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Topical Enviroxime Against Rhinovirus Infection

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Enviroxime, an inhibitor of rhinovirus replication, was studied in a double-blind, placebo-controlled trial with 99 volunteers. The efficacy of a nasal-spray formulation of enviroxime was tested as pretreatment or as postchallenge treatment for rhinovirus type 4 infection. In the regimens used, drug administration neither prevented infection nor reduced the frequency of specific colds. The mean concentration of enviroxime in nasal washes (12 h after a dose) differentiated two groups of responders. Those in whom the drug concentration exceeded 100 ng/ml had some benefits, although these were statistically insignificant.

Enviroxime, a substituted benzimidazole derivative, is virustatic for rhinoviruses at concentrations of 10 to 40 ng/ml in tissue cultures (1, 6). To study the clinical efficacy of enviroxime given as a nasal spray, a placebo-controlled, double-blind study of rhinovirus type 4 (RV4) infection was performed in volunteers. The study was designed to observe the effects of prophylactic and therapeutic treatment.

MATERIALS AND METHODS

A total of 101 healthy young adult volunteers from among the students and staff of the University of Illinois Medical Center in Chicago participated in the investigation. Subjects were excluded from the study if there was a history of respiratory, allergic, or cardiovascular diseases or an upper respiratory infection in the preceeding 3 weeks. A possibility of pregnancy was excluded in each of the 47 female participants by assays of urine for human chorionic gonadotrophin.

All volunteers were prescreened for specific serum neutralizing antibody to RV4. A total of 81 were seronegative (antibody titer less than 1:4), and 20 were seropositive (antibody titer greater than or equal to 1:4). The seronegative volunteers were assigned by a table of random numbers into one of four unequal treatment groups in which all subjects received the same intranasal RV4 challenge and the following treatment: (i) 30 received placebo medication for the entire 7 days; (ii) 21 received enviroxime 1 h before viral challenge and for 7 days after; (iii) 20 received the placebo for 1 day and enviroxime for the next 6 days; and (iv) 10 received the placebo for 3 days and enviroxime for 4 days. The seropositive volunteers received a noninfectious challenge and were randomly distributed into treatment groups in which 11 received enviroxime and 9 received placebo medication for 7 days. Throughout the study, all volunteers and investigators were blind as to the antibody status, challenge, and treatment group of the subjects.

The challenge strain of RV4 (NS494) was isolated from a person with a common cold and was identified by biological properties and specific neutralizing antibody. The second tissue culture passage in WI-38 human diploid fibroblasts (HEM Research Laboratories, Rockville, Md.) was free of bacteria and fungi by culture on appropriate media. No extraneous viruses were found by culture on WI-38, HeLa, and HEp-2 cell lines in which RV4 was neutralized by specific hyperimmune antisera. After dilution by a factor of 1:1,000, the harvest was used as the challenge inoculum. A nasal wash was obtained from each subject on the day the study began before viral challenge or administration of medication. None yielded a virus in tissue cultures. Each seronegative volunteer received as nose drops 3.2 times the tissue culture infective dose for 50% of cultures of RV4 in a volume of 1 ml. Control subjects received a noninfectious solution of freeze-thawed harvest from uninfected WI-38 fibroblasts.

A metered nasal spray delivered either enviroxime or a placebo as an alcoholic solution with a freon propellant (Eli Lilly & Co., Indianapolis, Ind.). Each application of enviroxime delivered 284 μg to each nostril. The placebo consisted of the vehicle constituents of the treatment material and was identical in appearance and properties to the solution containing the drug. If the subject sneezed immediately after a dose, the spray was repeated at once. This was observed 136 times with 1,536 doses of enviroxime and 100 times with 1,292 doses of placebo. All treatments were self-administered (initially under supervision) and given daily at 9:00 a.m., 1:00 p.m., 5:00 p.m., and 9:00 p.m. for 7 days. Volunteers were questioned daily about missed doses; 19 enviroxime and 10 placebo applications were missed. All subjects were given new treatment cannisters on days 1 and 3 postchallenge and were unaware of the nature of the contents.

Each morning over a 10-day period (except on days 1, 2, 8, and 9), approximately 12 h after the 9:00 p.m. dose of medication and before any other treatment was done, nasal washes were collected with 10 ml of antibiotic-free buffered saline and were divided into samples for virus isolation and enviroxime analysis.

