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Tolerance of One-Month Intranasal Interferon

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Under double-blind conditions, groups of volunteers (68 in total) were allocated at random to take intranasal solutions of placebo or one of three doses of highly purified leucocyte interferon by intranasal spray twice a day for 28 days. The highest dose would have been expected to protect against experimental colds. Treatment was discontinued because of upper respiratory symptoms as often in each of the interferon groups as in the placebo group. However, it was possible to distinguish clinically between "colds" on placebo and low-dose interferon and "reactions to treatment" on high-dose interferon. The features of the reactions to treatment were a protracted build-up of local symptoms and minor epistaxis. None of the volunteers on the high-dose interferon were thought to have a definite cold, but viruses were isolated from four out of six volunteers on low-dose interferon who had definite colds. Previous experiments had also shown this dose to be insufficient to protect against experimental rhinovirus challenge.

The dose of interferon that appeared to protect against virus infection caused significant unwanted effects. It is essential to find interferon preparations with less inflammatory activity before interferon can be considered for use as a long-term prophylactic against the common cold.

Key words: rhinovirus, coronavirus, paramyxovirus, volunteers, leucocyte interferon

INTRODUCTION

Various preparations of leucocyte-derived or rDNA-derived human alpha interferons (IFNα) when given intranasally can protect against rhinovirus and coronavirus colds in volunteers [Merigan et al, 1973; Scott et al, 1982; Higgins et al, 1983; Phillpotts et al, 1983]. In these studies, interferon was given before virus challenge, and there is no evidence yet that exogenous interferon can affect the clinical course of a cold once symptoms have begun. If interferon is to be used in prophylaxis,

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treatment would have to be taken on a regular basis during periods of risk. However, in controlled trials at the MRC Common Cold Unit (Salisbury, UK), volunteers treated with various interferon preparations and challenged with saline instead of virus had more local upper respiratory symptoms than those treated with placebo, suggesting that interferons themselves may cause local reactions [Scott, 1982]. This is supported by observations of local inflammation after intradermal injections of monoclonal antibody-purified interferon and influenza-like reactions after parenteral interferons [Priestman, 1980; Scott et al, 1981].

Dosing once a day with rDNA interferon-α can prevent rhinovirus colds, but more frequent dosing is required for consistent protection against experimental virus challenge at any time of the day [Phillpotts et al, 1983]. As a preliminary to large-scale prophylaxis studies, therefore, the tolerance of a schedule of intranasal interferon sufficient to protect against experimental virus challenge was studied in healthy volunteers for four weeks.

Volunteers

The study protocol was approved by the Ethical Committee at Northwick Park Hospital. Volunteers were recruited from the staff at the Clinical Research Centre, Northwick Park Hospital, and staff and students at Harrow College of Higher Education (Middlesex, UK). Volunteers were screened by medical history, examination of the upper respiratory tract, pulse and blood pressure, and by routine haematological and biochemical tests. In the females, urine was screened for human chorionic gonadotrophin. Volunteers with any clinically significant illness or abnormality were excluded. The most common exclusion criterion was perennial rhinitis, although volunteers with hay fever, but not expecting active disease during the period of study (March–April), were included in the study.

INTERFERON

Interferon was induced in pooled buffy-coat leucocytes using Sendai virus and was partially purified as previously described [Cantell et al, 1981]. Further purification was done by affinity adsorption chromatography on a monoclonal antibody (NK2) bound to Sepharose 4B [Secher and Burke, 1980]. Interferon was stabilized by the addition of twice-reprecipitated human serum albumin (1 mg/ml). The placebo solution was phosphate-buffered saline with albumin at the same concentration. Active and placebo preparations were tested extensively for contamination by adventitious agents and for acute toxicity in groups of mice and guinea pigs. The final preparations given to volunteers contained only 0.4–0.8 ng/ml bacterial endotoxin by limulus lysate assay. No preservative was added.

Interferon activity was assayed on human and bovine cells and by immunoradiometric assay using ^{125}I-NK2 [Secher, 1981]. Dilutions of the eluate from the NK2 column were made to give concentrations of 10, 3.3, and 1.0 million units (Mu) per ml. Back-titration of these dilutions in several assays against the leucocyte interferon standard MRC69/19B showed them to contain 11, 3.8, and 1.1 Mu/ml, respectively. All the solutions including placebo were indistinguishable.

