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Failure to demonstrate synergy between interferon-α and a synthetic antiviral, enviroxime, in rhinovirus infections in volunteers

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Summary

Marked synergy between the antirhinoviral effect of rHuIFNα and enviroxime has been observed in vitro but an attempt to demonstrate it in volunteers was unsuccessful. The sub-optimal intranasal dose of rHuIFNα (0.18 Mu four times daily for $4\frac{1}{4}$ days) used prophylactically in the trial did reduce the severity of colds induced by RV9 and 14, but the difference did not reach statistical significance and was not enhanced by the administration of enviroxime (0.28 mg six times daily for six days). The main reason for failure is thought to be the rapid removal of enviroxime from the nose when given intranasally.

Synergy; Interferon α; Enviroxime; Rhinovirus; Prophylaxis

Introduction

Many synthetic compounds prevent the infection of tissue cultures with rhinoviruses but none has been shown to have a significant effect in protecting volunteers against infection with this group of viruses. Until 1987 the most encouraging results have been achieved with enviroxime, a benzimidazole derivative. In a previous study at the Common Cold Unit, enviroxime given orally and intranasally

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was shown to reduce the number of colds, the mean daily clinical score (a measure of the severity of symptoms) and the mean nasal secretion weight compared with placebo, but none of these differences reached statistical significance at the $P < 0.05$ level (Phillpotts et al., 1981). When intranasal enviroxime was given in the incubation period of rhinovirus infection in a subsequent investigation there was a significant benefit to volunteers who cleared the drug slowly (Phillpotts et al., 1983b). Although we had shown a consistent benefit with enviroxime, this was too small to be detected in other studies elsewhere (Hayden and Gwaltney, 1982; Levandowski et al., 1982).

Intranasal interferon (IFN) α, on the other hand, completely protected volunteers from infection with rhinovirus type 9 (RV9) and 14 (RV14), but the amount required and the frequency of administration were critical. To be effective, a daily dose of 7.5 Mu IFN α given in two, or preferably three, divided doses was required in our studies (Phillpotts et al., 1983a). Unfortunately at this dosage at least 50% of recipients develop nasal stuffiness and blood stained discharge after one month's application (Hayden et al., 1983; Scott et al., 1985).

There is, therefore, a need to enhance the antiviral effect either of synthetic compounds such as enviroxime so that a significant clinical action can be demonstrated, or of IFN so that a smaller dose can be used with a reduction in its adverse effects.

In vitro studies have shown there is a marked synergistic effect between a number of synthetic antiviral compounds and different IFNs as there is between different IFNs themselves, in protecting tissue cultures against infection with rhinoviruses (Ahmad and Tyrrell, 1986).

More detailed studies of the combination of enviroxime and IFN α showed that antirhinoviral activity increased 100-fold when they were combined. Furthermore, since this synergy occurred in organ cultures of human respiratory epithelium as well as in tissue culture (Ahmad and Tyrrell, 1986), it might well be effective in human volunteers. A combination of antiviral agents, acting in different ways, would also have the added advantage that drug resistant mutants would be less likely to occur than during treatment with a single drug.

It was considered that the findings from these volunteer and laboratory experiments warranted a study in volunteers to determine whether synergy between enviroxime and IFN α could be demonstrated in man. The study was intended to discover whether the prevention of colds was markedly enhanced by combining treatments rather than using them separately. To make a clear contrast with the effects of combined medication, the dose of the drugs was reduced; slightly in the case of enviroxime and substantially in that of interferon, so that neither of them would be expected to have a significant effect on the incidence or severity of colds when given alone. If the number of colds and the severity of symptoms in those receiving combined medication were reduced by 90% compared with those given a single drug, the results of the trial would be statistically significant. The results of the study are reported here.

Materials and Methods

These trials were approved by the Harrow District Ethical Committee.

Healthy volunteers of either sex, aged between 18 and 50 years were recruited and housed in isolation in groups of two or three according to our normal practice (Beare and Reed, 1979). All volunteers completed questionnaires to assess their introversion/extroversion and certain obsessional factors as these have been shown to influence the outcome of virus challenge (Totman et al., 1980; Broadbent et al., 1984).

