

Prophylactic intranasal α_2 interferon and viral exacerbations of chronic respiratory disease

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Abstract

Background As respiratory virus infections often lead to exacerbations of chronic bronchitis and asthma an effective antiviral drug may be helpful in such patients. Alpha₂ interferon has been shown to give protection against rhinovirus infections in field studies.

Methods Patients with chronic respiratory disease exposed to close contacts with symptoms of upper respiratory tract infection were randomly allocated to receive nasal sprays of recombinant α_2 interferon (3×10^6 IU) or placebo twice daily for five days. Of the 123 patients recruited into the study, 69 took 117 courses of medication; 11 courses were excluded from analysis.

Results No important side effects were recorded and the incidence of possible adverse effects was similar in the two groups. Interferon treatment did not reduce the number or severity of symptomatic episodes; 11 of 48 patients given interferon and 16 of 58 given placebo developed lower respiratory symptoms. There were no differences in mean symptom scores (51 interferon and 52 placebo), number of symptomatic days (3.3 interferon and 5.0 placebo), peak flow values, number of general practitioner consultations, or use of antibiotics.

Conclusion Alpha₂ interferon 3×10^6 IU taken twice daily for five days does not protect patients with chronic respiratory disease from exacerbations after they have been in contact with an upper respiratory tract infection.

though little effect on colds due to other viruses.¹⁹⁻²³ Short courses of intranasal interferon are well tolerated, but treatment for over two weeks leads to nasal discharge and bleeding.^{15 19 21 24-26} A randomised, double blind, placebo controlled trial was designed to determine whether a five day course of prophylactic intranasal interferon would reduce the number and severity of respiratory infections in patients with chronic respiratory disease exposed to naturally occurring respiratory virus infection.

Methods

PATIENTS

Subjects were adults of either sex with a history of chronic airways disease. Asthma was defined as variable wheezy breathlessness with a documented FEV₁/FVC of less than 60% at least once and a change in FEV₁ or peak flow of at least 15% either spontaneously or as a result of bronchodilator treatment. Chronic bronchitis was defined as a history of sputum production on most days for at least three consecutive months for at least two successive years, an FEV₁ of less than 60%, and a change of less than 15% in peak flow or FEV₁ either spontaneously or as a result of bronchodilator treatment. Patients with bronchiectasis had a characteristic history of chronic purulent cough usually accompanied by radiographic changes. Patients were excluded if they had other medical conditions, nasal polyps or deformity, or penicillin hypersensitivity. Women of childbearing age were entered if they were using adequate contraception. Subjects were examined and baseline full blood count, electrolyte concentrations, liver function values, peak flow, and FEV₁/FVC were recorded. A chest radiograph was obtained if no recent film was available.

MEDICATION

Identical bottles of nasal spray containing either interferon or placebo were provided by the manufacturer (Boehringer Ingelheim). Each bottle was identified by a code number and the randomisation was not revealed until the trial and outcome analysis were completed. The active spray contained a solution of recombinant α_2 interferon administered as a metered aerosol delivering 0.5×10^6 IU per actuation. A course consisted of three sprays applied to each nostril twice daily (6×10^6 IU day) for five days. Subjects stored the bottles at 4°C. To confirm interferon activity unused medication spray was analysed independently

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Accepted 13 August 1991

Respiratory virus infections are frequently associated with exacerbations of chronic bronchitis¹⁻⁴ and asthma.⁵⁻¹⁰ Antiviral drugs that prevent or alleviate infection might benefit patients with chronic respiratory disease. Prophylactic intranasal interferon prevents experimental rhinovirus infection in healthy volunteers.¹¹ Subsequent studies have established that intranasal interferon, $3-10 \times 10^6$ IU daily, prevents symptoms in normal individuals inoculated with rhinovirus.¹²⁻¹⁷ Interferon may also protect against experimental coronavirus infection¹⁸ but has little or no effect against influenza virus.^{11 17} Field trials have confirmed the benefit of prophylactic intranasal interferon, which has about 80% efficacy against natural rhinovirus infection,

after completion of the trial. Fourteen bottles, selected at random, were sent to the National Institute for Biological Standards and Control for antiviral assay and immunoassay. Seven bottles contained active medication with no detectable loss of potency by comparison with standard samples supplied by Boehringer Ingelheim. The remaining seven bottles contained placebo and had no detectable activity.