Sufficient solution for fourteen days of treatment was dispensed in a sterile bottle fitted with a nasal spray pump. The dose was 0.1 ml per nostril each morning

and evening. Thus the intended daily dose of interferon was nil [placebo (group A), 0.44 Mu (group B), 1.52 Mu (group C), or 4.4 Mu (group D)]. The sprays were kept at home in the domestic refrigerator.

METHODS

Volunteers were allocated at random to receive placebo or one of the three interferon solutions and assessed under double-blind conditions. Those with colds during the two weeks before the start of the study were excluded. All volunteers were seen at the start of treatment and then at 14, 28, and 42 days.

In addition, volunteers were asked to report if they developed three or more upper respiratory symptoms for 48 hours. Volunteers kept a daily record of symptoms from nil (0) through mild (1), moderate (2) to severe (3). For 24 hours before each visit, including the pretrial assessment, each volunteer used standard paper tissues to blow the nose and these were collected, counted and weighed. At each visit, volunteers were assessed clinically according to the schedule used at the MRC Common Cold Unit [Beare and Reed, 1977]. Because it was suspected that the interferon itself might cause reactions and because it was not known in advance whether it would be possible to distinguish between colds and reactions, treatment was discontinued when symptoms had been present for 48 hours. At this time two clinical assessors (G.M.S. and J.K.O.) judged whether the syndrome was like or unlike a classical cold on the basis of clinical experience. Such an assessment, independent of the clinical score, is standard practice at the MRC Common Cold Unit [Beare and Reed, 1977]. Nasal washings and throat swabs were cultured in HeLa, primary baboon kidney, MRC5, and MRC C16 cells in the presence of 2×10^3 neutralising units of calf antilymphoblastoid interferon. Negative specimens were passaged once. Throat swabs were cultured for streptococci. Pre- and post-trial sera from all volunteers were examined for interferon neutralising activity. Because the treatment solutions contained no preservative, the remnants were cultured to assess the frequency and level of bacterial contamination. A random sample of treatment remnants were also assayed for antiviral activity to establish whether treatment had lost activity.

RESULTS

Clinical Reactions

Of seventy-three volunteers considered suitable for the trial, five had colds within two weeks of the start of treatment and were excluded. Table I shows some characteristics of the four groups of volunteers. Random allocation produced some imbalance in characteristics that might have influenced outcome (sex, age, history of hay fever, smoking, previous exposure to intranasal interferon). However, the numbers are small and there was no obvious influence of these factors on outcome in terms of the number of volunteers who subsequently discontinued treatment. In the case of two volunteers in group C, inadvertent premature ending of therapy was followed shortly by upper respiratory symptoms. One completed 22 days of treatment and developed symptoms one day afterwards: the other had a break of six days after ten days of treatment and developed upper respiratory symptoms on the day that the new bottle was dispensed. The other volunteers completed 28 days of treatment unless they had significant symptoms.

TABLE I. Characteristics of Volunteer Groups and Dropout Rates From the Trial According to Colds or Reactions*

Group	IFN dose Mu/day[a] A (placebo)	B (0.44)	C (1.52)	D (4.4)
Entered	17	18	17	16
Incomplete treatment	0	0	2	0
Male:female ratio	9:8 (1:3)	12:6 (5:2)	10:7 (3:2)	4:12 (1:7)
Age (years)	36	33	35	34
Mean range	19–60	21–57	19–56	18–57
History of				
Seasonal rhinitis	4 (1)	0	6 (3)	1 (1)
Smoking cigarettes < 10/day	2 (0)	2 (0)	1 (0)	0
> 10/day	2 (1)	1 (1)	2 (0)	5 (3)
Previous intranasal IFN	3 (0)	2 (0)	3 (3)	1 (1)
Outcome				
Continued 28 days	13	11	10	8
Discontinued because of				
Cold	3	6	4	0
Possible cold	1	1	0	3
"Reaction"	0	0	1	5
Virus isolated				
Coronavirus 229E	0	2	0	0
Rhinovirus	0	2	0	0
Mild reaction, treatment				
not discontinued	2	1	2	4
Posttreatment cold	5	2	3	0
Virus isolated				
Rhinovirus	1	0	0	0
Parainfluenza III	1	0	1	0

*Figures in brackets are numbers of volunteers who discontinued treatment early.
[a]IFN, interferon; Mu, million units.

