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Therapeutic Activity of Enviroxime Against Rhinovirus Infection in Volunteers

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The therapeutic effect of intranasal 2-amino-1-(isopropylsulphonyl)-6-benzimidazole phenyl ketone oxime (enviroxime) against human rhinovirus type 9 was evaluated in a double-blind, placebo-controlled volunteer trial. Enviroxime given 6 times a day was well tolerated, producing a reduction in clinical evidence of infection which coincided with the start of medication (44 h after virus challenge). Although there was a statistically significant reduction in clinical score in the enviroxime group on day 5 after virus challenge, reductions in total clinical score accumulated during the trial and reductions in the quantity of nasal secretion were not significant. A separate analysis was performed on data from volunteers who did not have symptoms when medication began (and who might have been expected to benefit more from enviroxime therapy). No apparent enhancement of the effects of enviroxime could be demonstrated in this group.

The compound 2-amino-1-(isopropylsulphonyl)-6-benzimidazole phenyl ketone oxime (enviroxime) is highly active in vitro against a number of rhinovirus serotypes (3; D. C. DeLong, J. W. Nelson, C. Y. E. Wu, B. Warren, J. Wikel, J. Chamberlin, D. Montgomery, and C. J. Paget, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S128, p. 234; C. Y. E. Wu, J. D. Nelson, B. R. Warren, and D. C. DeLong, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S129, p. 234). Enviroxime given four times a day has also been evaluated for its prophylactic effect against human rhinovirus type 9 (HRV9) infection in volunteers (5). There were some adverse reactions to oral medication (mild nausea and abdominal pains), but the intranasal preparation was well tolerated, apart from a mild and transient stinging on insufflation. Enviroxime had a significant protective effect, reducing both clinical and laboratory evidence of infection with HRV9. There was a negative correlation of clinical score, nasal-secretion weight, and quantity of virus excreted with the amount of enviroxime present in nasal wash. As enviroxime cannot be detected in nasal secretions after oral dosing, this relationship suggests that the drug given intranasally may have been principally responsible for protection. Locally administerd compounds are rapidly cleared from the nose (1), and therefore the protective effect of enviroxime might be enhanced by an increased frequency of intranasal medication.

In the present study, enviroxime was tested for its therapeutic effect in volunteers challenged with HRV9. The drug was given intranasally only and at the increased frequency of six times per day. Medication started 44 h after virus challenge, regardless of whether volunteers had developed symptoms of a cold.

MATERIALS AND METHODS

The study was approved by the Ethical Committee of Northwick Park Hospital, Harrow, London, England.

Volunteers. Healthy volunteers of both sexes, aged 18 to 50 years, were recruited, screened for suitability, and housed in isolation at the Common Cold Unit according to our usual procedures (2). Initial blood samples were taken for routine hematological and biochemical tests, including electrolytes and basic tests of hepatic and renal function, and to provide serum for the determination of neutralizing antibody to HRV9. Volunteers were allocated into two groups matched for age, sex, and antibody titer to HRV9. One group was given enviroxime and the other was given placebo. Those with antibody titers of >1:16 in the screening test were challenged with saline (to ensure the double-blind status of volunteers and the clinical observer), and the rest were given nasal drops containing 10 to 40 50% tissue culture infective doses of a virulent strain of HRV9 propagated by intranasal passage in volunteers. Six days after virus challenge, a further blood sample was taken for hematology and biochemistry tests to check for any effects of enviroxime.

The clinical effects were monitored by an observer who was unaware of the allocations of drug and virus. Each volunteer was assessed daily and assigned a daily score on the basis of clinical signs and cold symptoms, and clinical responses were graded as nil or

doubtful, very mild, mild, moderate, or severe colds (2). The time at which symptoms began in each volunteer was recorded. Paper handkerchiefs of known weight were issued to volunteers and were weighed after use in order to estimate the weights of daily nasal secretions (2). Nasal washings for virus isolation, titration, and enviroxime assay (by high-pressure liquid chromatography) were collected on the day before and on days 2 to 6 after virus challenge. A further serum sample was requested from each volunteer 10 to 11 days after leaving the unit and titrated in parallel with the initial sample for neutralizing antibody to HRV9. Volunteers were excluded from the trial if they developed signs of a cold before virus challenge or were excluded retrospectively if wild-type rhinovirus strains were recovered from their prechallenge nasal washes.

Medication. Enviroxime was given by an intranasal spray that delivered a metered dose of 284 μg per activation in an alcoholic solution. The placebo was alcoholic solvent. One activation per nostril was given six times per day (at 8:00 and 11:00 a.m. and 2:00, 5:00, 8:00, and 11:00 p.m.) starting 44 h after virus challenge and continuing for 5 days. The final dose was given at 8:00 a.m. on day 5.