STUDY DESIGN

The trial took place from October 1986 to April 1987 and from September 1987 to April 1988. Patients initiated treatment if they had been in close contact for six hours or more with someone having either one respiratory symptom for two days (unless sneezing was the only complaint), two symptoms for one day (rhinorrhoea, nasal congestion, sore throat, cough, hoarseness), or the appearance of symptoms suggesting influenza (fever, chills, muscle aches). On starting treatment patients kept a diary of upper respiratory and chest symptoms and recorded twice daily peak flow measurements for 10 days, or longer if symptoms persisted. Symptoms were also recorded for the index case and any secondary cases for 10 days. Compliance was monitored by checking the contents of the spray bottles after completion of treatment.

The subject and index case were seen within 24 hours of starting medication for the collection of nasal and throat swabs and blood samples. Swabs were placed in virus transport medium, frozen immediately in liquid nitrogen, and stored at -70°C . The trial organisers were notified when lower respiratory tract symptoms developed or secondary cases occurred; further visits were then made to confirm symptoms and take diagnostic samples. Subjects were reviewed seven and 21 days after starting medication and convalescent blood samples were taken from the patient and from the index case and any secondary cases on day 21. Symptom cards were collected and a new bottle of trial medication was issued on day 21. A nasal speculum examination was performed and the findings recorded at each visit.

The approval of the local ethics committee was obtained, and subjects gave written informed consent.

VIROLOGY

Respiratory virus infection was established by isolation of virus from nasal or throat swabs or by comparison of acute and convalescent serum samples. Nasal swabs were obtained from high in the anterior nares and throat swabs were passed firmly over the pharynx and tonsils. Swabs were placed together in 2.5 ml of viral transport medium containing nutrient broth with 10% fetal calf serum, penicillin, streptomycin, and amphotericin B. Samples were stored at -70°C and analysed in batches with anti-interferon antibody sufficient to neutralise 10 000 units of α_2 interferon added while they were defrosting. Volumes of 0.2 ml were inoculated on to monolayers of Ohio HeLa

cells, MRC-5 human lung fibroblasts, C16 cells (a cell line derived from MRC-5 fibroblasts, susceptible to coronavirus), and Madin Darby canine kidney (MDCK) cells (susceptible to influenza and parainfluenza virus). All cell lines were cultured in roller tubes at 33°C with 5% carbon dioxide and observed for 14 days. Specimens inoculated on to Ohio HeLa cells were routinely passaged once after seven days and equivocal specimens were passaged up to three times. Rhinovirus infection was diagnosed after observation of characteristic cytopathic effect. Inhibition of cytopathic effect at 37°C was not performed routinely but isolations of rhinovirus were confirmed by demonstrating characteristic acid lability at pH 3. Influenza and parainfluenza viruses were identified by haemadsorption inhibition on MDCK cells.

Acute and convalescent paired serum samples were tested for complement fixing antibodies to adenovirus, influenza A and B viruses, respiratory syncytial virus, *Mycoplasma pneumoniae*, *Coxiella burnetii*, and *Chlamydia psittaci*. An enzyme linked immunosorbent assay (ELISA) was used to detect a rise in titre of antibody to coronavirus 229E²⁷ and an identical ELISA was used to detect antibodies to coronavirus OC 43 with antigen prepared from the brains of infected suckling mice.

