Antiviral Research, 10 (1988) 225–234
Elsevier

AVR 00319

Intranasal tolerance and histopathologic effects of a novel synthetic interferon, rIFN-αCon$_1$

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(Received 23 June 1988; accepted 22 September 1988)

Summary

In a double-blind trial 119 adults were randomly assigned to receive daily sprays of placebo (N=30) or rIFN-αCon$_1$ 3 MU (N=29), 9 MU (N=30), or 30 MU (N=30) per day for 25 consecutive days. Fifty-nine subjects were removed from treatment because of abnormal nasal exams (N=56) or irritative symptoms (N=3). The fraction of drop-outs in the placebo group (30%) was significantly different (P<0.05) from that in the 3 MU (55%), 9 MU (57%), or 30 MU (67%) groups. Nasal mucosal biopsies collected 1–2 days after completing spray use detected moderate or marked lymphocytic infiltration in 10% of placebo (N=10), 90% of 3 MU (N=9), 85% of 9 MU (N=13), and 70% of 30 MU (N=10) subjects (P<0.05, placebo vs each rIFN-αCon$_1$ group). All 3 dose levels of rIFN-αCon$_1$ were associated with significant clinical and histopathologic signs of nasal irritation. The findings suggest that intranasal rIFN-αCon$_1$ does not have a more favorable therapeutic index than rIFN-α2 and that the risk of nasal irritation relates more closely to the antiviral activity than the protein content of the rIFN-α administered.

rIFN-αCon$_1$; Intranasal toxicity

Introduction

Intranasal recombinant interferon-α2b is protective against natural rhinovirus colds when used for seasonal prophylaxis in open populations (Farr et al., 1984; Hayden et al., 1985; Douglas et al., 1985; Monto et al., 1986, 1988) or for post-

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exposure prophylaxis in the family setting (Hayden et al., 1986; Douglas et al., 1986). However, both natural and recombinant interferon-αs are associated with substantial rates of nasal irritation, when used for periods longer than 1–2 weeks (Hayden et al., 1983, 1985; Samo et al., 1984; Scott et al., 1985; Farr et al., 1984; Douglas et al., 1985). Tolerance studies of rIFN-α2a and rIFN-α2b have also found that one-half or more of recipients develop nasal histologic alterations, specifically subepithelial lymphocytic infiltration (Hayden et al., 1983, 1987a,b). Lymphocytic infiltration can occur as early as 4 days after initiating exposure and appears to be a precursor to the development of clinical intolerance. In order to be an effective agent for the seasonal (long-term) prophylaxis of respiratory viral infections, an interferon would have to have a significantly improved therapeutic index compared to the recombinant and natural interferon-αs tested to date.

A family of related interferon-α subtypes exist, which have different relative antiviral and anti-proliferative activities (Weck et al., 1981; Alton et al., 1983; Fish et al., 1983). A synthetic gene has been constructed which codes for a novel consensus or average analog of all the known alpha-interferon subtypes (Alton et al., 1983). In general, this interferon incorporates the most frequently observed amino acid residue at each position. Designated rIFN-αCon_1, this consensus alpha interferon is most closely related to interferon-αF from which it differs in just 10 positions and is also more closely related to IFN-β than are any of the natural IFN-α subtypes.

The most interesting biological property of this molecule is its high specific activity. Relative to leukocyte-derived IFN-α, rIFN-αCon_1 has approximately 5- to 10-fold greater activity against EMC virus in Vero cells, but 8-fold lower activity against VSV in MDBK cells (Alton et al., 1983). Using IFN-αCon_1 purified to homogeneity, specific activity in WISH cells challenged with EMC or vesicular stomatitis virus is greater than 2×10^9 units/mg protein (Alton et al., 1983), many fold higher than other interferon-α subtypes in human cells (Weck et al., 1981; Lee et al., 1982). Antiviral activity has also been observed in other cell-virus systems and in several animal model systems at doses that are comparable on a units basis to other IFN-αs (Stebbing et al., 1985a,b; Van der Meide et al., 1985; Fish et al., 1985, 1986; Trousdale et al., 1985). Limited evidence suggests that pyrogenicity in rabbits is lower for rIFN-αCon_1 than for comparable doses of rIFN-α2b (Stebbing et al., 1985b). The high specific activity of rIFN-αCon_1 indicates that on a weight basis this recombinant interferon has greater antiviral activity than other interferon alpha species. If nasal inflammation correlates with interferon protein content and not antiviral activity, then this interferon could be associated with an improved therapeutic index.

