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Treatment of Primary Sjögren's Syndrome with Low-Dose Natural Human Interferon-α Administered by the Oral Mucosal Route: A Phase II Clinical Trial

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ABSTRACT

The purpose of this investigation was to examine the safety and efficacy of four dosages of natural human interferon-α (nHuIFN-α) delivered over a 12-week period orally in lozenges (150 IU and 450 IU, once [QD] or three times [TID] daily) compared to placebo in subjects with primary Sjögren's syndrome. This randomized, double-blinded clinical trial demonstrated that nHuIFN-α at a dose of 150 IU administered TID by oral lozenge significantly improved stimulated whole saliva output compared to placebo after 12 weeks of treatment. The 150 IU TID dose also was suggestive of benefit for 5 of 7 subjective measures of oral and ocular comfort. IFN lozenges demonstrated a good safety profile, with no serious adverse events found in any treatment group. There were no significant differences between the placebo and the four doses of IFN for adverse events by total number, organ system, severity, dropouts, and number judged to be related to treatment. In conclusion, these results demonstrated that the use of 150 IU IFN lozenges TID for 12 weeks in subjects with primary Sjögren's syndrome improved salivary output and decreased complaints of xerostomia without causing significant adverse medical events.

INTRODUCTION

SJÖGREN'S SYNDROME is an idiopathic autoimmune disorder characterized by lymphocytic infiltration of multiple organs and tissues as well as by the presence of autoantibodies, hypergammaglobulinemia, and immunoregulatory abnormalities.[1–3] Sjögren's syndrome is primarily a disease of women, with nearly 90% of all patients being female.[4,5] The disease has a typical onset during the fourth or fifth decade of life,[6] and it may be as prevalent as 1 out of every 2,500 females.[7]

Clinically, Sjögren's syndrome presents in either primary or secondary forms. Primary Sjögren's syndrome is characterized by xerostomia (dry mouth) and xerophthalmia (dry eyes) which are the result of a progressive loss of salivary and lacrimal function.[1,8] Secondary Sjögren's syndrome includes involvement of one or both of these exocrine sites in the presence of another connective tissue disease such as rheumatoid arthritis, systemic sclerosis, or systemic lupus erythematosus.[2,3,9] In both primary and secondary forms, there are commonly multiple extraglandular manifestations and serologic evidence of autoimmunity.

The presence of a focal, periductal mononuclear cell infiltrate is the characteristic histopathologic finding in salivary glands in Sjögren's syndrome.[10–12] Lymphocytic infiltrates of exocrine glands increase as the inflammatory disease pro-

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gresses, ultimately producing acinar gland degeneration, necrosis, and atrophy.[10]

Management of the dry eyes and mouth in subjects with Sjögren's syndrome is problematic.[9,13,14] Dry eyes are treated by protective measures, frequent ophthalmologic evaluations, and wetting agents to sustain a normal ocular surface.[15] The treatment of xerostomia is more difficult. Preventive measures such as supplemental fluoride, avoiding sugar or medications known to cause a dry mouth, and frequent dental checkups are recommended.[8,13,14] Artificial saliva or frequent ingestion of non-sugared liquids may provide symptomatic benefit for some subjects. Pharmacologic agents designed to increase salivary flow (secretogogues), including bromhexine, anetholetrithione, and pilocarpine, have been tested in subjects with Sjögren's syndrome. They have been shown to increase salivary output transiently, however, none have demonstrated sustained benefit in controlled clinical trials.[2,14]

IFNs have potent antiviral and immunomodulating effects,[16] and five distinct classes of IFN have been designated; alpha, beta, gamma, omega, and tau.[17] IFN has been shown to enhance phagocytic antigen processing and immune regulatory activity of macrophages,[17] regulate specific cytotoxicity of lymphocytes for target cells, and enhance natural killer cell activity.[18]

The therapeutic utility of IFN-α by the parenteral route has been well established. One natural and two recombinant forms of human IFN are approved for clinical use in the United States[19] in doses ranging from 0.25×10^6 IU/lesion administered twice weekly in condyloma acuminata up to 36×10^6 IU given daily in AIDS-related Kaposi's sarcoma. Other approved indications for IFN treatment include hairy cell leukemia, chronic hepatitis B, and chronic hepatitis C.[20] The adverse experiences associated with parenteral IFN appear to be dose related, with the majority of subjects developing one or multiple flu-like symptoms at doses greater than 1×10^6 IU/day.[19]