SYMPTOM ASSESSMENT AND DATA ANALYSIS

Treatment courses were included in the analysis if medication was started within 72 hours of initial contact with the index patient, if the entire course of medication was taken, and if the index patient had a definite upper respiratory tract infection documented on the symptom chart. The outcome was analysed by assessing the effect of interferon on the number and severity of respiratory episodes. Subjects scored each possible symptom daily on diary cards as 0 = nil, 1 = mild, 2 = moderate, or 3 = severe. When a patient had constant symptoms (for example, cough) only a change in the severity of that symptom was recorded. Only the upper or lower respiratory tract episodes that started during the 10 days after contact with the index case were included in the analysis. Each course was classified in terms of one of four possible outcomes; (a) "nil"—subject was symptomless; (b) "doubtful cold"—symptoms scoring no more than 1 or in only one of the categories nose, throat, and cough; (c) "upper respiratory tract infection"—symptoms in two or more of the categories nose, throat, cough, and systemic features with at least one symptom scoring 2 or more; (d) "lower respiratory tract infection"—symptoms in at least two of the categories cough, sputum, wheeze, and chest tightness lasting at least two days. If the patient normally complained of chest symptoms only an increase in severity of these symptoms was regarded as an indication of a lower respiratory tract exacerbation. Appearance of cough alone did not count as an upper or lower respiratory episode. "Symptomatic days" were defined as days in which two or more symptoms were recorded with at least one symptom moderately

severe or worse. Change in peak flow associated with each episode of medication use was estimated by comparing the initial peak flow with the mean of the peak flow on the worst day for each episode.

STATISTICAL ANALYSIS

Unpaired *t* tests were used to compare characteristics of patients taking interferon and placebo. χ^2 analysis was used to compare the number of respiratory infections and general practitioner consultations and the use of antibiotics and Fisher's exact test to compare the small numbers of hospital admissions. Mann-Whitney U tests were used to compare symptom scores, number of symptomatic days, and change in PEF associated with each episode. Values of *p* below 0.05 were considered significant.

Results

STUDY POPULATION AND TREATMENT EPISODES

Of the 123 subjects recruited, only 69 used the nasal spray during the study period. The mean

age of the 69 participants was 46.9 years, 33 were male, 44 had asthma, 20 had chronic bronchitis, and five had bronchiectasis. The mean duration of respiratory disease was 20.5 years and the mean period of chest clinic attendance was 10.7 years.

One hundred and seventeen courses of treatment were taken by the 69 patients (41 had one course, 15 had two courses, seven had three courses, five had four courses, and one had five courses). Eleven episodes were excluded, five because patients took medication when they developed symptoms in the absence of an index case, four because data sheets were not filled in correctly, and two because the patients lost their record cards. Although the exclusions were not included in the analysis of outcome they provided information on possible adverse effects.

Data on the remaining 106 episodes were analysed. Interferon (*n* = 48) and placebo (*n* = 58) groups were closely matched with no significant differences in terms of underlying diagnosis, length or severity of illness, or length of contact with infection (table 1). Medication

Table 1 Comparison of interferon and placebo groups

	Interferon				Placebo			
	No	(%)	Range	SD	No	(%)	Range	SD
Male	19	(40)			26	(45)		
Mean age (y)	47.4		19-73	15.1	48.4		17-72	14.0
Asthma	33	(69)			35	(60)		
Chronic bronchitis	13	(27)			15	(26)		
Bronchiectasis	2	(4)			8	(14)		
Mean years of illness	20.25		1-66		20.7		1-51	
History of smoking	31	(65)			36	(62)		
Present smoker	5	(10)			4	(7)		
Mean peak flow (l/min)	303		70-530	102	289		80-505	95
Mean FVC (ml)	2550		800-4600	765	2308		800-3650	673
Mean FEV ₁ /FVC ratio	0.68		0.25-0.93	0.20	0.67		0.33-0.93	0.20
Mean No in household	3.9		1-8		3.6		1-9	
Mean No in house aged < 16	0.85		0-4		0.84		0-4	
Mean hours in contact with index case	26.4		6-72	19.1	30.4		6-72	20.1
Mean hours between initial contact and starting treatment	34.7		6-72	24.6	36.1		6-72	20.5
Total No evaluable	48				58			