Consequently, we conducted a randomized, double-blind and placebo-controlled trial to determine the dose-related tolerance and safety of intranasal rIFN-αCon_1 in healthy adults. A subset of patients also had nasal biopsies taken after exposure to determine the histopathologic response to this interferon.

Materials and Methods

Participants

After screening 140 subjects, 119 healthy adults who had had no prior exogenous interferon exposure were enrolled into the study. Subjects were excluded if they had had recent upper respiratory tract illness or fever of uncertain cause within one week prior to initiation of the study; history of clinically significant medical disease or need for concomitant medication, nasal polyps, acute sinusitis, chronic or allergic rhinitis; or other respiratory disease, requirement for chronic concomitant medication which could interfere with the evaluation of interferon (e.g., aspirin, nonsteroidal anti-inflammatory drugs); exposure to any investigational drug within 28 days; pregnancy or lactation. The first 10–12 assigned to each group were asked to have punch nasal biopsies performed at the completion of drug administration. Written informed consent approved by the University of Virginia Human Investigation Committee was obtained from all subjects.

Drug administration

Recombinant IFN-αCon_1 (Lot no. 403H5) was supplied as a sterile solution (0.225 mg/ml; specific activity approx. 6×10^8 U/mg protein) by Amgen, Inc. (Thousand Oaks, California). The solution was diluted in phosphate buffered saline containing human serum albumin (0.25% final concentration) for distribution into the spray devices. The vehicle which contained 0.25% human serum albumin in phosphate buffered saline without preservative served as placebo. Although stable for up to 24 h at room temperature or at 4°C, the study drugs were prepared and used within a period of 4 hours.

The subjects were randomized into four treatment groups: 30 MU/day, 9 MU/day, 3 MU/day, or placebo. The lower dose level (3 MU/day) was selected because the results of earlier field studies indicated that it is consistently efficacious in the seasonal prophylaxis of natural rhinovirus infections (Douglas et al., 1985; Monto et al., 1986, 1988). Subjects self-administered the study drugs once daily for 25 consecutive days under the direct observation of a monitor. A metered pump spray device, designed to deliver 0.1 ml per activation, was used to deliver two sprays per nostril. A new spray device was used for each subject each day.

Monitoring

Subjects visited the study center daily for recording of symptoms and administration of the study drug. Nasal examinations were performed routinely before and on days 5, 12, 19 and 25 of treatment. In the event of an abnormal nasal examination leading to withdrawal from the study, a prospective judgement was made by one of the investigators (FGH) regarding the possible cause (IFN exposure or sprayer trauma). Routine hematology studies were done before, on day 8 or 9, and again on day 25 of the study. Standard blood chemistries and urinanalysis were done before and after dosing. If a subject consented to have a punch nasal biopsy, it was collected within 2 days of the last drug dose. Nose and throat swabs were collected from subjects reporting symptoms of respiratory tract illness for isolation

of viruses in standard cell culture systems. If the subject was dropped from the study early, the schedule of activities listed for the last day of dosing was completed, including, if appropriate, a nasal biopsy.

Nasal biopsies

Nasal biopsy samples were collected and processed by previously described techniques (Winther et al., 1987; Hayden et al., 1987a). Sections were stained by immunoperoxidase methods with monoclonal antibodies directed against T and B lymphocyte markers, and the overall degree of lymphocytic infiltration assessed independently by two pathologists under blinded conditions. The overall interpretation of each pathologist was reviewed, and the opinion expressing the more severe degree of lymphocytic infiltration was used in analysis. When a pathologist gave an opinion which bridged two categories, i.e., mild to moderate or moderate to severe, the more severe of the two ratings was used.

