

## Controlled Trial of Enviroxime Against Natural Rhinovirus Infections in a Community

F. DEWOLFE MILLER,<sup>1†</sup> ARNOLD S. MONTO,<sup>1\*</sup> DONALD C. DELONG,<sup>2</sup> ALICE EXELBY,<sup>1</sup> ELZA R. BRYAN,<sup>1</sup>  
AND SUJAN SRIVASTAVA<sup>1</sup>

*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109,<sup>1</sup> and Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, Indiana 47285<sup>2</sup>*

Received 30 April 1984/Accepted 14 October 1984

**The therapeutic effect of intranasally administered enviroxime was tested against naturally occurring common colds. The double-blind evaluation was carried out in Tecumseh, Mich., during a period when rhinoviruses are usually the principal pathogen. Rhinovirus transmission followed the typical pattern during this period of study. Although there were trends indicating greater therapeutic effectiveness for enviroxime when certain nasal symptoms were considered, there were no consistent statistically significant differences between treated and untreated groups. Results were unchanged when illnesses in different periods or associated with rhinovirus isolation were examined. It was concluded that no therapeutic effect of enviroxime was demonstrated.**

Although enviroxime [2-amino-1-(isopropylsulfonyl)-6-benzimidazole phenyl ketone oxime] has been shown to be effective against rhinovirus replication *in vitro* (1), conflicting evidence has been presented on its efficacy in preventing or treating human infections. For example, Phillipotts et al. (5) showed a reduction in rhinorrhea and shedding of rhinovirus type 9 in a prophylactic challenge trial during which enviroxime was administered to volunteers both intranasally (284 µg) and orally (25 mg) four times a day. In contrast, Hayden and Gwaltney (2) did not find differences in infection or illness in a prophylactic trial during which 284 µg of enviroxime was given intranasally five times a day against a challenge of rhinovirus type 39; in this study, no oral drug was given. In a recent report in which intranasal enviroxime was tested in therapy against rhinovirus challenge, Phillipotts et al. (6) administered six daily intranasal doses and found a significant improvement on day 5 of the trial. They suggested that larger numbers of individuals might be needed before a significant therapeutic effect could be unequivocally demonstrated and that a large-scale field trial to evaluate conditions of natural infection would be more realistic in testing enviroxime.

For this reason, a therapeutic trial of enviroxime was carried out against natural infection in an open population. The location of the trial was Tecumseh, Mich., a community of over 10,000 which has been the site of continuous epidemiological investigations for almost 20 years. Considerable epidemiological detail is available about respiratory infections in this community (4), including information on the circulation of viruses which cause common colds. During the early fall months, a consistent increase in acute upper respiratory illness has been observed in parallel with the recovery of rhinoviruses as the predominant isolate (4). Thus, we decided to test the value of enviroxime in therapy for respiratory infections occurring during the period of September to early October.

### MATERIALS AND METHODS

**Population and preparations used.** In August 1982, families living in Tecumseh, Mich., were recruited by telephone. Families who agreed to participate were stratified into four groups before being randomly grouped to receive either placebo or enviroxime. All members of a family were assigned to the same treatment group. The strata were as follows: (i) four or fewer members, all 8 years of age or older; (ii) four or fewer members, some less than 8 years of age; (iii) more than four members, all 8 years of age or older; (iv) more than four members, some less than 8 years of age.

The treatments were double blind. Neither the families nor those supplying the treatment kits and collecting the data were aware of the nature of the treatment. Nor were families aware that each member within the family was receiving the same treatment. This was accomplished by making up individual kits for each family member, away from the investigation site, and placing the name of the participant on the kit before delivery to the family. Within the families, asthmatics, pregnant and lactating women, and individuals less than 8 or above 64 years of age were excluded from taking enviroxime or placebo. Individuals taking medication for seasonal allergies were also excluded. Surveillance, which included all members of the participating families, eligible for treatment or not, began in the last week of August, 1982, and continued through 19 October 1982. Each family was contacted once a week by telephone to determine whether any member had cold symptoms.