Medication

A highly purified preparation of human IFN α 2A produced by genetic engineering, Roferon-A, made by Hoffman-La Roche, Switzerland, was provided by Roche Products Ltd., Welwyn Garden City. It was supplied as an aqueous solution containing 2 Mu/ml in sprays which delivered 0.09 ml per activation. An interferon placebo (PIFN) was obtained from the same source and consisted of the aqueous vehicle alone in identical sprays.

Micronised 2-amino-1-(isopropylsulphonyl)-6-benzimidazole phenyl ketone oxime, enviroxime, was supplied as a metered dose inhaler delivering 0.142 mg per activation. Tween-85 with trichloromonofluromethane and dichlorodifluromethane acted as propellant. The propellant alone in identical inhalers constituted the enviroxime placebo (PEN) and both were supplied by Lilly Research Centre Ltd., Windlesham.

Virus challenge

The virus challenge consisted of two filtered, bacteriologically sterile nasal wash pools, one containing RV9 and the other RV14 and both propagated by serial intranasal passage in human volunteers at the Common Cold Unit, Salisbury.

Study design

Basically the study was designed to compare the effects of either one half the previously used dose of enviroxime or approximately one fifth the optimal dose of IFN α with that of both treatments together in volunteers exposed to rhinovirus challenge.

The actual dosage regimen chosen was self-administered under supervision of one spray of IFN α to each nostril, four times a day for 17 doses ($4\frac{1}{4}$ days, total dose 6.12 Mu) and for enviroxime one spray to each nostril, 30 to 60 minutes after IFN, for 24 doses (6 days, total dose 6.816 mg). Volunteers used to assess IFN or enviroxime alone received PEN or PIFN respectively to maintain the double blind nature of the trial.

The tolerance of volunteers to larger doses of both IFN α and enviroxime has been established previously but that of giving the combination of drugs needed to be investigated. A small number of volunteers were given the regimen of combined IFN and enviroxime described above and the effects compared with those resulting from a similar number of volunteers given both PIFN and PEN. The vol-

unteers were treated and monitored as in the challenge study (see below) but no virus was given and no virological studies undertaken.

In the challenge study volunteers were arranged in three groups and randomly allocated to receive IFN and PEN, PIFN and enviroxime or IFN and enviroxime. After a 48 h quarantine period (day 2) those volunteers without symptoms commenced medication and were challenged with an estimated 100 $TCID_{50}$ of rhinovirus type 9 2 h after the fifth dose of IFN or PIFN (day 3) and a similar dose of rhinovirus type 14 1 h later. A few volunteers were given saline instead of virus and these served to maintain the double-blind nature of the trial and provide further data on tolerance. Both volunteers and observer were blind as to the medication given and to virus or saline challenge. Each volunteer was assessed daily and a score given according to the signs and symptoms present. In addition, the amount of nasal secretion produced each day was weighed. At the end of the trial the clinical observer assessed each volunteer as having suffered no cold, a doubtful cold (not significant), or a significant cold of a mild, moderate or severe nature. Nasal washings were collected from each volunteer prior to inoculation and on days 5 to 9 inclusive. Blood samples for haematological and biochemical tests were collected before and at the end of medication.

Virological procedures

Virus isolation was attempted by inoculating cultures of Ohio HeLa cells with nasal washings. Serum neutralising antibody titres were assayed by a micro-neutralisation test in Ohio HeLa cells on the initial blood sample and on a further sample collected approximately three weeks after virus challenge. A 4-fold or greater rise in antibody titre to either of the challenge viruses or their isolation was taken as evidence of infection.

Statistical analysis

Differences in the frequency of the colds and infection between the groups were tested for significance using the Chi-squared test with Yates' correction. Clinical scores and nasal secretion weight data were tested by rank analysis of variance with each variable divided into three strata according to the lower of the pre-challenge neutralizing titres to the two challenge viruses of each volunteer ($<$1:2, 1:2 to 1:8 and $>$1:8).