Results

Participants

The groups were comparable in demographic characteristics (Table 1). Approximately two-thirds of the participants in each group were female. Smoking, which may reduce the risk of interferon-induced nasal inflammation (Hayden et al., 1987b), was infrequent. Less than 1% of the scheduled doses were missed in each group. Although the use of concomitant medications was common (Table 1), subjects were asked to avoid nasal use of any drugs and to use acetaminophen instead of aspirin or nonsteroidal anti-inflammatory medications.

Withdrawals

This study was associated with a high dropout rate relative to previous tolerance studies of intranasal interferons (Hayden et al., 1983, 1987a,b). Sixty-two subjects

TABLE 1

Demographic characteristics of the treatment groups

Parameter	Treatment group			
	Placebo	3 MU	9 MU	30 MU
No. subjects	30	29	30	30
No. females	19	22	20	21
Mean age (yrs)	26	28	28	32
No. smokers	5	4	3	4
No. missed doses	5	2	4	3
No. concomitant				
ASA, NSAIDS[a]	3	0	1	1
Acetaminophen	13	9	11	12
Decongestant/antihistamine	3	0	0	1

[a]NSAIDS = nonsteroidal, anti-inflammatory drugs.

TABLE 2

Abnormal nasal examination findings

Cause of withdrawal[a]	Number of subjects			
	Placebo (N=30)	3 MU (N=29)	9 MU (N=30)	30 MU (N=30)
Total with abnormal exams	7	16[b]	16[b]	17[b]
Sprayer-related[a]	4	6	4	6
Drug-related[c]	3	10[b]	12[b]	11[b]

[a]Determination of the possible cause was made at the time of withdrawal under blinded conditions by one of the investigators (FGH). Sprayer-related abnormalities were usually small (≤1 mm) superficial erosions of the mid or anterior septum mucosa without associated inflammation.

[b]$P<0.05$, versus placebo group, 2-tailed Fisher's exact test.

[c]Friable mucosa, erosions, ulcers.

failed to complete the treatment regimen, 56 of whom were dropped because of abnormal nasal examinations (Table 2). Three subjects were dropped from treatment because of persistent nasal symptoms, typically mild to moderate nasal obstruction and associated sore throat (1 placebo, two 30 MU). Three other subjects were dropped for other reasons (1 placebo with intercurrent illness, one 9 MU with persistent leukopenia present at baseline, one 30 MU with leukopenia developing on treatment). As shown in Fig. 1, excess withdrawals occurred in each of the interferon groups compared to placebo during the second week of the study. By the end of the 25-day treatment period, the proportion of withdrawals for any reason in 3 MU (55% of subjects), 9 MU (57%), and 30 MU (67%) interferon groups differed significantly ($P<0.05$) from placebo (30%).

For 20 of the 56 dropped because of abnormal exam findings, mucosal changes consistent with sprayer-related trauma were judged to be present at the time treatment was discontinued (Table 2). These changes were typically small (1 mm or

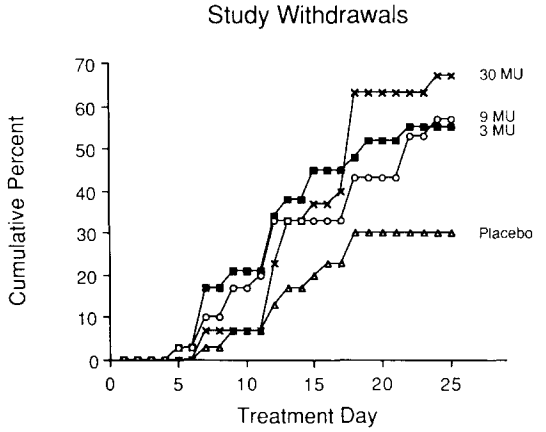

Fig. 1. Cumulative percent of subjects withdrawn from each of the treatment groups over the entire treatment period. Each of the interferon groups differed significantly ($P<0.05$) from the placebo group.