Virological procedures. Nasal washes were performed with 10 ml of Hanks balanced salt solution, and samples were stored at −20°C for enviroxime assay or at −70°C after the addition of an equal volume of nutrient broth. Samples stored at −70°C were inoculated into both human diploid fetal tonsil cell cultures and rhinovirus-sensitive Ohio HeLa cell cultures from which the maintenance medium had been removed. After 90 min of adsorption at 33°C, cell cultures were washed three times with phosphate-buffered saline (to remove residual enviroxime) and replenished with maintenance medium. A fresh sample of each virus-positive nasal wash was titrated by quantal assay in O-HeLa cells by the same procedure. All cell cultures were incubated in a roller tube apparatus at 33°C. At least one isolate from each volunteer was identified as HRV9 by inhibition with a specific antiserum. Wild-type rhinovirus strains isolated from prechallenge nasal wash samples were identified by the characteristic pattern of cell destruction and sensitivity to low pH.

Serum neutralizing antibody titers to HRV9 were estimated by a micro-method in O-HeLa cells. Sufficient virus was used in the screening test to give an easily recognizable cytopathic effect in controls within 48 h, and paired serum samples were tested with a virus dose of 100 50% tissue culture infective doses. A 50% reduction in cytopathic effect was taken as the endpoint in each titration, and a fourfold or greater rise in antibody titer was considered significant.

Statistical analysis. Differences in the frequency of colds, virus isolations, and antibody rises between the enviroxime and placebo groups were tested for significance by Fisher's exact test. Clinical score, nasal-secretion weight, and virus excretion data was evaluated by a nonparametric analysis of variance in which each sample was divided into three strata according to prechallenge serum neutralizing antibody titers to HRV9 (<1:2, 1:2 to 1:8, and >1:8) (4). Correlations of clinical score, nasal secretion, and virus excretion with the quantity of enviroxime present in nasal washes were determined by Kendall's rank test.

RESULTS

Fifty-five volunteers took part in the trial. No abnormal values were encountered in hematological or biochemical tests performed on blood samples from 27 volunteers given enviroxime. There were no exclusions due to quarantine colds, but five volunteers (two given enviroxime and three given placebo) were excluded retrospectively because wild-type rhinovirus strains were isolated from their prechallenge nasal-wash samples. One volunteer taking placebo developed a transient macular rash which was attributed to medication. This patient did not take the last three doses of medication but was included in the analysis.

Results from 50 volunteers were analyzed, 9 of whom were challenged with saline (five given

TABLE 1. Clinical and laboratory findings in all volunteers challenged with HRV9

Group	Pretrial antibody titer	No.	No. with clinically diagnosed colds		Laboratory findings		
			Mild or worse	Very mild or absent	Antibody rises	Virus isolated	Either or both
Enviroxime	<2	9	3	6	3	9	9
	2–8	8	3	5	6	5	7
	>8	4	1	3	1	4	4
	Total (%)	21	7 (33)[a]	14	10 (48)[a,b]	18 (86)[a]	20 (95)[a]
Placebo	<2	10	8	2	7	10	10
	2–8	7	3	4	6	5	7
	>8	3	0	3	2	2	2
	Total (%)	20	11 (55)[a]	9	15 (75)[a]	17 (85)[a]	19 (95)[a]

[a] $P > 0.05$.
[b] One second serum sample not returned.

placebo and four given enviroxime). There was no increase in clinical score or nasal secretion among volunteers given enviroxime, and the maximum total clinical score allocated was 2. Of 41 volunteers challenged with HRV9, 21 were given enviroxime and 20 were given placebo. The results of virus challenge are shown in Table 1. Mild or worse colds were considered significant, as it was always possible to find laboratory evidence of infection; either virus was isolated or a rise in antibody was demonstrated or both. In addition, such illnesses did not occur in volunteers challenged with saline. There were reductions in the number of mild or worse colds and antibody rises in the enviroxime-treated group as compared with the placebo-treated group, but these differences were not statistically significant. There was no apparent difference between the two groups when the number of volunteers excreting virus or with laboratory evidence of infection were considered. The reduction in the frequency of colds in the enviroxime-treated group as compared with the placebo-treated group was apparently greater when only those volunteers without detectable serum antibody were considered, but this difference was not significant ($P = 0.11$).

The effects of enviroxime on rhinorrhoea, clinical score, and quantity of virus present in nasal-wash samples are shown in Fig. 1. After the start of medication, there were reductions on the last 3 days in both clinical score and rhinorrhoea in the enviroxime-treated group as compared with the placebo-treated group. Clinical score was significantly reduced on day 5 after virus challenge ($P = 0.04$); the reductions in nasal secretion were not significant. There was no significant difference between the enviroxime- and the placebo-treated groups when virus excretion, total clinical scores, or mean daily weight of nasal secretion produced by each volunteer during the medication period were analyzed.