The use of high-dose injectable IFN-α for subjects with Sjögren's syndrome was first described in 1993. Shiozawa *et al.*[21] reported that weekly intramuscular (i.m.) injections of IFN (1×10^6 IU/dose) resulted in a significant increase of saliva secretion in subjects with primary Sjögren's syndrome. Ferraccioli *et al.*[22] successfully treated Sjögren's syndrome subjects with 3×10^6 IU IFN parenterally three times weekly. These investigators reported significant increases in lacrimal and salivary function with IFN compared to subjects treated with hydroxychloroquine. They also noted improvement in minor salivary gland histopathology on repeat biopsy in 3 of the IFN-treated subjects. Although few subjects experienced adverse effects at these high doses, it is well established that higher doses can cause a consistent and high percent of untoward effects.[23,24–26]

Shiozawa and colleagues subsequently examined the potential benefit of mHu IFN-α in a low-dose lozenge form in two open-label studies.[27,28] Using 150 IU IFN lozenges three times daily, 5 of 7 subjects[27] and 13 of 24 subjects with Sjögren's syndrome[28] had >30% increase in stimulated whole saliva output following treatment between 9 and 37 weeks (mean duration = 22 weeks). Recently, these investigators reported the effectiveness of 150 IU IFN lozenges given TID in 30 subjects with Sjögren's syndrome (28 with the primary form).[29] At 24 weeks, 15 (50%) of 30 subjects treated with 150 IU IFN lozenges TID had a ≥100% increase from baseline in stimulated whole salivary flow, compared to only 1 (3%) of 30 control subjects given sucralfate ($p < 0.001$). No clinically significant adverse experiences were reported in this clinical trial. Additionally, the investigators examined minor salivary glands at the completion of the trial in 9 subjects who had responded to treatment with at least a two-fold increase in saliva output. A significant improvement in pathologic changes, including reduced mononuclear infiltration and increased histologically normal epithelial tissue, was found compared to pretherapy biopsies.

Therefore, it appears that low-dose IFN-α administered via the oral–mucosal route may significantly increase salivary secretions in subjects with Sjögren's syndrome without causing the troublesome adverse effects associated with high dose parenteral IFN administration. The purpose of the present investigation was to examine the safety and efficacy of four different dosages of IFN lozenges compared to placebo in subjects with primary Sjögren's syndrome over a 12-week period.

MATERIALS AND METHODS

Study population

Eligible subjects were ≥18 years old, not pregnant, and had a diagnosis of primary Sjögren's syndrome according to the revised European Community (EC) proposed criteria.[30,31] All had a stimulated whole salivary flow rate of ≥0.05 g/min, a dentition free from acute odontogenic or periodontal infections, and completed an Institutional Review Board (IRB)-approved written consent form. Exclusionary criteria included current or anticipated use of a secretogogue (e.g., bromhexine, anetholetrithione, pilocarpine), exposure to systemic steroids within 30 days of initiation of study treatment, and a history of antineoplastic chemotherapy or radiotherapy for treatment of head and neck tumors. A total of 180 subjects were screened for inclusion in the study. Of the 119 who met eligibility criteria, 111 chose to enter the study and were randomized at baseline from 12 clinical sites within the United States. Of these, 109 were evaluable at baseline and 1 week, 102 at week 4, 96 at week 8, and 90 were evaluable at week 12.

Experimental design

This was a randomized, parallel group, double-blind, placebo-controlled clinical trial. At baseline, subjects were randomized to 12 weeks of daily treatment in one of 5 groups (Table 1), and underwent an oral examination, measurement of vital signs, review of medical history and concomitant medications, pregnancy test (if applicable), collection of saliva, and completion of a series of 100-mm visual analog scales concerning oral and ocular symptoms. Subjects returned for evaluation at 1, 4, 8, and 12 weeks.

Saliva collection

Whole salivary flow rates were determined at baseline, 4, 8, and 12 weeks. Subjects were instructed not to eat, drink, smoke, chew gum, or perform oral hygiene for at leat 60 min prior to

TABLE 1. STUDY POPULATION AT BASELINE AND TREATMENT SCHEDULE

Group (n)	*Age*[a]	*Sex (F/M)*	*8 am*	*2 pm*	*8 pm*	*Daily dose*	*Total dose/person*[b]
Placebo (22)	58.3 ± 2.1	19/3	0	0	0	0	0
150 IU[c] once daily (21)	55.8 ± 2.9	21/0	150	0	0	150	12,600
150 IU three times daily (22)	63.3 ± 3.1	22/0	150	150	150	450	37,800
450 IU once daily (23)	59.2 ± 2.7	21/2	450	0	0	450	37,800
450 IU three times daily (21)	56.7 ± 3.0	18/3	450	450	450	1,350	113,400

[a]Age in years, mean ± SEM values.
[b]Total dose/person for 12 weeks of treatment.
[c]All doses in International Units (IU).

saliva collection. Saliva collections were conducted within a specified time period of the day (preferably between 8 and 11 am) at each visit. Each subject was seated in a comfortable examination chair for a least 5 min before initiation of saliva collection.