less in diameter) superficial erosions, occurring on the mucosa of the mid or anterior septum, usually without associated erythema or surrounding inflammation. No differences existed between the groups for withdrawals due to such findings. Overall, possible sprayer-related trauma was observed in 13 placebo, eight 3 MU, six 9 MU, and ten 30 MU recipients at some point in the study. In contrast, a total of 36 subjects were dropped because of nasal abnormalities felt to be consistent with interferon-related changes. These included friable mucosa with bleeding, large erosions or ulcerations, and abnormalities involving the turbinates or posterior septum. The proportion of subjects withdrawn for such changes was significantly higher in each of the interferon groups (34–40% of subjects) than placebo (10%), but no clear dose-related effect was apparent (Table 2).

Symptoms

Irritative nasal symptoms (obstruction, dryness, burning, blood in mucus) occurred in similar overall proportions of each group. Specifically, blood in mucus occurring during or within 3 days of stopping treatment, was reported by 50% of placebo, 34% of 3 MU, 53% of 9 MU, and 70% of 30 MU recipients. Possible systemic side effects during intranasal rIFN-αCon$_1$ were infrequently observed. Fatigue tended to be reported more often in the 9 MU (5 subjects) and 30 MU (4 subjects) than in the placebo (1 subject) or 3 MU (none) groups, although there were no statistically significant differences in this or other constitutional complaints compared to placebo. The only laboratory abnormalities of possible clinical consequence were two instances of leukopenia (WBC <4000 cells/mm^3) in the 30 MU group and one subject with mild eosinophilia in the 9 MU group. These laboratory abnormalities resolved shortly after cessation of interferon administration.

Nasal biopsies

Nasal biopsy samples were collected from approximately 9–13 subjects in each of the groups within two days of their last treatment. In the placebo group, 90% of subjects had post-exposure biopsies that were interpreted as being normal, that is showing mild degrees of lymphocytic infiltration (Figure 2). In contrast, in each of the interferon groups, 30% or less had post-exposure biopsies that were interpreted as being normal. A significantly higher proportion of subjects in each of the interferon groups had biopsies that showed moderate or marked degrees of lymphocytic infiltration (Figure 2), as compared to placebo. However, no differences were observed among the interferon groups to suggest a dose–response effect.

Respiratory illness

Nose and throat swabs were collected for viral isolation during study drug administration in 9 placebo, four 3 MU, six 9 MU, and nine 30 MU participants. Rhinoviruses were the only isolates recovered from these specimens. Rhinovirus-proven illnesses occurred in only 3 placebo and 3 interferon (1 in each dose group) recipients.

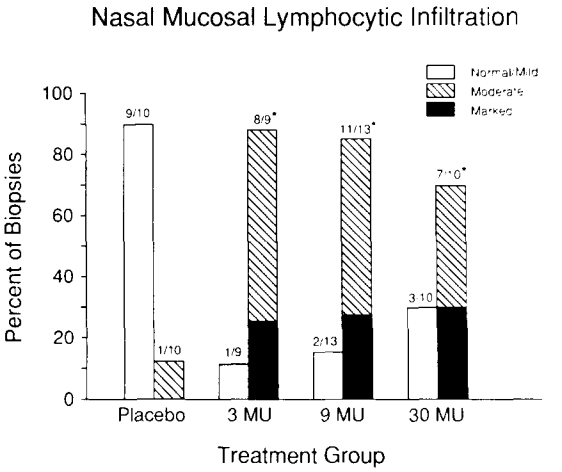

Fig. 2. Proportions of nasal biopsy samples taken after interferon exposure that showed normal (mild) or abnormal (moderate/marked) degrees of lymphocytic infiltration. Each interferon group (*) differed significantly ($P<0.05$) from the placebo group.