Nasal washes were taken approximately 2 h after an intranasal spray and assayed for enviroxime. The quantity present ranged from 0 to 46 μg/ml (mean ± standard deviation, 6.52 ± 8.8 μg/ml). A negative correlation was found between the quantity of enviroxime present in nasal wash and the mean daily nasal secretion during the time of treatment ($P = 0.016$), total clinical score ($P = 0.06$), and mean virus titer present in nasal wash ($P = 0.049$).

These findings suggested that enviroxime produced a small reduction in the clinical evidence of infection. Five volunteers in the enviroxime group and three in the placebo group developed signs of a cold before medication began. If enviroxime is effective, it might be expected that a greater reduction in symptoms would result from early treatment. Therefore, an analysis of the results of virus challenge was performed, restricted to those volunteers who had not developed symptoms at the time medication began (Table 2). The reduction in the number of colds in the enviroxime-treated group as compared with the placebo-treated group was greater than when all volunteers were considered but was not statistically significant. There was an apparently greater reduction in the frequency of colds in the enviroxime group when only volunteers with undetectable serum antibody were considered which approached significance ($P = 0.06$). The proportion of volunteers with antibody rises, virus excretion, or laboratory evidence of infection in each group remained about the same as when all volunteers were considered. An analysis of the daily clinical score, nasal secretion, and virus excretion data is shown in Fig. 2. Consistent reductions in rhinorrhoea (not significant) and clinical score (significant on day 3 after virus challenge; $P = 0.02$) were found in the enviroxime-treated group. These reductions were greater than those shown in Fig. 1 (data from all volunteers). There were no significant differences between the enviroxime- and the placebo-treated groups in virus excretion, total clinical scores, or mean daily weight of nasal

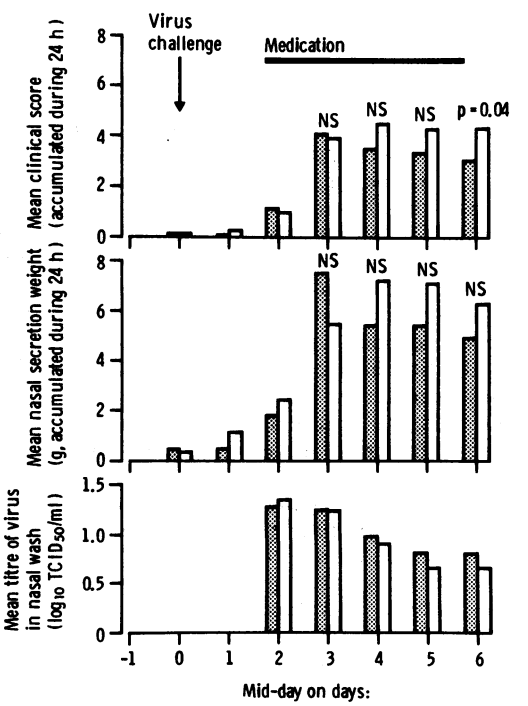

FIG. 1. Clinical score, nasal secretion, and virus excretion in all volunteers challenged with HRV9 and treated with enviroxime (▨) and placebo (□). NS, Not significant.

TABLE 2. Clinical and laboratory findings in volunteers who developed symptoms after medication began

Group	Pretrial antibody titer	No.	No. with clinically diagnosed colds		Laboratory findings		
			Mild or worse	Very mild or absent	Antibody rises	Virus isolated	Either or both
Enviroxime	<2	7	1	6	3	7	7
	2–8	7	2	5	6	4	6
	>8	2	0	2	0	2	2
	Total (%)	16	3 (19)	13	9 (56)[a]	13 (81)[a]	15 (94)[a]
Placebo	<2	8	6	2	5	8	8
	2–8	6	2	4	5	4	6
	>8	3	0	3	2	2	2
	Total (%)	17	8 (47)	9	12 (71)[a]	14 (82)[a]	16 (94)[a]

[a] $P > 0.05$.

secretion produced by each volunteer during the medication period.

Intranasal enviroxime had no discernible effect on rhinovirus infection in volunteers who developed symptoms before the start of medication.

DISCUSSION

In the present study, 21 volunteers were given intranasal enviroxime six times daily, starting 44 h after challenge with a virulent strain of HRV9. The mean concentration of enviroxime present in nasal washes taken approximately 2 h after the administration of an intranasal spray (6.52 μg/ml) was over 100 times the 50% inhibitory concentration for HRV9, and a concentration of greater than 10 times the 50% inhibitory concentration for HRV9 was exceeded in 88% of the nasal-wash samples. It therefore seems likely that a concentration of drug in excess of the 50% inhibitory concentration for HRV9 would have been in contact with the nasal mucosa for most of the time in the majority of volunteers. The intranasal sprays were well tolerated, although the mild and transient stinging experienced on insufflation may prove to be a limiting factor upon any increase in the frequency of medication. Stinging was caused by both the placebo and the enviroxime preparation, and no side effects could be attributed to enviroxime alone in volunteers challenged with saline, although the number of volunteers in this group was small.