First, unstimulated whole saliva (UWS) was collected for 5 min utilizing the spitting technique, according to established methods.[32,33] Next, stimulated whole saliva (SWS) was collected for a total of 5 min. The subject chewed a preweighed piece of unflavored chewing gum base 60 times/min (timed with a metronome), and accumulated saliva was spit into a preweighed vial every minute, as previously described.[32,33] All flow rates were determined gravimetrically, assuming a specific gravity of saliva of 1.0, and expressed as g/5 min.

Visual analog scales for oral and ocular symptoms

At each visit, subjects were instructed to provide a rating for each of the following seven items using a 100-mm visual analog scale (VAS): (1) oral dryness, (2) oral comfort, (3) difficulty swallowing dry food without any additional liquids, (4) difficulty swallowing any food without any additional liquids, (5) difficulty speaking without drinking liquids, (6) dry eyes, and (7) burning eyes.

Safety parameters and adverse events

Subjects returned for oral (weeks 1, 4, 8, 12) and physical (week 12) examination, measurement of vital signs (weeks 1, 4, 8, 12), review of medical history and concomitant medications (weeks 1, 4, 8, 12), and hematology and blood chemistry tests (weeks 4, 12). The safety variables assessed were divided into seven main categories: (1) hematologic variables of RBC, hemoglobin, hematocrit, platelets, WBC, segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils, and ESR; (2) body weight; (3) vital signs of temperature, heart rate, and blood pressure; (4) blood chemistry variables of total bilirubin, LDH, ALT, alkaline phosphatase, total protein, IgG, albumin, calcium, inorganic phosphorous, uric acid, total cholesterol, and creatinine; (5) medical events as reported by each subject; (6) clinical symptoms including nausea, vomiting, diarrhea, dizziness, fatigue, fever, headache, myalgia, arthralgia, appetite loss, dry cough, dry throat, dry nasal passages, and poor sleep; and (7) oral pathoses including fungal infections and episodes of salivary gland enlargement.

Medication and placebo

All lozenges were identical in appearance, taste, and smell, and contained 200 mg of a pharmaceutical grade anhydrous crystalline maltose (carrier for the IFN) and magnesium stearate (lubricant/excipient). IFN-α was produced by Hayashibara Biochemical Laboratories (HBL; Okayama, Japan) by *in vitro* induction of a human cell line (BALL-1) using hemagglutinating virus of Japan (also called Sendai virus) according to previously described criteria.[34] HBL IFN-α is greater than 98% pure and has a specific activity of approximately 2×10^8 IU/mg protein, which is comparable to other FDA-approved IFN products. IFN and identical-appearing placebo lozenges were individually strip-wrapped in plastic-lined foil, and subjects were asked to keep them refrigerated.

Subjects were instructed to take the lozenges at approximately 8 am, 2 pm, and 8 pm. Subjects were instructed to allow the lozenge to dissolve in their mouth and not to chew or swallow the lozenge while it was dissolving. They refrained from eating, drinking, using oral wetting agents, or brushing their teeth for 30 min before and after taking the lozenge. Lozenges were not used within 60 min of saliva collection during study visits.

Analysis

The primary efficacy end point was a combination based on changes from baseline in both the VAS for oral dryness and the UWS flow. Response was defined as complete if the patient experienced at least a 25-mm increase (improvement) in the VAS for oral dryness and an increase of unstimulated whole salivary flow of at least 0.05 g/min. A partial response was achieved if the patient experienced either of these improvements, but not both. Seven secondary outcome measures were analyzed. These were one additional objective measure (SWS), and six additional VAS items (see above).

All statistical assessments of efficacy and safety were directed at contrasts between each of the four treated groups (150 IU QD, 150 IU TID, 450 IU QD, 450 IU TID) and the placebo group. All statistical tests were two-sided and conducted at the 5% level of significance. SAS™ software was used for all analyses and graphics.

The statistical analyses of the primary efficacy variable were conducted with Chi-square tests on each of three binary outcomes (complete versus none, partial versus none, any versus none) at weeks 4, 8, and 12. Statistical analyses of continuously distributed outcome variables (UWS, SWS, and VAS scores)

assessed the significance of treatment group differences with placebo on the average of within-subject differences from baseline to weeks 4, 8, and 12; pairwise contrasts with placebo were carried out using a linear model.