Each family was visited in their home before the trial began, to obtain written, informed consent. Family members were provided with appropriately labeled canisters and were instructed on how to administer therapy. They were to begin therapy as soon as they considered a cold to have started, by spraying each nostril twice, six times on the first two days and four times a day for an additional 5 days, and they were to notify the office. This office had a 24-h answering service to provide assistance and initiate data collection. Daily after the onset of a cold, the participant completed a diary form for respiratory symptoms. The following symptoms were included on this form: runny nose, irritated nose, stuffy or blocked nose, chills, headache, earache, general aches and pains, sore throat, swollen or tender glands, hoarseness,

\* Corresponding author.

† Present address: Department of Public Health Sciences, School of Public Health, University of Hawaii at Manoa, Honolulu, HI 96822.

cough, phlegm from the chest, wheezy breathing or breathing discomfort, nausea, vomiting, diarrhea, burning, aching, or redness of the eyes, and stiffness of the neck. Each symptom was scored by the participant as (i) none, (ii) mild, (iii) moderate, or (iv) severe. A mean severity score was calculated from the reported data. Also recorded were the presence of fever, number of days in bed, and degree of activity restriction and whether a physician was seen. Parents were instructed to assist children who had colds to administer the spray and complete the diaries. Individuals with colds were contacted daily by telephone, and they read data in the diary to the project interviewer, who recorded these results on identical forms. Individuals were asked not to use over-the-counter cold medications, but notation of any such medication that was actually used was made during the interview.

Enviroxime was supplied as a 1% suspension in a mixture of Tween 85 (10%) and propellant Freon 11 and 12. The canisters were designed to deliver at least 658  $\mu\text{g}$  of enviroxime with each activation. The placebo was identically supplied and delivered but lacked the enviroxime. Both the drug and placebo were labeled with a code number and supplied by Lilly Research Laboratories. None of the investigative field team nor the participants had knowledge of the code assignment.

After the trial, all of the canisters were collected or accounted for and returned to the Lilly Research Laboratories. Enviroxime canisters were weighed individually before and after distribution, and the net material consumed was calculated. These results were compared with the data collected by the interviewer on the number of times the respondent reported discharging the canister.

**Isolation of infecting viruses.** Specimens for viral isolation were collected when an individual eligible for treatment reported that he or she had started to use the spray because a cold had begun. The initial specimen was collected as soon as possible after treatment had started, generally within hours. A second specimen was collected 2 days later in the same manner. The specimen was collected by using nasal and oropharyngeal swabs. These swabs were separately placed into a single tube of veal infusion broth enriched with 0.5% bovine serum albumin and containing 500 U of penicillin and 2  $\mu\text{g}$  of amphotericin per ml. After each swab had been agitated in the material and excess fluid was expressed, the swab was discarded. The material was inoculated, generally without prior freezing, into two tubes of either WI-38 or MRC-5 cells obtained commercially or produced in the laboratory of D.C.D. The remaining specimen was divided into two samples and frozen at  $-70^{\circ}\text{C}$ . The inoculated tubes were placed at room temperature, and virus was allowed to absorb for 3 h. Thereafter, tubes were washed three times with Hanks balanced salt solution and fed with Earle minimum essential medium with 2% fetal bovine serum. This procedure was followed to remove any enviroxime that might be carried into the specimen. The tubes were placed on a roller drum and incubated at  $33^{\circ}\text{C}$ . They were regularly observed for the occurrence of cytopathic effect, until the controls had degenerated. Occasionally, there were problems with cell cultures from commercial sources, and in such cases, the frozen specimens were inoculated into fresh tubes, and the entire procedure was repeated. Rhinovirus isolates were identified as previously described (4).

Original specimens from which rhinoviruses were recovered were titrated to identify the quantity of virus contained. The original specimen and a half-log dilution of it were each inoculated into two tubes of WI-38 or MRC-5 cells. After

absorption and washing, the tubes were placed on the roller drum at  $33^{\circ}\text{C}$  and observed for 5 days. Endpoints were determined microscopically and calculated by the method of Reed and Muench (7).

## RESULTS

**Illness frequency and virus isolations during the study period.** A total of 301 families were recruited in Tecumseh and monitored weekly during the study period. The eligible family members who received either enviroxime or placebo totaled 1,020. There were an additional 288 family members who either did not meet the criteria necessary to receive treatment or elected not to receive therapy. They were also monitored weekly for respiratory symptoms and were termed the ineligible group.