TABLE 1. Distribution of volunteers with regard to challenge, treatment, and outcome in a trial of enviroxime nasal spray

Challenge	Treatment	Total no. of persons	[E][a] in nasal wash	No. of persons with:			Total no. of RV4-infected persons
				RV4-specific cold	RV4 in nasal wash[b]	RV4-specific antibody rise	
RV4	Placebo	30	1 ± 12	12	22 (28)	17	26
	Drug						
	Before challenge						
	[E][c] > 100 ng/ml	12	495 ± 152	4	11 (25)	6	11
	[E] < 100 ng/ml	9	9 ± 5	5	5 (28)	6	7
	After challenge						
	[E] > 100 ng/ml	15	1,102 ± 370	4	11 (24)	6	12
	[E] < 100 ng/ml	15	17 ± 5	11	13 (20)	7	13
Noninfectious	Placebo control	8	5 ± 17	0	0 (0)	0	0
	Drug control	10	395 ± 558	0	1 (5)	0	1

[a] [E], Concentration of enviroxime; expressed as mean ± standard error of the mean.
[b] Percentage of specimens is given within parentheses.
[c] Mean in nasal wash.

Nasal secretions for virus isolation were inoculated within 4 h of collection onto MRC-5 or WI-38 human diploid fibroblast cell lines (HEM Research Laboratories). After a 60- to 90-min incubation to allow virus attachment, the tissue cultures were washed with 1 to 2 ml of Hanks balanced salt solution three times to remove residual drug (1) and then incubated in maintenance medium on roller drums at 33°C. Cells were examined for cytopathic effect daily for 10 days, during which time the tissue cultures received one additional change of medium. Two subjects in the seropositive group were excluded from the analysis because of a respiratory infection with a rhinovirus other than the challenge virus. No other extraneous viruses were recovered. Nasal washes for enviroxime assay were collected on 3 separate days, and under blind code the enviroxime was quantified by high-performance liquid chromatography at Eli Lilly & Co. (6).

Blood specimens for neutralizing antibody against RV4 and for toxicology studies were collected before the virus challenge and at 7 and 42 days after challenge. Serum neutralizing antibody titers specific for RV4 were determined by serial twofold dilutions of serum with 32 times the tissue culture infective dose for 50% of cultures of virus.

Thirteen clinical symptoms were monitored daily for 7 days and graded as absent, mild, moderate, severe, or very severe (scored numerically as 0, 1, 2, 3, and 4, respectively) (3). The numerical values of symptoms were considered only to the extent that they increased above the level reported before the initiation of the study. A common cold was designated in subjects who fulfilled two of the following three criteria: (i) a total symptom score greater than 14 for the symptoms of sneezing, headache, sore throat, nasal discharge, nasal obstruction, and cough; (ii) increased nasal discharge on 3 of the 7 days after virus challenge; and (iii) a subjective evaluation by the volunteer at the conclusion of the study that a common cold had occurred. Transient symptoms temporally related to drug or placebo administration were not included in the analysis. The illness was considered to be RV4 specific if there was a fourfold rise in serum antibody or if RV4 was recovered from more than one nasal wash specimen. In the analysis, the two post-RV4 challenge treatment groups were not significantly different in outcome and were combined.

RESULTS

Enviroxime at a concentration of 125 ng/ml completely inhibited 100 times the tissue culture infective dose for 50% of cultures of the challenge strain of RV4 in tissue cultures and reduced the virus yield by 50% at a concentration of 60 ng/ml.

The concentration of enviroxime in nasal secretions measured 12 h after the 9:00 p.m. dose of nasal spray on days 3, 4, and 5 ranged from undetectable to 4,662 ng/ml. Volunteers composed a bimodal population according to the mean drug concentration in nasal washes. In one group the concentration was 798 ± 310 ng/ml (mean ± standard error of the mean), and in the other group the concentration was 13 ± 5 ng/ml. Levels of 0 to 30 ng/ml were recorded in volunteers who received the placebo, and these levels represent the limits of the assay.

In Table 1 the enviroxime-treated, RV4-challenged volunteers are tabulated according to the time of initial drug administration and whether or not the observed mean drug concentration in the nasal washes was greater than 100 ng/ml. Clinical, virological, and serological measurements are shown. Colds occurred in 27 to 73% of the volunteers in the different test groups. The rate was lowest in the combined treated groups among subjects with mean measured drug levels of >100 ng of enviroxime per ml in the nasal washes (8 of 27 subjects) and highest in treated