Statistical analyses of continuously distributed safety variables assessed the significance of treatment group differences with placebo on the average of within-subject differences from screening to weeks 4 and 12; pairwise contrasts with placebo were carried out using a linear model. Continuously distributed laboratory variables were additionally analyzed using Chi-square tests on matched-pair tables. Changes from abnormally low to normal and conversely, abnormally high to normal and conversely, and abnormally low to abnormally high and conversely were assessed. Body weight was analyzed based on pairwise contrasts with placebo on averages of within subject changes from week 0 to week 12. The incidence and severity of oral pathoses and medical events were analyzed with Chi-square tests. Changes from baseline in clinical symptoms were analyzed using Chi-square tests on matched-pair counts (present at baseline, not present at subsequent evaluation; not present at baseline, present at subsequent evaluation). A search for outliers was conducted using standard methodology.[35]

RESULTS

All groups were similar with respect to number, age, sex, and race (Table 1). Most subjects were female (92.6%) and white (95.4%) and they ranged in age from 30 to 86 years, with a mean age of 58.7 years. Loss to follow-up was not significantly associated with treatment group at weeks 1, 4, 8, or 12. Of the 21 subjects who were not evaluable at the conclusion of the study, 8 were withdrawn due to various protocol violations (noncompliance), 7 experienced nonserious medical events (3 in the 150 IU IFN QD group and 1 in each of the other groups),

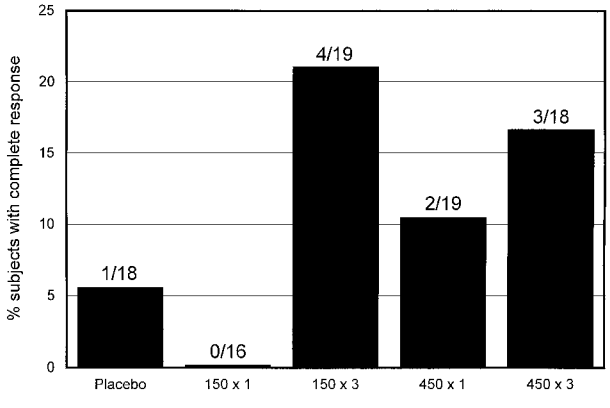

FIG. 1. Percentage of subjects in each group demonstrating a complete response on the primary outcome measure at week 12. Numbers above columns represent the number of individuals who were responders in each group/total number of subjects in the group. The one responder in the placebo group was determined to be a statistical outlier (see Materials and Methods and Results).

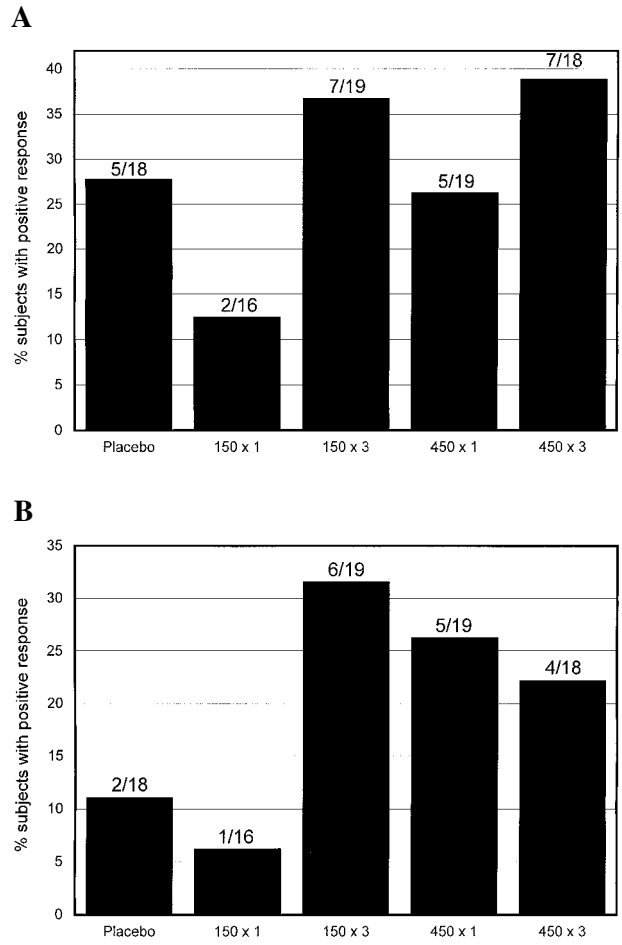

FIG. 2. Individual components comprising the primary outcome measure at week 12. **(A)** Percentage of subjects in each group demonstrating an increase of >0.05 g/min of unstimulated whole saliva. After removal of the statistical outlier in the placebo group, 23.5% of subjects (4 of 17) had a positive response. **(B)** Percentage of subjects in each group demonstrating >25 mm improvement in VAS for oral dryness. Numbers above columns represent the number of individuals who were responders in each group/total number of subjects in the group.

and 6 were terminated for starting a prohibited medication during the study (4 used a systemic corticosteroid and 2 an antidepressant).