Daily reporting of illnesses with respiratory symptoms began in the first week of the study, increased to a peak of 22 colds on 19 September, and then decreased gradually until the study was terminated ca. 6 weeks later on 19 October 1982. The epidemic curves for all individuals reporting and for the eligible and ineligible groups are shown in Fig. 1. The occurrences of illness in the eligible and ineligible groups were roughly parallel. The absolute number of ineligibles reporting symptoms was smaller than the number of eligibles reporting symptoms because of the much smaller size of the former group. The numbers of daily rhinovirus isolates are included as bars in Fig. 1 and also paralleled the number of illnesses.

The overall attack rate during the study period for the eligible group was 28.4 per 100 ( $290/1,020 \times 100$ ). The overall attack rate in the ineligible group was 54.2 per 100 ( $156/288 \times 100$ ). This difference in attack rate was most likely due to a difference in age distribution between the two groups. As expected, the mean age for the eligible group was significantly higher, 25.9 years compared with 12.8 years for the ineligible group.

There were 290 eligibles who reported respiratory symptoms and had been assigned to either the enviroxime or the placebo group. Treatment with enviroxime and placebo was divided equally among the 290, i.e., there were 145 individuals in each treatment group. The mean age and sex compositions of these two groups were not significantly different ( $P > 0.05$ ).

Characteristics of the illness in the eligible and ineligible groups are shown in Table 1. The frequency of respiratory symptoms is also shown for those from whom rhinoviruses were isolated. Note that the eligible and ineligible groups are mutually exclusive. However, the rhinovirus isolates were taken only from individuals who were in the eligible group. Respiratory symptoms were similar between the enviroxime and placebo groups. Stuffy nose and rhinorrhea were the most commonly reported symptoms for the eligible and ineligible groups, although at lower frequency in those individuals from whom rhinovirus was isolated. Cough, sore throat, and headache were the next most frequently seen respiratory symptoms and were parallel in occurrence in all groups except the ineligible group, who had fewer headaches and more fevers. This and other differences are probably due to the younger mean age of the ineligible group. Also noteworthy in the ineligible group is the near absence of irritated noses, which may also be related to their not using nasal spray.

The effect of enviroxime versus that of placebo on stuffy nose in illnesses of eligible individuals on a day-by-day basis is shown in Fig. 2. Comparison was made between mean severity scores for enviroxime and placebo groups. It should