Discussion

This study found that each dose level of intranasal rIFN-αCon_1 resulted in significantly higher dropout rates due to adverse nasal effects compared to placebo. Each dose level was also associated with significantly higher rates of histologic changes, specifically lymphocytic infiltration, in nasal mucosal biopsies compared to placebo. None of the dose levels completely protected against rhinovirus infections, and even the lowest dose of rIFN-αCon_1 studied (3 MU/day) was associated with nasal irritation and mucosal inflammation. Because this dose corresponds to the minimally effective one of rIFN-α2b needed for prevention of natural rhinovirus colds (Monto et al., 1988), the results suggest that rIFN-αCon_1 does not have a more favorable therapeutic index for intranasal use than previously tested natural and recombinant IFN-αs. However, this conclusion is limited by the fact that the current study did not directly compare rIFN-αCon_1 with other IFN-αs.

This study confirms earlier observations with rIFN-α2a or α2b which found that intranasal application was associated with the development of lymphocytic infiltration in nasal biopsies in 50% or more of recipients (Hayden et al., 1983, 1987a,b). In accord with earlier findings, no clear dose-response relationship was apparent in regard to histopathologic alterations, perhaps because the dose–effect relationship may be a threshold type and because host factors may be important in determining responsiveness. The specificity of this effect is demonstrated by the infrequent finding of comparable histopathologic changes in placebo recipients and by the abolition of the effect with the use of anti-interferon antisera in a rodent model of cutaneous inflammation (Issekutz et al., 1986). Although the exact mechanism of this effect is undefined, the development of lymphocytic infiltration

may relate to interferon's effect on lymphocyte migration in exposed tissues (Gresser et al., 1981; Korngold et al., 1983; Issekutz et al., 1986). In mice, injected interferon is associated with generalized lymphadenopathy resulting from decreased movement of lymphocytes out of lymph nodes (Gresser et al., 1981). Issekutz et al. (1986) have proposed that interferon acts on endothelial cells to express receptors for lymphocytes, which causes them to adhere and migrate out of the blood. In in vitro testing, rIFN-αCon_1 has shown immunomodulating activities similar to human IFN -αs (Neubauer et al., 1985; Fish et al., 1985). It also appears to have effects on nasal histopathology comparable to rIFN-α2.

This study was designed in part to examine the relationship between interferon antiviral activity, protein content and nasal inflammation. The lowest dose of rIFN-αCon_1 tested (3 MU/day) had an interferon protein content similar to that of recombinant or natural rIFN-α doses (0.5–0.7 MU/day) that appeared to be well tolerated when administered on a prolonged basis, although ineffective in preventing rhinovirus colds (Samo et al., 1984; Scott et al., 1985). On the other hand, an rIFN-α2 dose of 3 MU/day is associated with clinical and histologic evidence of nasal inflammation (Samo et al., 1984; Hayden et al., 1987a; Douglas et al., 1985; Monto et al., 1986). The findings of these various studies suggest that the risk of interferon-induced nasal inflammation appears to relate more closely to the antiviral activity of the administered interferon rather than its protein content.

Since this was the first human trial using intranasal rIFN-αCon_1, subjects were monitored closely for the development of possible local or systemic side effects and withdrawn from the study as soon as possible problems were identified. This, in part, accounts for the high withdrawal rate in this trial relative to previous tolerance studies of intranasal interferons. However, a relatively high proportion of subjects in the placebo group was also withdrawn from the study because of abnormal nasal findings. Erosions often occurred on the mid or anterior portion of the nasal septum, sites at which the sprayer tip or perhaps blast of the spray could have induced mechanical trauma. Of note, earlier field studies also found that placebo recipients have substantial rates of signs and symptoms indicating minor degrees of nasal irritation (Hayden et al., 1985; Douglas et al., 1985; Monto et al., 1988). One non-blinded study found that placebo sprayers had higher rates of reporting blood-tinged mucus than nonspraying control subjects who were observed concurrently (Monto et al., 1988). Although possibly due to reporting bias in a non-blinded study, it is likely that such differences relate to other factors such as the vehicle or spray device (Monto et al., 1988).

