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The efficacy of intranasal interferonα-2a in respiratory syncytial virus infection in volunteers

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Summary

In a double-blind, placebo-controlled study, self-administered intranasal interferonα-2a or placebo was given both before and after challenge with respiratory syncytial virus. The incidence of colds and the severity of signs and symptoms were reduced in those receiving interferonα-2a as compared with those given placebo.

In a further double-blind, placebo-controlled study, self-administered interferonα-2a or placebo was given only to those volunteers who developed colds following challenge with respiratory syncytial virus. There was no evidence that interferonα-2a reduced the severity of the signs and symptoms or shortened the duration of the illness.

The similarity of these results to the effect of interferonα-2a in rhinovirus infections in volunteers is discussed.

Interferon α-2a; RSV infection; Prophylaxis; Therapy

Introduction

Respiratory syncytial virus (RSV) is an important pathogen particularly in infants. Primary infection can result in bronchiolitis, pneumonia or even death especially in premature babies and those with pre-existing pulmonary or cardiac abnormalities. No effective vaccine is currently available and ribavirin, the only therapy that has been shown to influence beneficially the course of the disease, is costly and complicated to administer.

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The interferon response to RSV infection in children and adults is minimal compared with that to influenza virus (Hall et al., 1978; McIntosh, 1978; Isaacs, 1989; Hall et al., 1981) although in vitro, RSV replication is readily inhibited by interferon (Moehring and Forsyth, 1971). We have, therefore, studied the prophylactic and therapeutic effects of exogenous recombinant interferonα-2a on RSV infections in adult volunteers and the results are reported here. Although the illness following re-infection with RSV in adults, a common cold, differs from that of a primary infection in babies and affects a different region of the respiratory tract, the results obtained could give an indication of the response to be expected in children.

Materials and Methods

These studies were approved by the Harrow District Ethical Committee at Northwick Park.

Healthy volunteers between the ages of 18 and 55 years were recruited and housed in isolation at the Common Cold Unit in groups of two or three according to our usual practice. They were observed daily clinically under double-blind conditions. The symptoms and signs were used to make a diagnosis of a common cold and to construct a clinical score. They used standard paper handkerchiefs which were collected and weighed to give a daily nasal secretion weight (Beare and Reed, 1977). Psychological scores for extroversion/introversion and obsessionality of each volunteer were determined as these can influence the clinical and virological response to infection (Totman et al., 1980; Broadbent et al., 1984).

Lyophilised interferonα-2a (Roferon-A, Roche) was reconstituted to contain 10 million units/ml in 0.5% human serum albumin in phosphate buffered saline which, on its own, constituted the placebo. Both interferon and placebo were prepared fresh on alternate days and dispensed in a Mistette Mark II spray which delivered 0.1 ml per activation.

The virus challenge consisted of a bacteriologically sterile MRC_5 tissue culture fluid of the RSS-2 strain of RSV of the 11th passage containing $10^{5.5}$ $TCID_{50}$/ml (McKay et al., 1988). $10^{4.8}$ $TCID_{50}$ failed to produce symptoms in any of 10 volunteers challenged so the dose chosen represents less than 10 cold-producing doses.

Virus was isolated by inoculation of nasal washings into roller tube cultures of HEp-2 cells in the presence of anti IFNα antiserum (2000 units/ml when receiving medication and 200 units/ml when medication ceased). Virus was detected and identified by specific immunofluorescence of cells scraped from the tubes.

Antiviral antibody was detected by an ELISA test based on a test for coronavirus antibody previously described (Callow, 1985). Frozen and thawed virus-infected tissue culture, clarified by centrifugation, was used as antigen and similarly treated, uninfected cultures acted as a control. Both were bound directly to microtitre plates. Suitable dilutions of serum were bound and IgG detected with a phosphataselinked antibody probe. Antibody titres were calculated by a computer programme and expressed in terms of arbitrary units assigned to a reference human

serum which was titrated in parallel in every set of assays. A 1.5-fold rise represented an increase twice that of the standard error of the test and was regarded as significant. Antibody measured in this way is not a good predictor of susceptibility to infection.

Study design

Prophylactic trial. Volunteers were divided into two groups, balanced for age and sex, to receive either interferonα-2a or placebo. After a 36 h quarantine period volunteers self-administered, under supervision, 0.1 ml of medication to each nostril, three times a day, for 13 doses (a total of 26 million units). Four hours after the fourth dose of medication volunteers were challenged with virus or saline, 0.5 ml to each nostril. Volunteers were assessed clinically each day. Nasal washings for virus isolation were collected on day 1 and daily from day 5 to 9 inclusive and volunteers were requested to supply a convalescent sample of blood.

