normal volunteers. In preliminary experiments in our laboratory, the compound had been shown to

induce interferon in tissue cultures which was active against rhinovirus type 13. In addition, rhinovirus type 13 has been shown to be sensitive to interferon induced by other means (12). The dose of virus used to inoculate the volunteers, 80 HID_{50} , was small in order to simulate natural transmission. The results indicate that detectable levels of interferon developed in nasal secretions in 67% of CP-20,961-treated subjects. Administration of CP-20,961 did not prevent illness or infection, but physical findings were significantly diminished as compared with placebo-treated control subjects.

In a previously reported study, Gatmaitan et al. (5) tested the effect of 2 days of CP-20,961 treatment on rhinovirus type 21 infection. Their results were similar to ours in that they demonstrated no difference in rate of infection or incidence of illness, but reduced severity of symptoms was observed. Since interferon might not be expected to protect against infection but rather exert its maximal effect in recovery from infection (1), we used a longer course of treatment which extended 7 days, through the beginning of the usual recovery phase from rhinovirus infection.

Previous studies have shown that the presence and severity of illness correlated with peak virus quantities in nasal wash specimens (3). Thus, drugs which reduce total symptom and sign scores might be expected to also reduce virus titers. In the present study, a slight but not statistically significant reduction in quantities of virus shed was noted among CP-20,961-treated subjects as compared with placebo-treated subjects. This finding is in keeping with the minimal effect observed on illness.

Interferon was detected in none of 14 volunteers receiving placebo before infection, in 2 of 11 receiving placebo who became infected, in 3 of 15 receiving CP-20,961 before infection, and in 10 of 14 receiving CP-20,961 who became infected. This data suggests that the combination of viral infection plus CP-20,961 is a more potent stimulator of interferon than either infection or CP-20,961 alone. An alternate explanation may be that several days are necessary for optimal drug effect, since the 6th day of CP-20,961 administration was the day of highest frequency and quantity of interferon in nasal secretions. In contrast to animal models (13), a lag phase of 1 to 5 days was noted before interferon was detected, and exhaustion of interferon response was not observed.

Although not statistically significant because of the small numbers, it is of interest that all three late infections occurred in the CP-20,961 group (3/4 versus 0/4 susceptible subjects in the

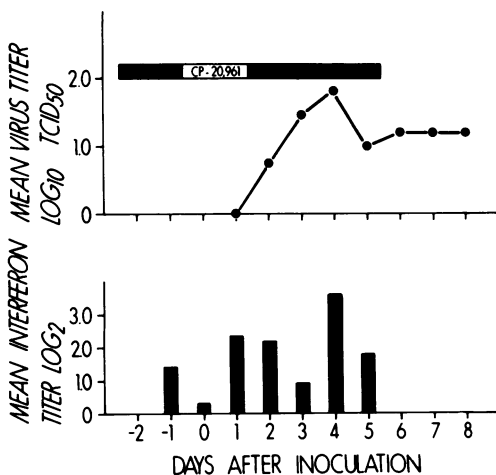


FIG. 4. Relationship of CP-20,961 administration, appearance of interferon in nasal secretions, and detection of virus in nasal secretions in 10 volunteers.

placebo group). In our prior experience with several hundred volunteers, onsets of shedding invariably occur between days 1 and 4. Late onset of shedding may have been due to suppression of virus replication due to CP-20,961 administration or perhaps to enhanced susceptibility to person-to-person transmission after prolonged administration of an interferon inducer.

In the present study, levels of interferon detected were in the same order of magnitude as observed in naturally occurring rhinovirus infections (1). Although the majority of CP-20,961-treated subjects developed detectable interferon in nasal secretions, they were not protected against experimental rhinovirus infection. In fact, all three subjects who had developed interferon in nasal secretions before virus inoculation became infected, and two became ill. In addition, five of seven remaining volunteers who developed detectable interferon did so before detection of rhinovirus type 13. In addition to the failure of induced interferon to protect against infection, as noted above, only mild effects were observed on severity of illness, and the presence of interferon was not associated with a more rapid decrease in quantities of virus in nasal secretions, decreased duration of shedding, or decreased frequency or duration of illness. Thus, recovery from illness and infection occurred at the same rate regardless of drug administration or presence of interferon. These findings suggest that host factors other than interferon may contribute to the recovery process.

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