In summary, intranasal rIFN-αCon_1 in doses ranging from 3 to 30 MU/day was associated with significantly greater rates of adverse nasal effects and abnormal histopathologic responses than placebo. The results suggest that the risk of interferon-induced nasal inflammation appears more closely related to the antiviral activity than the interferon protein content of the rIFN-α administered.

Acknowledgements

The authors thank the staff of the Respiratory Disease Study Unit and the ENT Clinic of the University of Virginia for help in conducting this study, Dr. R. Royds of Theradex, Inc. for assistance in data analysis, and Margaret Sipe for clerical help. This study was supported in part by a grant from Amgen, Inc., Thousand Oaks, California.

References

Alton, K., Stabinsky, Y., Richards, R., Ferguson, B., Goldstein, L., Altrock, B., Miller, L. and Stebbing, N. (1983) Production, characterization and biological effects of recombinant DNA derived human IFN-α and IFN-λ analogs. In: E. DeMaeyer and H. Schellekens (Eds.), The Biology of the Interferon System 1983, pp. 119–128. Elsevier, Amsterdam.

Douglas, R.M., Albrecht, J.K., Miles, H.B., Moore, B.W., Read, R., Worswick, D.A. and Woodward, A.J. (1985) Intranasal interferon-α2 prophylaxis of natural respiratory virus infection. J. Infect. Dis. 151, 731–736.

Douglas, R.M., Moore, B.M., Miles, H.B., Davies, L.M., Graham, N.M.H., Ryan, P., Warswick, D.A. and Albrecht, J.K. (1986) Prophylactic efficacy of intranasal alpha2-interferon against rhinovirus infections in the family setting. N. Engl. J. Med. 314, 65–70.

Farr, B.M., Gwaltney, J.M. Jr., Adams, K.F. and Hayden, F.G. (1984) Intranasal interferon-α2 for prevention of natural rhinovirus colds. Antimicrob. Agents Chemother. 26, 31–34.

Fish, E.N., Banerjee, K. and Stebbing, N. (1983) Human leukocyte interferon subtypes have different antiproliferative and antiviral activities on human cells. Biochem. Biophys. Res. Commun. 112, 537–546.

Fish, E.N., Banerjee, K. and Stebbing, N. (1985) Efficacy of consensus interferon alpha against HSV-2 infections. In: A. Billiau, E. DeClercq and H. Schellekens (Eds.), Proc. 1st Int. TNO Conf. Antiviral Res. Rotterdam, Suppl. 1 pp. 191–197. Elsevier, Amsterdam.

Fish, E.N., Banerjee, K., Levine, H.L. and Stebbing, N. (1986) Antiherpetic effects of a human alpha interferon analog, IFN-αCon_1, in hamsters. Antimicrob. Agents Chemother. 30, 52–56.

Greser, I., Gry-Grand, D., Maury, C. and Maunoury, M.-T. (1981) Interferon induces peripheral lymphademopathy in mice. J. Immunol. 127, 1569–1574.

Hayden, F.G., Mills, S.E. and Johns, M.E. (1983) Human tolerance and histopathologic effects of long-term administration of intranasal interferon-α2. J. Infect. Dis. 148, 914–921.

Hayden, F.G., Gwaltney, J.M. Jr. and Johns, M.E. (1985) Prophylactic efficacy and tolerance of low-dose intranasal interferon-alpha2 in natural respiratory viral infections. Antiviral Res. 5, 111–116.

Hayden, F.G., Albrecht, J.K., Kaiser, D.L. and Gwaltney, J.M. Jr. (1986) Prevention of natural colds by contact prophylaxis with intranasal alpha2-interferon. N. Engl. J. Med. 314, 71–75.

Hayden, F.G., Mills, S.E., Innes, D.J., Darrach, A., Just, M., Linder-Chiccolunghi, S.N. and Gauci, L. (1987a) Tolerance and nasal histopathologic effects of long-term, low-dose intranasal recombinant interferon alpha-2A (Roferon-A). Antiviral Res. 8, 139–149.