Table 2 Viral diagnosis and clinical outcome in 106 episodes

	Clinical outcome in respiratory patients								
	Nil		Doubtful		URTI		Lower RTI		
Virus	Index case	Subject	Index case	Subject	Index case	Subject	Index case	Subject	Total (%)
None	23	36	13	18	13	11	21	18	153 (72)
Rhinovirus	6	1	4	2	0	0	1	1	15 (7)
Coronavirus OC 43	3	1	1	2	3	3	2	3	18 (8.5)
Coronavirus 229E	4	1	2	1	0	0	2	3	13 (6)
Influenza A virus	2	1	1	0	0	0	1	1	6 (3)
Influenza B virus	2	0	1	0	1	1	0	0	5 (2.5)
Respiratory syncytial virus	0	0	0	0	0	0	0	1	1 (0.5)
Mycoplasma pneumoniae	0	0	1	0	0	0	0	0	1 (0.5)
Total No of episodes	40	40	23	23	16	16	27	27	212 (100)

(U)RTI—(upper) respiratory tract infection.

Table 3 Outcome of courses of medication

Outcome	Interferon No (%)	Placebo No (%)	Total No (%)
No symptoms	19 (39)	21 (36)	40 (38)
Doubtful cold	11 (23)	12 (21)	23 (22)
URTI	7 (14)	9 (15)	16 (15)
Lower RTI	11 (23)	16 (27)	27 (25)
Total	48	58	106

All differences between the interferon and the placebo group were non-significant.

(U)RTI—(upper) respiratory tract infection.

was dispensed at random so subjects who had more than one course of medication are included in either or both groups depending on the number of courses of interferon or placebo taken.

VIRUS ISOLATIONS AND SEROLOGICAL STUDIES

The results of viral diagnosis are summarised in table 2. Respiratory viruses were implicated in 41 of the 106 episodes (39%) (index case or subject or both). There were 15 rhinovirus isolations; 13 of these were associated with no symptoms or a doubtful cold and only two isolations (13%) were implicated in lower respiratory tract infections. There were no cases of enterovirus, herpes simplex virus or adenovirus infection.

RESPIRATORY EPISODES

The clinical outcome of subjects taking interferon or placebo are summarised in table 3. In 40 of 106 episodes (38%) the subject did not develop any symptoms, 23 (22%) had a doubtful cold, 16 (15%) had an upper respiratory tract infection, and 27 (25%) developed a lower respiratory tract infection. The use of prophylactic intranasal interferon was not associated with a significant reduction in the proportion of upper or lower respiratory tract infections, 11/48 (23%) interferon users developing lower respiratory infection compared with 16/58 (27%) of those who used placebo ($p = 0.58$).

SYMPTOMATIC DAYS AND SYMPTOM SCORES

When symptomatic days were compared in all subjects interferon produced a non-significant reduction in the mean length of illness from 5.0 (median 2, range 0–33) days compared with 3.3 (median 1, range 0–12) days (Mann-Whitney test, $p = 0.63$). The mean symptom score after prophylaxis with interferon of 51.4 (median 37, range 0–207) was virtually identical to the mean score after placebo of 51.6 (median 26, range 0–316).

PEAK FLOW MEASUREMENTS

The overall mean initial peak flow on the first day of medication use was similar for those who used interferon and those who used placebo (table 1). There was no significant difference in the overall reduction in peak flow after interferon and after placebo use. The mean reduction was 63 (median 50, range 0–195) l/min for all interferon episodes compared with 48 (median 35, range 0–185) l/min for all placebo

episodes. When lower respiratory episodes only were compared there was similarly no significant difference in the reduction in peak flow after medication. The mean reduction in peak flow in lower respiratory episodes was 91 (median 75, range 0–190) l/min after interferon compared with 72 (median 55, range 10–185) l/min after placebo.