Results

Eleven volunteers took part in the initial tolerance experiment. IFN and PIFN were well tolerated; but the majority of volunteers found the nasal stinging and eye watering that followed the use of either enviroxime or PEN unpleasant, although only one volunteer withdrew from the study on account of this. Of the ten remaining volunteers five received IFN and enviroxime and five the two corresponding placebos. No other adverse effects were encountered and the mean total clinical score was very low and identical for the two groups at 0.8.

Fifty-seven volunteers took part in the prophylactic study of which two were subsequently found to have virus in their pre-challenge nasal wash and were excluded, retrospectively, from the final analysis. Of the remaining 55 volunteers four were challenged with saline, two received IFN α/PEN, one enviroxime/PIFN and one IFN α/enviroxime.

The 51 volunteers challenged with virus were divided equally among the three medication groups which were well balanced for sex, age and pre-challenge neutralizing antibody titre. Similarly introversion/extroversion scores were comparable between the groups and the difference in obsession scores is not statistically significant (Table 1).

The number of significant colds was lowest 4/17 (23.5%) in the IFN α/enviroxime group, next lowest 6/17 (35.3%) in the IFN α/PEN group and highest 8/17 (47%) in the enviroxime/PIFN group. These differences are not statistically significant;

TABLE 1

Clinical responses and evidence for infection among volunteers

Volunteer group and pretrial AB titre +	No. of volunteers	No. with clinically diagnosed colds (%)		Virological findings		
		Significant	Not significant	Rise in AB titre	Virus isolated	Either or both
Interferon						
< 2	15	5	10	8	8	10
2–8	0	0	0	0	0	0
> 8	2	1	1	0	0	0
	17	6 (35.3)	11	8	8	10
Enviroxime						
< 2	13	5	8	4	6	7
2–8	4	3	1	2	0	2
> 8	0	0	0	0	0	0
	17	8 (47.0)	9	6*	6	9
Interferon/ enviroxime						
< 2	14	3	11	4	4	6
2–8	2	1	1	2	1	2
> 8	1	0	1	0	0	0
	17	4 (23.5)	13	6*	5	8

* Only 15 convalescent sera received.

Interferon: 8 females, 9 males; mean age 33.18 ± 2.03 years; obsession 1.63 ± 1.20; introversion/extroversion 10.19 ± 4.46.

Enviroxime: 8 females, 9 males; mean age 33.77 ± 9.00 years; obsession 2.59 ± 1.73; introversion/extroversion 9.94 ± 4.05.

Interferon/ enviroxime: 8 females, 9 males; mean age 35.29 ± 7.56 years; obsession 2.35 ± 1.62; introversion/extroversion 10.35 ± 3.53.

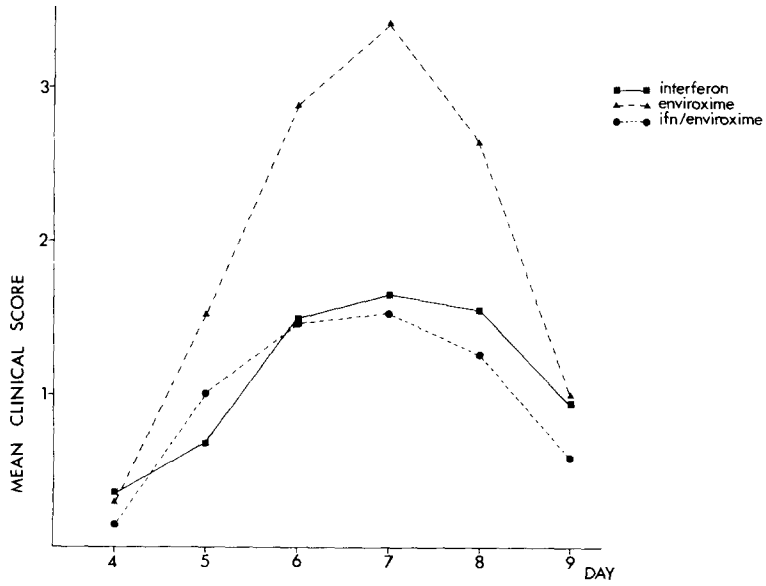

Fig. 1. Mean daily clinical scores in volunteers given interferon and enviroxime placebo, interferon placebo and enviroxime, and interferon and enviroxime and challenged with both RV9 and RV14.