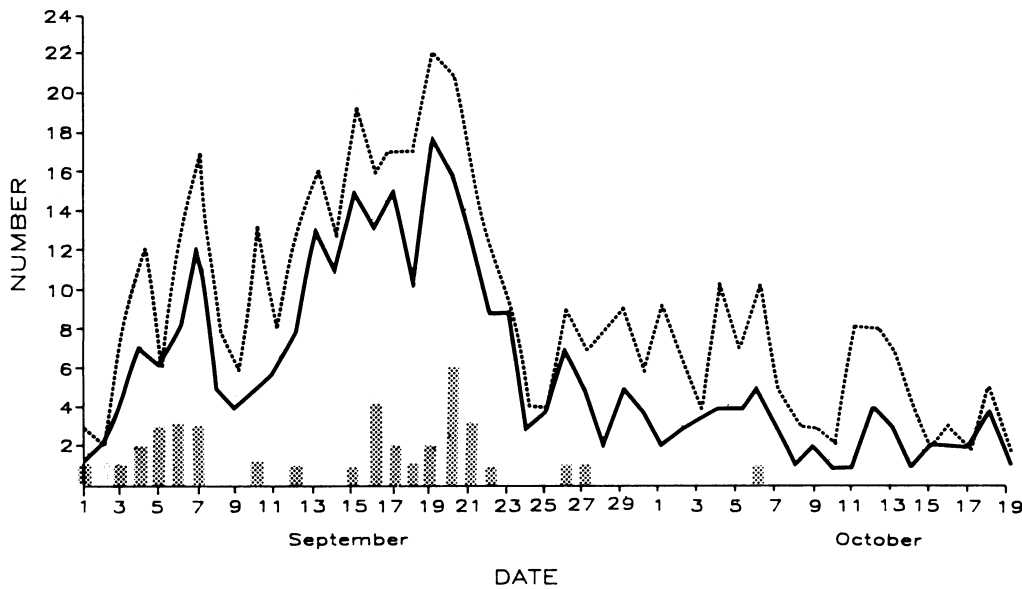


FIG. 1. Daily number of respiratory illnesses and rhinovirus isolations, Tecumseh, Mich., September to October 1982. Symbols: ..... , total respiratory illness; —, illness in those eligible for therapy; ■, rhinovirus isolates.

be noted that individuals started the treatment at different times on day 1. Therefore, the results seen on that day may not reflect a fair comparison. There was a pattern of higher mean severity scores for stuffy nose for the placebo group, except on days 1 and 3, when the outcome was reversed (Fig. 2). These differences were not statistically significant ( $P > 0.05$ ) for any day. All other cold symptoms were examined. The patterns of results for the other symptoms were sometimes similar to that seen in Fig. 2 for stuffy nose, but sometimes the patterns favored the placebo group.

Shedding of rhinovirus in specimens collected on days 1 and 3 of treatment was compared for the two eligible groups. Somewhat more participants receiving the placebo, who were positive for rhinovirus in specimens collected on day 1, were positive in specimens collected on day 3 (30%) than was the case for individuals receiving the drug (26.7%), but

these differences were not statistically significant. In addition, no differences in virus titer between the groups could be demonstrated for those situations in which first and second specimens were both positive, nor did the mean duration of illness or mean severity scores of symptoms differ significantly between the enviroxime and placebo groups, who were rhinovirus positive.

TABLE 1. Characteristics of illnesses in individuals in different treatment groups and in individuals positive for rhinovirus

Symptom	% With symptom in group:			
	Enviroxime (n = 145)	Placebo (n = 145)	Nontreated <sup>a</sup> (n = 156)	Rhinovirus positive <sup>b</sup> (n = 40)
Stuffy nose	91.0	89.7	57.7	95.0
Rhinorrhea	87.5	82.1	59.0	92.5
Irritated nose	46.2	35.9	1.3	52.5
Fever	9.7	8.0	29.5	2.5
Chills	25.5	22.8	10.3	35.0
Headache	63.8	46.9	15.4	60.0
Earache	18.6	19.3	1.9	25.0
General ache	35.9	29.7	10.3	35.0
Sore throat	62.1	62.8	40.4	70.0
Swollen glands	25.5	16.6	8.3	22.5
Hoarseness	39.3	39.3	12.8	55.0
Cough	71.7	59.3	44.9	77.5
Phlegm	35.2	37.9	12.8	50.0
Wheeze	21.4	14.5	9.6	30.0

<sup>a</sup> See text for criteria of eligibility.

<sup>b</sup> Includes individuals in enviroxime and placebo groups.