GENERAL PRACTITIONER AND CONSULTATIONS AND HOSPITALISATION

There were no significant differences in the general practitioner consultation rate or use of antibiotics in the two groups. The patient's general practitioner was consulted after 12 of 48 episodes when interferon was used compared with 16 of 58 when placebo was used ($p = 0.76$). Antibiotics were prescribed after nine of 48 interferon episodes compared with 14 of 58 placebo episodes ($p = 0.50$). One of the 11 lower respiratory episodes that followed the use of interferon required admission to hospital compared with four of the 16 of that followed placebo (Fisher's exact test, $p = 0.29$).

ADVERSE EFFECTS

Some mild symptoms were reported, including rhinorrhoea, nasal stuffiness, nasal dryness, and dry mouth, though none was significantly associated with the use of the active interferon spray. Bloodstained nasal discharge was reported by three subjects taking interferon and three using placebo.

Discussion

Patients with chronic respiratory disease may develop severe exacerbations if they are in close contact with someone who has a cold. A quarter of patients in this study developed lower respiratory complications after contact with a cold and a fifth of these required admission to hospital for treatment of complications. This reflects the importance of respiratory viruses in chronic respiratory disease and their undoubted morbidity, mortality, and economic importance.

Previous volunteer studies and field trials in normal individuals have shown that prophylactic α_2 interferon can prevent rhinovirus infections.^{11–17 19–23} Patients with chronic respiratory disease would be expected to gain particular benefit from the use of prophylactic antiviral medication. Our study showed no significant reduction in the number or severity of upper or lower respiratory tract episodes after intranasal α_2 interferon, however; there was a small reduction in the mean number of days with symptoms in subjects who used interferon, but this was not significant and the mean symptom scores were similar for the interferon and placebo recipients.

A respiratory virus was identified in 39% of episodes, which is similar to the proportion in other studies of this nature.^{19 21–23 26} The range of cell lines used was designed to allow isolation of all commonly occurring respiratory viruses, though only rhinoviruses were diagnosed by isolation, other infections being identified sero-

logically. The yield of viruses might have been greater if nasal washes or aspirates had been obtained, but this was considered impracticable. In addition, the isolation of fragile enveloped viruses (particularly respiratory syncytial virus) will have been reduced by freezing specimens before culture; logistically, however, fresh specimens could not be inoculated directly into tissue culture. There is no reason to suspect that these difficulties would result in any differences between the interferon and placebo groups.

There are several possible explanations for the lack of benefit associated with intranasal interferon, in contrast to the findings in previous studies performed in healthy family members.^{22 23} Rhinoviruses were isolated from only 15 patients and index cases, and the number of rhinovirus episodes was therefore too small to determine the efficacy of interferon against rhinovirus infection alone. Field studies in healthy subjects have consistently shown no effect of interferon on illness caused by viruses other than rhinovirus.^{22 23} The efficacy of interferon might have appeared greater if the study had been conducted in more individuals over a shorter time that coincided with rhinovirus activity. The timing of treatment is also likely to be of crucial importance as prophylactic interferon spray was found to be ineffective against established naturally occurring colds.²⁸ The mean period between initial contact with the index case and the start of interferon medication in our study was 34.7 hours. This may have been too late to prevent infection in many cases. The mean period between contact and starting medication was not documented in the family studies in Australia²² and the United States²³ but may have been less.

The development of lower respiratory symptoms after challenge with respiratory virus is thought to depend on inhalation of infective virus particles into the lung.²⁹ The pulmonary distribution of intranasal medication might be an important factor in patients with underlying chest disease. The spray used in this study was designed to give a mist of sufficiently large particle size to be distributed in the nasal passages with comparatively little deposition in large airways or alveoli. No significant differences were observed in the reduction of peak flow associated with respiratory infection after interferon and after placebo, indicating a similar degree of airway narrowing in the two groups. Particle size is obviously a crucial factor determining the penetration of the medication.³⁰ Aerosolised interferon delivered to the lungs by a nebuliser device might be more effective³¹ and is worthy of further study.