and if doubtful colds (i.e. those with upper respiratory symptoms but not sufficiently characteristic, persistent or severe to enable a firm diagnosis of a cold to be made) are included, the figures are 10, 11 and 10, respectively.

Evidence of infection, a 4-fold rise in antibody titre to, and/or isolation of one of the challenge viruses, was found in 10 (59%) in the IFN α/PEN group, 9 (53%) in the enviroxime/PIFN group and 8 (47%) in the IFN α/enviroxime group.

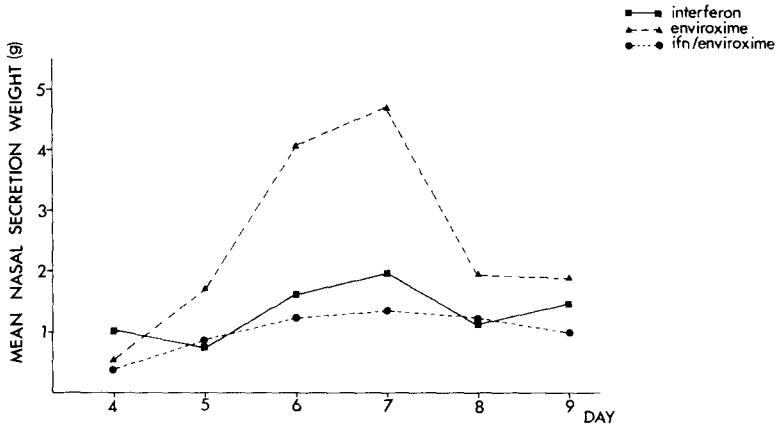

Fig. 2. Mean daily nasal secretion weights in volunteers given interferon and enviroxime placebo, interferon placebo and enviroxime, and interferon and enviroxime and challenged with both RV9 and RV14.

The mean daily clinical scores for the two groups receiving IFN were half those for the enviroxime/PIFN group, and the differences in mean daily nasal secretion weights followed the same pattern but were even more marked (Figs. 1 and 2). However, none of the differences between any of the groups reached statistical significance.

The mean total clinical score of the four volunteers challenged with saline, 0.75, confirmed the findings in the tolerance studies (0.8) that these drugs were well tolerated. There were no significant changes in the values for haematological and biochemical tests on blood collected from volunteers at the end of the trial compared with those on blood obtained prior to medication.

The proportion of volunteers excreting virus was low in all three groups. Virus recovery was most frequent in those given IFN α/PEN (Fig. 3) and the number of volunteers shedding virus doubled in this group on the day medication with IFN ceased compared with the previous two days. Only a slight increase was observed in the IFN α/enviroxime group, while the proportion of volunteers in the enviroxime/PIFN group never exceeded 17.5% for the duration of the trial.

Discussion

Some reduction in the frequency of colds was observed in both groups receiving IFN compared with the enviroxime/PIFN group. The differences do not reach statistical significance with either IFN α group singly or combined. However, it is likely that this reduction is genuine and it would be expected from previous experience