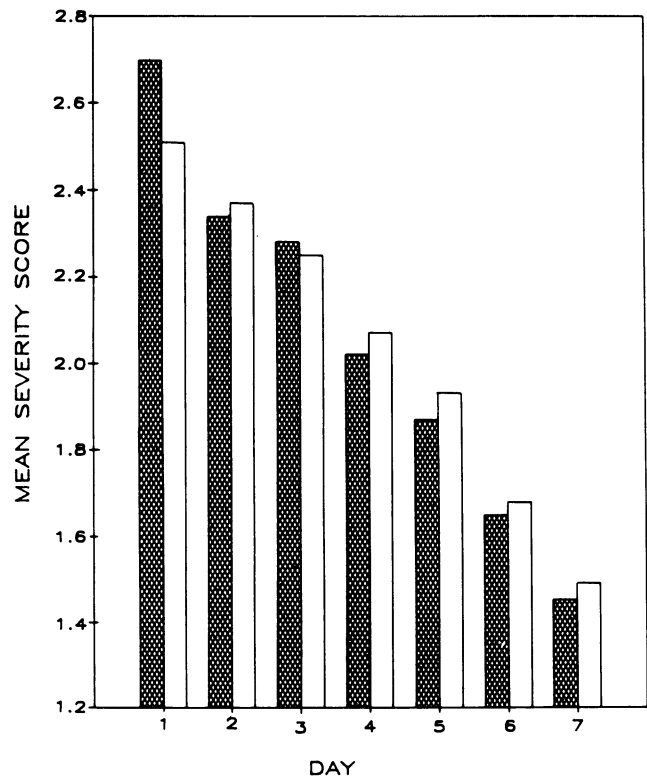


FIG. 2. Mean daily severity scores of stuffy nose in all eligible individuals. n = 145 in each group. Symbols: ■, enviroxime; □, placebo.

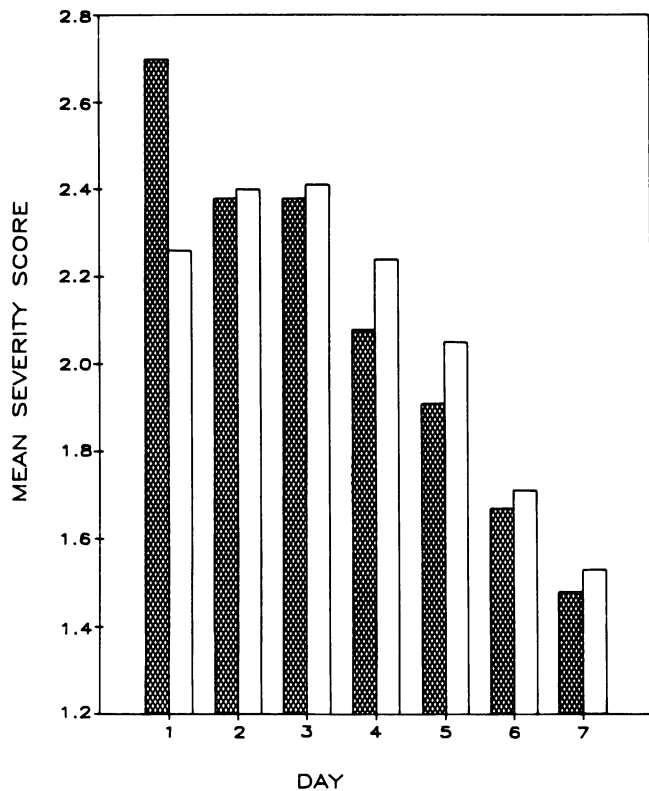




FIG. 3. Mean daily severity scores of stuffy nose in those using medication at least three times daily for 5 days.  $n = 78$  for enviroxime;  $n = 70$  for placebo. Symbols: , enviroxime; , placebo.

Eligible participants reported irritation, stinging, and other discomforts upon insufflation. It was important to determine whether these reports of discomfort influenced the reporting of how many times the individual canisters were actually used. The number of times a canister was reported as activated was regressed against the total material discharged as determined by weighing the canisters before and after they were distributed. The correlation ( $r = 0.57$ ;  $P < 0.0001$ ) indicated that the reported usage was in agreement with the amount of material discharged from the canister.

Numbers of times the participants reported that they had used the specific regimen were not uniform among all participants. After day 2, there was a decline in the number of individuals per day who performed the insufflations at the requested frequency. The decline in the number of insufflations was equal in the enviroxime and placebo groups. Further analysis was done to learn whether inadequate use had affected the results by eliminating participants who did not meet certain compliance criteria. These results are shown in Fig. 3, which is similar to Fig. 2 but includes only those individuals who administered the treatment at least three times a day for 5 days. A pattern for stuffy nose shows that the mean severity score for each day was consistently less in the enviroxime group, except on day 1. As explained previously, results from this day are difficult to interpret. However, the differences again were not statistically significant. All other symptoms, as well as fever and bed disability, were tested by using numerous other definitions of compliance, without significant differences being demonstrated. The number of participants was considerably reduced as the required number of uses was increased. Regardless of the specific compliance criteria used, the

resulting numbers of participants in drug and placebo groups were relatively even, suggesting that the type of therapy had not influenced the extent of use. Analyses were also carried out for those individuals with rhinovirus isolates and for the major portion of rhinovirus transmission in September. Again, no clearly significant differences could be demonstrated.