Hayden, F.G., Winther, B., Donowitz, G.R., Mills, S.E. and Innes, D.K. (1987b) Human nasal mucosal responses to topically applied recombinant leukocyte A interferon. J. Infect. Dis. 156, 64–72.

Issekutz, T.B., Stoltz, J.M. and Webster, D.M. (1986) Role of interferon in lymphocyte recruitment into the skin. Cell. Immunol. 99, 322–333.

Korngold, R., Blank, K.J. and Murasko, D.M. (1983) Effect of interferon on thoracic duct lymphocyte output: induction with either poly I:poly C or vaccinia virus. J. Immunol. 130, 2236–2240.

Monto, A.S., Shope, T.C., Schwartz, S.A. and Albrecht, J.K. (1986) Intranasal interferon-α2b for seasonal prophylaxis of respiratory infection. J. Infect. Dis. 154, 128–133.

Monto, A.S., Albrecht, J.K. and Schwartz, S.A. (1988) Demonstration of dose-response relationship in seasonal prophylaxis of respiratory infections with alpha-2b interferon. Antimicrob. Agents Chemother. 32, 47–50.

Neubauer, R.H., Goldstein, L., Rabin, H. and Stebbing, N. (1985) Stimulation of in vitro immunoglobulin production by interferon-α. J. Immunol. 134, 299–304.

Pope, R.M., McChesney, L., Stebbing, N., Goldstein, L. and Talal, N. (1985) Regulation of T cell proliferation by cloned interferon-α mediated by Leu-11b-positive cells. J. Immunol. 135, 4048–4053.

Samo, T.C., Greenberg, S.B., Palmer, J.M., Couch, R.B., Harmon, M.W. and Johnson, P.E. (1984) Intranasally applied recombinant leukocyte A interferon in normal volunteers. II. Determination of minimal effective and tolerable dose. J. Infect. Dis. 150, 181–188.

Scott, G.M., Onwubalili, J.K., Robinson, J.A., Dore, C., Secher, D.S. and Cantell, K. (1985) Tolerance of one-month intranasal interferon. J. Med. Virol. 17, 99–106.

Stebbing, N., Fagin, K., Altrock, B.W., Fish, E.N., Mackar, S. and Renton, K. (1985a) Experimental animal models for assessing clinical utility of human interferon: studies in hamsters. Program and Abstracts of the 1985 TNO-ISIR Meeting on the Interferon System, Clearwater Beach, Florida, October 13–18. p. 35.

Stebbing, N., Trousdale, M.D. and Fish, E.N. (1985b) Novel interferons obtained by recombinant DNA techniques: properties against herpes virus infections. In: R. Kono and A. Nakajima (Eds.), Herpes Viruses and Virus Chemotherapy: Pharmacological and Clinical Approaches, pp. 305–308, Elsevier, Amsterdam.

Trousdale, M.D., Goldstein, L., Stebbing, N., Perters, A.C.B., Schanzlin, D.J. and Robin, J.B. (1985) Human alpha and gamma interferon analogs in rabbits with herpetic keratitis. Invest. Opthalmol. Vis. Sci. 26, 1244–1251.

Van Der Meide, P.H., Harteveld, N., Stebbing, N. and Schellekens, H. (1985) The effects of recombinant and natural human interferons in vaccinia virus infected rhesus monkeys. In: A. Billiau, E. DeClercq and H. Schellekens (Eds.), Proc. 1st Int. TNO Conf. Antiviral Res. Rotterdam, Suppl. 1, pp. 199–207. Elsevier, Amsterdam.

Weck, P.K., Apperson, S., May, L. and Stebbing, N. (1981) Comparison of the antiviral activities of various cloned human interferon-α subtypes in mammalian cell cultures. J. Gen. Virol. 57, 233–237.

Winther, B., Innes, D.J., Mills, S.E., Mygind, N., Zito, D. and Hayden, F.G. (1987) Lymphocyte subsets in normal airway mucosa of the human nose. Arch. Otolaryngol. Head Neck Surg. 113, 59–62.