Therapeutic trial. After a 24 h quarantine period volunteers were challenged with virus or saline as in the prophylactic study. Subjects who were diagnosed as having developed a cold at any one of three inspection times between 9 a.m. on day 4 and 9 a.m. on day 6 were randomly allotted to receive interferonα-2a or placebo, 0.1 ml to each nostril hourly for the first 10 waking hours in order to expose as many mucosal cells as possible as soon as possible to interferon. Treatment was then continued four times a day for a further three days or until the end of the trial. Volunteers were assessed clinically and nasal washings and convalescent blood collected as in the prophylactic study.

Statistical methods. The frequency of colds and the proportion of volunteers infected in the two groups were compared by a χ^2 test. The significance of differences in clinical scores and nasal secretion weights was determined by rank analysis of variance with 'blocking' for the effect of pre-challenge antibody titre (Meddis, 1980).

Results

Prophylactic trial

Forty-six volunteers were enrolled in the prophylactic trial: of these three were excluded either because they developed a wild cold or were in contact with one and a further two because they failed to remain for the full length of the trial. Of the remaining 43 who took part in the study, three (two interferonα-2a and one placebo recipients) were challenged with saline. The two groups were well balanced for sex, age, pre-challenge antibody concentration and psychological scores (Table 1). There was a significant difference ($P < 0.05$) in the frequency of colds in the two groups as seven colds, one moderate and six mild, occurred in the 19 volunteers

TABLE 1

Volunteer group	Pretrial antibody*	No. of volunteers	No. with significant colds	Laboratory evidence of infection**	Virus isolated	Seroconversion
Interferonα-2a[a]	<2.5	6	1	4	4	4
	2.5-3.0	9	0	5	5	1
	>3.0	4	0	1	1	1
Total		19	1	10[c]		
Placebo[b]	<2.5	8	3	5	5	5
	2.5-3.0	4	2	4	4	2
	>3.0	7	2	5	5	1
Total		19	7	14[d]		

[a]Males 10, females 9, mean age 39.1 ± 10.6 years.Psychological scores: intro/extroversion 10.3 ± 5.3, obsessionality 2.1 ± 1.5.
[b]Males 10, females 9, mean age 38.9 ± 11.1 years.Psychological scores: intro/extroversion 10.3 ± 5.7, obsessionality 2.7 ± 2.2.
[c]Sixteen paired sera examined.
[d]Eighteen paired sera examined.
* Arbitrary units.
** Virus isolated and/or antibody rise; all subjects with colds had laboratory evidence of infection.

receiving placebo compared with one mild cold in those given interferon α-2a. Ten of the 19 in the interferonα-2a group showed laboratory evidence of infection compared with 14 of the 19 in the placebo group.

The mean daily clinical score (Fig. 1) was greater on days 3, 4 and 5 in the interferonα-2a group than the placebo group; however, this was statistically significant ($P < 0.05$) on day 3 only. When colds were apparent clinically the mean daily clinical score was appreciably greater in the placebo group and this reached statistical significance on two days, on day 8 ($P < 0.001$) and on day 9 ($P < 0.05$). A similar pattern is seen in the mean daily nasal secretion weights with those of the placebo group being significantly greater than those of the interferonα-2a group on three days, on days 7 and 9 ($P < 0.05$) and on day 8 ($P < 0.01$). The mean total clinical score, 12 ± 15, and mean total nasal secretion weight, 17 ± 21, in the placebo group were significantly greater ($P < 0.05$) than those in the interferonα-2a group, 3 ± 6 and 7 ± 12, respectively.

No colds occurred among the three saline recipients and the mean total clinical score and mean total nasal secretion weight for the one subject given placebo were 0.5 and 0.36 g respectively, compared with 1.3 and 3.2 g in the two receiving interferonα-2a.

Therapeutic trial

Sixty volunteers were enrolled in the therapeutic study and only one was excluded; the result of being medically unfit. Two were given saline and 57 were challenged with RSV of whom 21 developed colds. Eleven of the volunteers with colds (two moderate, nine mild) were given placebo and 10 (three moderate, seven

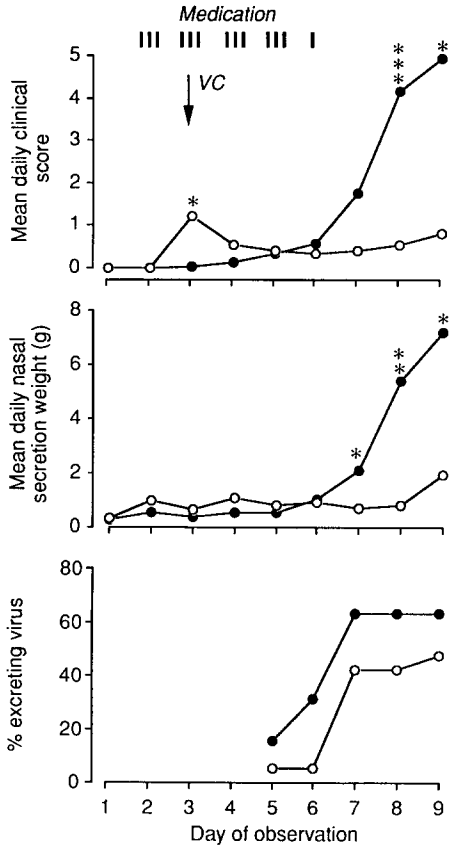

Fig. 1. Prophylaxis of RSV infection with interferonα-2a; •—•: placebo, ○—○: interferonα-2a. VC, virus challenge *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