With the various compliance rules, an analysis of time to cure was carried out. Cure was defined as at least a decrease in severity from severe to mild or from moderate to none for a specific symptom. All symptoms were tested by using the method described by Landis et al. (3). The enviroxime group did not have a significantly different time to cure than the placebo group for any of these symptoms.

## DISCUSSION

In an open field trial of enviroxime to test its use in therapy for natural rhinovirus infection, no consistent statistically significant differences in effectiveness over placebo were found. Although there were trends favoring the drug for treatment of certain symptoms, especially in the most compliant individuals, in general the mean severity in both enviroxime and placebo groups was similar, as was the time to cure for all symptoms measured. When the analysis specified the number of times the therapy was actually used, no significant differences were detected in symptoms, nor were differences seen when the measure was defined in terms of time to cure or to improvement.

The descriptive epidemiological data suggest that the September to October 1982 period was characteristic and typical for acute upper respiratory illness expected in this community at this time. The characteristics of common cold-like illness were similar in all groups, and there was a typical pattern of upper respiratory illness outbreak in the Tecumseh community for the period of the field trial. Rhinoviruses were circulating in parallel with the outbreak, as shown by the viral recovery rates.

Although compliance by the participants with therapeutic regimens varied, results were in good agreement with laboratory-determined usage values. It can be safely assumed that individuals did not discharge the canisters in the air and then report that the therapy had been applied since participation in the study was entirely voluntary. The dosage used was 680  $\mu\text{g}$  per spray and two sprays per nostril (2, 5, 6). Studies on the nasopharyngeal clearance of enviroxime (2) suggest that this dosage would be sufficient to maintain a virologically active residual. However, it is possible that the vehicle used to propel the spray for either enviroxime or placebo was itself irritating to the nasal mucosa of the user. The resulting response by the mucosa may have obscured any subtle benefits in relieving the most common cold symptoms, which were stuffy nose, rhinorrhea, and irritated nose. There was no evidence of a reduction of any non-nasopharyngeal symptoms by enviroxime.

Thus, although enviroxime has been shown to be a strong *in vitro* inhibitor of rhinovirus replication, in keeping with previous reports (2, 6), intranasal administration alone of enviroxime has limited benefit, if any. Testing enviroxime and similar antiviral agents in an open, community-based trial is feasible and has the advantage of demonstrating efficacy against natural challenge. Although some favorable differences were seen between enviroxime and the placebo, there was no clear reproducible result. Therefore, if differences do exist, they are of little practical importance.

## ACKNOWLEDGMENT

This work was supported in part by a grant from Eli Lilly & Co.

## LITERATURE CITED

1. **DeLong, D. C., and S. E. Reed.** 1980. Inhibition of rhinovirus replication in organ culture by a potential antiviral drug. *J. Infect. Dis.* **141**:87-91.
2. **Hayden, F. G., and J. M. Gwaltney, Jr.** 1982. Prophylactic activity of intranasal enivoxime against experimentally induced rhinovirus type 39 infection. *Antimicrob. Agents Chemother.* **21**:892-897.
3. **Landis, J. R., M. M. Cooper, T. Kennedy, and G. G. Koch.** 1979. A computer program for testing average partial association in three-way contingency tables (PARCAT). *Comput. Programs Biomed.* **9**:223-246.
4. **Monto, A. S., and J. J. Cavallaro.** 1972. The Tecumseh study of respiratory illness. IV. Prevalence of rhinovirus serotypes, 1966-1969. *Am. J. Epidemiol.* **96**:352-360.
5. **Phillpotts, R. J., D. C. DeLong, J. Wallace, R. W. Jones, S. E. Reed, and D. A. J. Tyrrell.** 1981. The activity of enivoxime against rhinovirus infection in man. *Lancet* **ii**:1342-1344.
6. **Phillpotts, R. J., J. Wallace, D. A. J. Tyrrell, and V. B. Tagart.** 1983. Therapeutic activity of enivoxime against rhinovirus infection in volunteers. *Antimicrob. Agents Chemother.* **23**:671-675.
7. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* **27**:493-497.