Definite reactions thought to be unlike a cold were diagnosed in 5 out of 16 given the highest dose of interferon, but in none of 17 given placebo ($P = 0.02$, Fisher's exact test, two-tailed). However, the differences between the groups in the proportion of volunteers who stopped treatment because of possible or definite colds as opposed to reactions do not achieve statistical significance. The times at which volunteers discontinued treatment in each group are presented using life tables in Figure 1. Although each interferon group appeared to do worse than the placebo group, log rank analysis revealed no significant differences between the groups ($P = 0.49$), nor a significant trend with increasing dose ($P = 0.22$).

Nine volunteers had mild symptoms but did not discontinue treatment, either because they were thought by the volunteers too trivial to merit a special visit or because they did not fulfill the criteria for withdrawing treatment. The cluster of symptoms over a few days in some of these indicates that they may have had very mild colds. Others had one or two symptoms only that were suggestive of perennial rhinitis.

Virus Isolation

Viruses were isolated from four volunteers with definite colds while on low-dose interferon. Viral isolation was negative in all those who stopped the highest dose

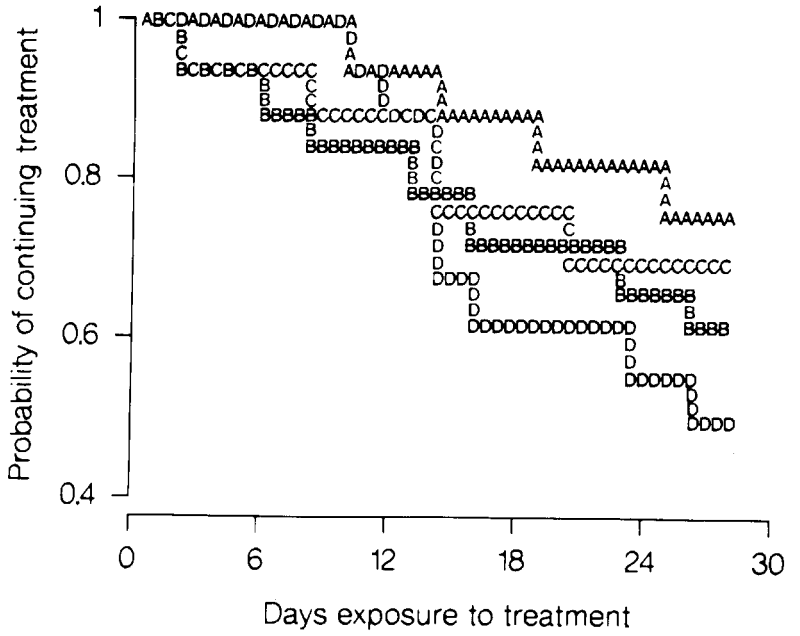

Fig. 1 Life table of withdrawal from treatment because of upper respiratory symptoms according to treatment group. A. placebo; B, 0.44; C, 1.52; D, 4.4 Mu interferon per day.

of interferon, although β-haemolytic streptococcus, Lancefield group G, was isolated from the throat of one.

In addition, four viruses were isolated from twelve volunteers (including two in group C who stopped treatment prematurely) who had colds after completion of or withdrawal from treatment. In three volunteers there were two discrete upper respiratory illnesses, but no volunteers on high-dose interferon (group D) developed further upper respiratory symptoms after stopping treatment.

Differences Between Colds and Reactions to Treatment

The symptoms of nine volunteers in the placebo and low-dose interferon groups thought to have colds were compared with those of five on high-dose interferon who were assessed as having reactions (Table II). The latter had mild symptoms, particularly nasal discomfort, on many days, whereas those with colds showed high clinical scores over one or two days. Secondly, seven of nine patients with colds had a clear nasal discharge compared with only one of five with reactions. Thirdly, all of five patients with reactions had prolonged bloodstained nasal secretions, two with frank epistaxis, compared with only one of nine of these with colds. This latter volunteer reported a tendency towards having nose bleeds before the trial.

In the volunteers who did not stop treatment, there were no changes in the mean number of tissues used over 24 hours during the trial, except in group D on the highest dose of interferon, where a rise from 2.3 pretrial to 7.1 (at two weeks) and 5.5 (at four weeks) was observed. There was only a small parallel rise in mean 24-hour nasal secretion weight.