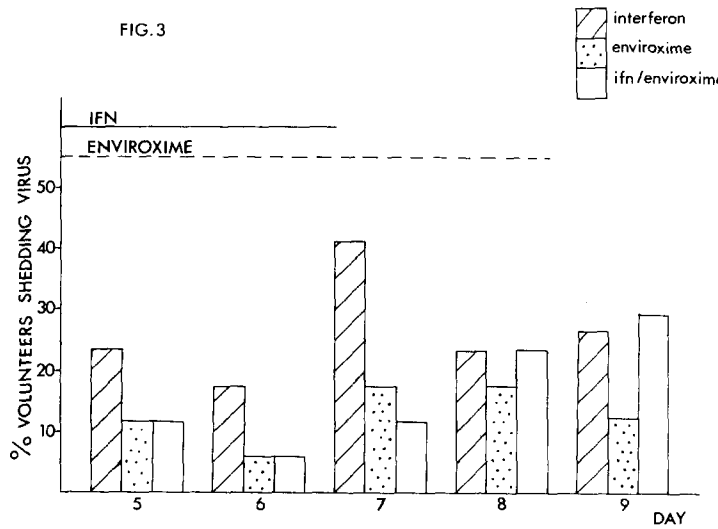

Fig. 3. Proportion of volunteers excreting virus in nasal washings given interferon and enviroxime placebo, interferon placebo and enviroxime, and interferon and enviroxime and challenged with both RV9 and RV14.

that one fifth of the optimal dose of IFN α would produce some effect short of total protection especially as it was administered in four, rather than three, divided doses (Phillpotts et al., 1983a).

There was a corresponding non-significant reduction in mean daily clinical scores and mean daily nasal secretion weights in the two IFN α groups. Possibly, the reduction in these statistics resulted in some infections which would otherwise have been diagnosed as significant colds being labelled as doubtful colds. The frequency of combined colds and doubtful colds is virtually identical for all three groups. Furthermore, no significant differences were observed in the proportion of volunteers infected in the three groups, and what differences there are could well have been less had sera from all 17 volunteers been obtained, as in the IFN α/PEN group, instead of the 15 in both the enviroxime/PIFN and enviroxime/IFN α groups. This regime of intranasal IFN α appears to afford no protection against infection but does reduce the severity of the cold. Had even a minor amount of synergy occurred, a highly significant degree of, if not complete, protection would have been expected and this clearly has not been demonstrated.

In previous studies when IFN α has been used, anti-IFN α has been added to the culture media during assay of the nasal washings (Phillpotts et al., 1983a). Similarly, when enviroxime has been employed the nasal washing has been allowed in contact with tissue cultures for 90 min to permit adsorption of virus, before being washed three times and fresh media added (Phillpotts et al., 1983b). In the absence of clinically demonstrable synergism the additional labour to include these refinements was considered unwarranted and was omitted. It is not surprising, therefore, that the recovery rate of virus was low because of the presence of antiviral activity in tissue cultures transferred from the nasal washings. This is supported by the large increase in the proportion of volunteers in the IFN α/PEN group from whom virus was isolated when the nasal washings were collected after IFN medication ceased. Inhibiting concentrations of enviroxime, up to 1000 MIC, occur in washings after intranasal use (Phillpotts et al., 1981) and the persistence of medication with this drug up to and including the penultimate day of the trial would account for the small number of volunteers from whom virus could be recovered in the enviroxime/PIFN and IFN α/enviroxime groups.

The failure to demonstrate synergy between these two antivirals in volunteers in the face of strong laboratory evidence to the contrary is probably no more than an extension of the reason why so many synthetic antivirals fail to fulfil their in vitro promise. In tissue or organ culture both drugs are in contact with the host cells throughout the experiment. In human subjects IFN α has an advantage in that there are receptors on the cells of the nasal mucosa and after attachment IFN induces an antiviral state in the cell which persists for several hours. Enviroxime, on the other hand, is probably rapidly removed by the ciliary–mucous mechanism and is only available to exert an effect, either singly or synergistically with IFN α, for a very limited period. The drug recovered during nasal washing is likely to be that deposited on the stratified epithelium of the external nares. For these reasons the in vitro and in vivo experiments are not strictly comparable. It would probably be necessary to have a synthetic drug taken orally and continuously excreted in the

nasal secretions so as to provide a constant presence of drug in the region of the host cells to be used in conjunction with IFN in order to stimulate better, in volunteers, the laboratory experiments. Unfortunately no such antiviral, active against rhinoviruses, is currently available.

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