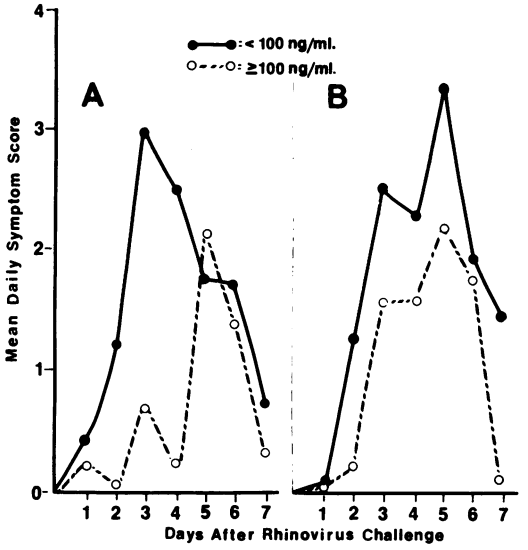

FIG. 1 Comparison of symptom scores after RV4 challenge for enviroxime-treated volunteers grouped on the basis of nasal wash enviroxime levels. (A) Scores for volunteers who began enviroxime treatment 1 h before RV4 challenge (>100 ng/ml, 12 subjects; <100 ng/ml, 9 subjects). (B) Scores for those who began enviroxime treatment 24 to 72 h after RV4 challenge (>100 ng/ml, 15 subjects; <100 ng/ml, 15 subjects). The scores represent the values after subtraction of the scores of placebo or drug-treated subjects given a noninfectious challenge.

subjects with mean levels of <100 ng/ml (16 of 24 subjects). The difference is statistically significant, but neither result is significantly different from that of the untreated controls (12 of 30 subjects).

Persons treated with enviroxime before RV4 challenge and who had observed mean enviroxime concentrations of >100 ng/ml in their nasal washes experienced fewer symptoms during the first 4 days after challenge (Fig. 1A). A surge of symptoms appeared in these individuals on days 5 and 6, to the extent of symptoms occurring concomitantly in volunteers whose nasal wash enviroxime levels were <100 ng/ml. A slight beneficial influence on symptoms was observed in volunteers treated after the viral challenge in whom enviroxime levels of >100 ng/ml were present in the nasal washes (Fig. 1B).

A total of 73% of the untreated subjects shed RV4, and 28% of all nasal washes collected from these subjects over a 10-day period yielded RV4. There was no significant reduction in the proportion of volunteers or the proportion of specimens from each group that yielded RV4 isolates. An appreciable but statistically insignificant reduction occurred in the number of treated versus untreated persons who shed virus on 3 or more postchallenge days (14 versus 23%). The period when the delayed appearance of symptoms occurred (Fig. 1A) was not associated with renewed virus shedding. A similar proportion of fourfold or greatest rises in specific antibody titer was found in the RV4-challenged volunteers in the different groups. The magnitude of the rise expressed as the geometric mean was diminished among enviroxime-treated persons to the extent of 2-fold and 1.6-fold when the nasal drug concentration was >100 ng/ml or <100 ng/ml, respectively.

A total of 20 of 38 placebo-treated and 47 of 61 enviroxime-treated volunteers reported transient mild nasal stinging for the first few minutes after spray application. No RV4-specific illness occurred in subjects challenged with noninfectious fluid, but 2 of 10 persons given enviroxime had nasal symptoms that persisted and were severe enough to satisfy the defined criteria of a cold. No abnormalities of the total or differential leukocyte count, hemoglobin concentrations, or tests of renal or hepatic function were encountered that were attributable to nasal-spray administration of enviroxime.

DISCUSSION

Although enviroxime was virustatic in tissue cultures, it did not prevent or eliminate infection after a rhinovirus challenge that caused infection in 85% of volunteers without specific antibody. In a subgroup of treated persons, the observed delay or amelioration of symptoms, reduced persistence of virus in nasal secretions, and smaller increment of rise in the serum antibody titer were trends that might be expected from antiviral chemotherapy (5). The late expression of symptoms in the pretreatment group was disappointing, but it could not be shown to be the result of persistent or recurrent virus shedding. The diminished virus shedding in persons with higher enviroxime levels was not considered to be a drug carry-over effect since the tissue culture procedure used allowed virus attachment, which was followed by repeated washes to remove residual drug (1).

The delivery and retention of the drug at the site of infection is a major problem in the practicality of topical nasal chemotherapy. The most consistent factor that correlated with the reduction of symptoms and virus shedding was whether the subjects were in the group that had a higher drug level in the nasal washes. When the treatment was not associated with higher nasal drug levels, the symptoms and the apparent number of colds increased. Noncompliance in taking the medication did not appear to be the cause of the difference; at least the report of missed doses did not correlate with higher or lower nasal levels. Also, as determined by the scores for nasal discharge, the difference was

cause of the difference; at least the report of missed doses did not correlate with higher or lower nasal levels. Also, as determined by the scores for nasal discharge, the difference was not from increased nasal secretions causing more rapid drug washout. It is possible that variation in the technique of self-administration of the drug was a factor. The more definite effect shown in a study in which both oral and nasal-spray enviroxime were administered and the lack of effect noted in another study in which only nasal-spray enviroxime was given also suggest an inconsistency of drug levels from the nasal route of delivery (2, 4).

Although the results of the trial were negative in most respects, the trends observed in the context of obtaining adequate nasal concentrations are suggestive of some viral inhibition accompanied by a clinical benefit. Further investigation is required for the elucidation of these effects and the formulation of enviroxime in a delivery system to maximize the potential chemotherapeutic benefit.

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