Comparisons with a placebo-treated control group having a similar immune status to HRV9 showed that there were small reductions in clinical score and rhinorrhoea in the enviroxime-treated group. The reduction in clinical score apparently caused by enviroxime reached statistical significance only on day 5 after virus challenge, and the reductions in total clinical scores and rhinorrhoea were not significant. Neither the quantity of virus present in nasal washes nor the number of antibody rises to HRV9 were

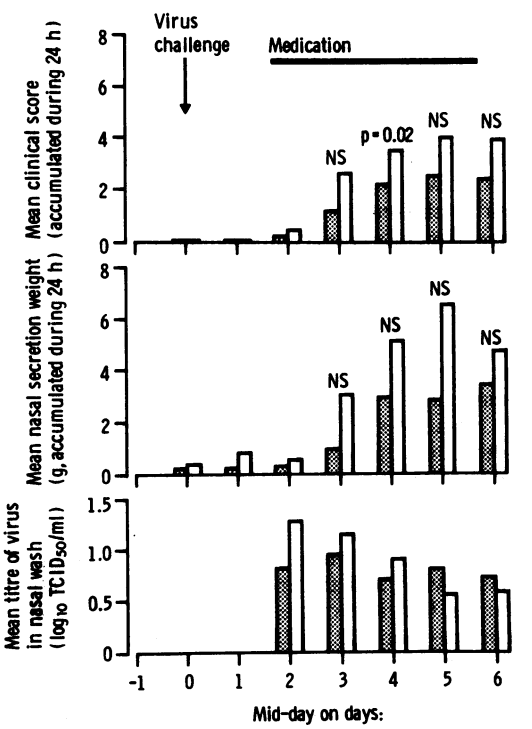

FIG. 2. Clinical score, nasal secretion, and virus excretion in volunteers who did not develop cold symptoms until after medication had begun. ▨, Enviroxime-treated group; □, placebo-treated group; NS, not significant.

significantly reduced in the enviroxime-treated group as compared with the placebo-treated group. It is nevertheless striking that consistent reductions in clinical scores and rhinorrhoea followed the start of medication. A negative correlation between the quantity of drug present in nasal washes and clinical and laboratory evidence of infection would be expected if enviroxime is effective. However, it should be borne in mind that rhinorrhoea may lead to a more rapid clearance of the drug. As both clinical and laboratory evidence of rhinovirus infection are highly correlated, this mechanism could have been responsible for the observed association.

A separate analysis of volunteers who did not have clinical symptoms at the start of medication might have been expected to reveal an increased effect of enviroxime, but such an increase was not obvious. Although the proportion of colds in the enviroxime-treated group decreased relative to that in the placebo-treated group, the difference was not statistically significant. There was a significant reduction in daily clinical score in the enviroxime-treated group on day 3 after virus challenge, but this observation was not supported by a significant reduction in total clinical scores or in the mean daily weight of nasal secretions produced by each volunteer during the medication period. In both analyses, there was an apparently greater effect of enviroxime in the absence of detectable serum neutralizing antibody to the challenge virus. This difference could have been due to a diminished effect of the drug in the presence of preexisting immunity. However, this explanation seems unlikely, and there was no evidence to suggest that the observed differences were not purely random.

Subtraction from the analysis of volunteers who developed symptoms before medication began reduced the number in the placebo-treated group to 17 and that in the enviroxime-treated group to 16. Calculations of the statistical power of trials conducted at the Common Cold Unit after HRV9 challenge have shown that relatively large effects may be missed, even when numbers in excess of 20 volunteers per group are used (6).

In conclusion, intranasal therapeutic enviroxime seems to have some beneficial effect in volunteers challenged with HRV9. Perhaps the most striking evidence of this conclusion is the consistent decrease in clinical symptoms observed after medication began (Fig. 1). The reductions in clinical and laboratory evidence of infection (with the exception of antibody response) were less than those produced by combined oral and intranasal enviroxime given prophylactically (5). Considerably larger numbers would be required to prove conclusively that enviroxime has a therapeutic effect, and such large trials could not be conducted with volunteers housed in isolation. In addition, although our experiments are designed to simulate natural infection, we do not know how close the approximation is. For these reasons, it would seem appropriate to test enviroxime against rhinovirus infection in a large-scale field trial in order to further investigate its value.

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