mild) received interferonα-2a. The two groups were well balanced for age, sex and psychological scores (Fig. 2). The severity of the colds at onset, as measured by the mean nasal secretion weights, were similar as was the progress of the disease in the two groups. They were also well matched for the interval between the onset of symptoms and the beginning of treatment. The only significant difference between the two groups was an increased mean clinical score in the interferon group on day 2 ($P < 0.05$). The pattern of virus excretion was also very similar in the two groups.

Discussion

The most striking feature of this study is the similarity to the results obtained with intranasal interferonα-2 in rhinovirus infections (Scott et al., 1982; Scott and Tyrrell, 1985). In both models about half of the volunteers infected with the virus develop colds. In both instances there was a marked prophylactic effect but no evi-

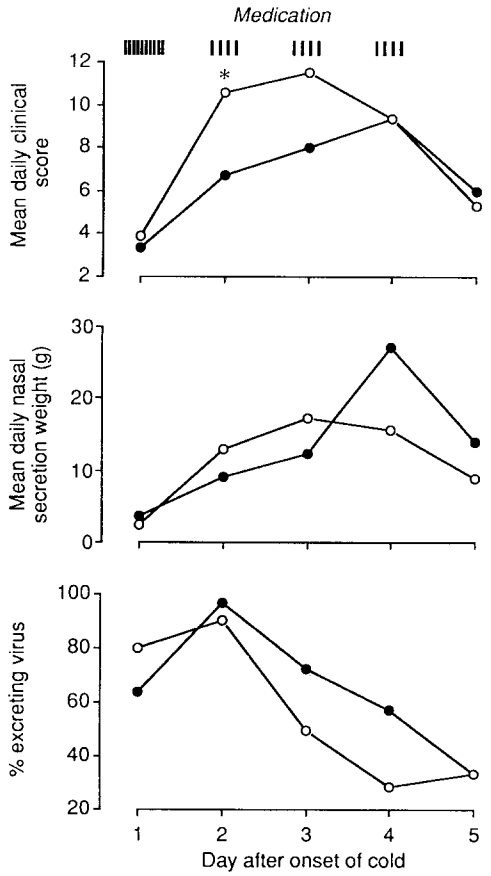

Fig. 2. Therapy of RSV induced colds with interferonα-2a; ●—●, placebo, $N=11$ (3 M, 8 F), mean age 40.0 ± 10.9 years; introversion/extroversion = 9.55, obsessionality = 1.91; ○—○ interferonα-2a, $N=10$ (3 M, 7 F) mean age 39.3 ± 13.1 years; introversion/extroversion = 9.60, obsessionality = 2.00; *, $P<0.05$.

dence of a beneficial action when given therapeutically. Furthermore, the significant increase in mean daily clinical score in the interferonα-2a group compared with the placebo group, before colds developed in the prophylactic study and during medication in the therapeutic trial, provide further evidence that interferon, when given for even short periods, acts as an irritant (Hayden et al., 1983; Scott et al., 1985).

These findings are predictable in rhinovirus infections as interferonα-2a has no direct effect on the virus particle and requires time to induce an antiviral state in the host cells. As RSV is such a poor inducer of interferon and so sensitive to its action it was hoped that it might be possible to use interferon to treat RSV infections. There is no evidence from this study to support the therapeutic use of interferon in RSV infections, although it is still possible that interferon might be effective in treating primary RSV infections in infants.

It is possible that the results obtained in these trials and the similarity to those in rhinovirus infections occur because the clinical illness is the same, a cold, as is the site of infection, the nose and nasopharynx. It is not possible from these data, to say what would be result of treating bronchiolitis with intrapulmonary interferonα-2a. However, our results together with the failure of systemic interferon to influence the course of severe RSV infection in seven children (Chiba et al., 1988) offers little encouragement. On the other hand the efficacy of interferonα-2a in preventing RSV infections in adults suggests that a contact prophylaxis study for preventing RSV infection in children at high risk for severe infection (e.g. babies with cystic fibrosis or bronchopulmonary dysplasia) analogous to those performed by Hayden et al. (1986) and Douglas et al. (1986) for rhinovirus infections might be effective, even though there was no trend in these studies indicating a beneficial effect on RSV infections.

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