Some mild symptoms were reported in this study but were not clearly associated with active interferon medication and many of the nasal and upper respiratory symptoms could have been due to virus infection. These findings are similar to those reported in other studies, where short term intranasal interferon has been well tolerated.^{12 20 22 23 25}

This study showed that patients with chronic respiratory disease are at definite risk of

developing severe exacerbations of their condition if they are in close contact with someone who has a cold. Unfortunately prophylactic intranasal interferon spray did not prevent or ameliorate these exacerbations. Studies might be considered with combinations of antiviral medication and comparing intranasal and nebulised delivery.

MJW was supported by the British Medical Association HC Roscoe Fund. We gratefully acknowledge the assistance of Miss Morag Forsythe, senior technician, and other staff at the Common Cold Unit, for supplies of tissue culture cell lines and assistance with virus isolation; Dr Carol Jagger, lecturer in medical statistics, Department of Community Health, Leicester University, for statistical advice; and Dr A Meager, National Institute for Biological Standards and Control, for performing interferon assays.

- 1 Stuart-Harris CH. The role of bacterial and viral infection in chronic bronchitis. *Arch Environ Health* 1968;16:586-95.
- 2 Stott EJ, Grist NR, Eadie MB. Rhinovirus infections in chronic bronchitis: isolation of eight possibly new rhinovirus serotypes. *J Med Microbiol* 1968;1:109-17.
- 3 Monto AS, Higgins MW, Ross HW. The Tecumseh study of respiratory illness. VIII. Acute infection in chronic respiratory disease and comparison groups. *Am Rev Infect Dis* 1975;111:27-36.
- 4 Buscho RO, Saxton D, Shultz PS, Finch E, Mufson MA. Infections with viruses and *mycoplasma pneumoniae* during exacerbations of chronic bronchitis. *J Infect Dis* 1978;137:377-83.
- 5 Horn MEC, Gregg I. 1973. Role of viral infection and host factors in acute episodes of asthma and chronic bronchitis. *Chest* 1973;63:44-8.
- 6 Minor TE, Dick EC, DeMeo AN, et al. Viruses as precipitants of asthmatic attacks in children. *JAMA* 1974;227:292-8.
- 7 Minor TE, Dick EC, Baker JW, et al. Rhinovirus and influenza type A infections as precipitants of asthma. *Am Rev Respir Dis* 1976;113:149-53.
- 8 Mitchell I, Inglis H, Simpson H. Viral infection in wheezy bronchitis and asthma in children. *Arch Dis Child* 1976;51:707-11.
- 9 Carlsen KH, Orstavik I, Leegaard J, Hoeg H. Respiratory virus infection and aeroallergens in acute bronchial asthma. *Arch Dis Child* 1984;59:310-5.
- 10 Busse WW. The precipitation of asthma by upper respiratory tract infections. *Chest* 1985;87:44-9.
- 11 Merigan TC, Reed SE, Hall TS, Tyrrell DAJ. Inhibition of respiratory virus infection by locally applied interferon. *Lancet* 1973;i:563-7.
- 12 Greenberg SB, Marmon MW, Couch RB, et al. Prophylactic effect of low doses of human leucocyte interferon against infection with rhinovirus. *J Infect Dis* 1982;145:542-6.
- 13 Scott GM, Phillpotts RJ, Wallace J, et al. Purified interferon as protection against rhinovirus infection. *BMJ* 1982;284:1822-5.
- 14 Scott GM, Phillpotts RJ, Wallace J, Gauci CL, Greiner J, Tyrrell DAJ. Prevention of rhinovirus colds by human interferon alpha-2 from *Escherichia coli*. *Lancet* 1982;ii:186-8.
- 15 Samo TC, Greenburg SB, Couch RB, et al. Efficacy and tolerance of intranasally applied recombinant leucocyte A interferon in normal volunteers. *J Infect Dis* 1983;148:535-42.
- 16 Hayden FG, Gwaltney JM jun. Intranasal interferon alpha-2 for prevention of rhinovirus infection and illness. *J Infect Dis* 1983;148:543-50.
- 17 Phillpotts RJ, Higgins PG, Willman JS, Tyrrell DAJ, Freestone DS, Shepherd WM. Intranasal lymphoblastoid interferon ("Wellferon") prophylaxis against rhinovirus and influenza virus in volunteers. *J Interferon Res* 1984;4:535-541.
- 18 Turner RB, Felton A, Kosak K, Kelsey DK, Meschievitz CK. Prevention of coronavirus colds with intranasal alpha-2b interferon. *J Infect Dis* 1986;154:443-7.
- 19 Farr BM, Gwaltney JM jun, Adams KF, Hayden FG. Intranasal interferon alpha 2 for prevention of natural rhinovirus colds. *Antimicrob Agents Chemother* 1984;26:31-4.
- 20 Herzog C, Berger R, Fernex M. Intranasal interferon (rIFN alpha-2a, Ro 22-8181) for contact prophylaxis against common cold: a randomised, double-blind and placebo controlled field study. *Antiviral Res* 1986;6:171-6.
- 21 Monto AS, Shope TC, Schwartz SA, Albrecht JK. Intranasal interferon alpha-2b for seasonal prophylaxis of respiratory infection. *J Infect Dis* 1986;154:128-33.
- 22 Douglas RM, Moore BW, Miles HB, et al. Prophylactic efficacy of prophylactic alpha2-interferon in the family setting. *N Engl J Med* 1986;314:65-70.
- 23 Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM jun.