TABLE II. Analysis of Symptoms Experienced by Nine Volunteers With Colds in Groups A (Placebo) and B (Low-Dose Interferon) Compared With Those of Five Volunteers in Group D (High-Dose Interferon) Assessed as Having Reactions to Treatment

a. Duration of symptoms before treatment was stopped (no. of volunteers)

Day before stopping treatment	13	12	11	10	9	8	7	6	5	4	3	2	1
Colds (n = 9)							1	1	1	2	5	8	9
Reactions (n = 5)	2	2	2	3	3	3	3	5	5	5	5	5	5

b. Symptoms

	Colds (n = 9)	Reactions (n = 5)
Local		
Runny nose	8	3
Clear discharge	7	1
Purulent discharge	1	0
Nosebleed or bloodstained secretions	1	5
Sneezing	7	4
Blocked nose	5	3
Postnasal discharge	6	2
Watering/itching eyes	4	2
Sore throat	6	3
Hoarseness	4	1
Cough	6	1
General		
Headache	6	3
Malaise	8	3
Myalgia	5	2
Fever	3	1
Chills	1	2
Clinical assessment of reactions		
Upper respiratory reaction graded		
Mild	5	1
Moderate	3	4
Severe	1	0
General reaction graded		
Nil	2	2
Mild	3	0
Moderate	4	2
Severe	0	1
Analysis of 24 hr nasal secretions		
Mean increase between assessment pretreatment and on discontinuing treatment:		
Number of tissues used (range)	5.6 (0–12)	2.8 (0–6)
Nasal secretion weight, gm (range)	2.1 (0–6.4)	0.6 (0–1.6)

Treatment Quality Control and Antibody Assays

Only four bottles used during treatment out of 118 cultured when they were returned to the laboratory grew any organisms. These were typical of commensal upper respiratory flora. The interferon solutions did not lose activity over the study period. The posttreatment sera failed to neutralise the antiviral effect of NK2-interferon in vitro.

DISCUSSION

During the six-week period of this study, there were more upper respiratory events in the 68 volunteers who entered this trial than had been expected. Only

seventeen reported no symptoms, and perhaps partly because of the introspection engendered by daily reporting of symptoms, another group recorded minor symptoms intermittently through the trial period. It is to be expected that regular spraying of any substance into the nose, however innocuous, might cause symptoms (if only a slight rhinorrhoea) for a short period after spraying.

The five clinical colds that occurred in the fortnight preceding the start of the trial suggested that 13 further volunteers might have been expected to develop colds over the subsequent six weeks. However, in addition to 13 volunters who had definite clinical colds, 5 others had possible colds while on treatment and a further 12 had posttreatment colds. Six volunteers had apparent reactions to treatment that manifested as protracted symptoms with nasal discomfort and nose bleeding but without much rhinorrhoea. It had been suggested that low doses of interferon might protect volunteers against wild as opposed to experimental virus exposure, but treatment with the lowest dose of interferon clearly failed to protect against clinical colds, as rhinoviruses or coronaviruses were isolated from four of these volunteers. The small numbers in this trial do not allow us to evaluate whether a small dose of interferon might ameliorate a cold rather than prevent it totally. However, the highest dose caused unwanted upper respiratory symptoms, particularly a dry uncomfortable nose with nose bleeding. From previous studies, this dose should have been sufficient to protect against most rhinovirus and coronavirus colds, and clinically there were no definite colds in this group nor viruses isolated, neither were there posttreatment colds during the period of observation.

Other groups have found that rDNA IFN-α_2, given by nasal spray in doses of 5–10 Mu per day, caused local symptoms after some weeks of administration, yet was highly effective in protecting against wild rhinovirus infection [Farr et al, 1983; Betts et al, 1983]. Histology of nasal mucosal biopsies revealed nonspecific inflammation with some ulceration [Hayden et al, 1983]. The purity of our material, together with these results with a single species of leucocyte interferon, suggest that exogenous interferon itself causes intranasal inflammation. Whether endogenous interferon produced during a cold contributes to the symptoms of a cold is a matter for speculation.

This preliminary study shows that the minimal effective dose of intranasal interferon necessary to prevent colds caused local inflammation when given for more than a week, so that fewer than half the volunteers could continue to take it for a month without significant discomfort. It is, therefore, important to search for preparations of interferon that have less inflammatory effect, but an alternative approach will be to use high-dose interferon for a short time after contact with colds [Herzog et al, 1983].

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