- Prevention of natural colds by contact prophylaxis with intranasal alpha2-interferon. *N Engl J Med* 1986;**314**: 71-5.
- 24 Hayden FG, Mills SE, Johns ME. Human tolerance and histopathologic effects of long-term administration of interferon-alpha 2. *J Infect Dis* 1983;**148**:914-21.
 - 25 Samo SC, Greenberg SB, Palmer JM, Couch RB, Harmon MW, Johnson PE. Intranasally applied recombinant leucocyte A interferon in normal volunteers II determination of minimal effective and tolerable dose. *J Infect Dis* 1984;**150**:181-8.
 - 26 Monto AS, Albrecht JK, Schwartz SA. Demonstration of dose-response relationship in seasonal prophylaxis of respiratory infections with alpha-2b interferon. *Antimicrob Agents Chemother* 1988;**32**:47-50.
 - 27 Kraaijeveld CA, Reed SE, Macnaughton MR. Enzyme-linked immunosorbent assay for detection of antibody in volunteers experimentally infected with human coronavirus strain 229E. *J Clin Microbiol* 1980;**12**:493-7.
 - 28 Hayden FG, Kaiser DL, Albrecht JK. Intranasal recombinant alfa-2b interferon treatment of naturally occurring common colds. *Antimicrob Agents Chemother* 1988;**32**: 224-30.
 - 29 Cate TR, Couch RB, Fleet WF, Griffith WR, Gerone PJ, Knight V. Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol. *Am J Epidemiol* 1964;**81**:95-105.
 - 30 Wyde PR, Wilson SZ, Kramer MJ, Sun CS, Knight V. Pulmonary deposition and clearance of aerosolized interferon. *Antimicrob Agents Chemother* 1984;**25**:729-34.
 - 31 Wyde PR, Sun CS, Wilson SZ, Knight V. Duration of effect of interferon aerosol prophylaxis of vesicular stomatitis virus infection in mice. *Antimicrob Agents Chemother* 1985;**27**:60-4.