Analysis of the primary end point failed to demonstrate a significant treatment effect based on complete responses. However, there was a suggestion of a benefit in group 150 IU IFN TID, in which 4 of 19 subjects (21.1%) experienced a complete response compared to only 1 of 18 subjects (5.5%) in the placebo group (Fig. 1).

The components of the primary end point were further analyzed independently. The UWS did not yield any significant treatment effects, yet groups 150 IU TID and 450 IU TID demonstrated a greater percentage of subjects with positive responses for UWS compared to the placebo group at 12 weeks (Fig. 2A). The UWS analyses were influenced by 1 subject in the placebo group who had an unusually large increase in UWS

flow. This response, as determined by influential point methodology, was considered to be an outlier. Removal of this single data point revealed a borderline significant ($p = 0.06$) increase in UWS in group IFN 450 IU TID (0.08 ± 0.03 g/5 min; mean ± sem) and a trend ($p = 0.13$) of an improvement in group IFN 150 IU TID (0.06 ± 0.02 g/5 min) at week 12 compared to placebo (0.02 ± 0.01 g/5 min). After removal of this outlier in the primary end point analysis, there were no subjects in the placebo group who demonstrated a complete response. Additionally, the pairwise comparison between group 150 IU TID (4/19 complete responses) and placebo (0/17) then yielded a trend toward significance ($p = 0.1$).

No significant treatment effects were found with regard to the VAS for oral dryness (Fig. 2B). Although not statistically significant, a trend was apparent for increasing benefit over time in groups 150 IU TID and 450 IU TID, and an apparent loss of response over time in the placebo group (data not shown).

SWS flow rates were significantly ($p = 0.04$) increased in the 150 IU IFN TID group compared to placebo at week 12 (Fig. 3). The mean SWS increase in group 150 IU TID was 0.79 ± 0.46 g/5 min, compared to 0.06 ± 0.11 g/5 min in the placebo group. Mean changes at other time points and doses were not significantly different among the groups. SWS flow rates diminished in the placebo group over the study period, while the 150 IU TID group maintained an increased output over the same time period (Fig. 3).

The VAS for oral comfort (Fig. 4A), difficulty swallowing any food without additional liquids (Fig. 4B), difficulty speaking without drinking (Fig. 4C), and eye dryness (Fig. 4D) did not demonstrate significant differences among the treatment groups. However, results demonstrated consistent trends over time for the active dosages compared to placebo (Fig. 4). Specifically, the placebo group showed smaller responses over time, while the 150 IU TID group showed an increasing improvement. Further, the 150 IU TID group appeared to provide the greatest benefit for these four measures compared to other groups. The VAS for the ability to swallow dry food without additional liquids and for burning eyes did not demonstrate any significant differences nor trends among the treatment groups (data not shown).

Responses of the individual subjects in the 150 IU TID group for oral and ocular symptoms are shown in Fig. 5. Responses on the VAS for oral dryness (Fig. 5A) were ranked from the highest (most improved) to lowest for all subjects at 12 weeks. Positive changes on the VAS denote a reduction in the symptom. Responses on the other VAS measures of oral symptoms (Fig. 5B, oral comfort; Fig. 5C, difficulty swallowing any food; Fig. 5D, difficulty speaking; Fig. 5E, difficulty swallowing dry food) were consistent, with the same individuals, in general, reporting improvement on each measure.

There were no serious adverse events encountered. Results of the safety analyses revealed no significant differences between groups for adverse events by total number, by organ system, severity, dropouts, or by number judged to be related to treatment (data not shown). The average number of adverse events (per subject) over the 12-week investigation was not significantly different among the five treatment groups (Fig. 6). Laboratory and clinical safety measures demonstrated no clinically significant changes in any of the groups over the duration of the study (data not shown).

DISCUSSION

Results from this 12-week randomized, double-blind clinical trial demonstrated that IFN delivered in lozenges at a dose of 150 IU administered TID significantly increased SWS flow rates in subjects with primary Sjögren's syndrome. This is consistent with earlier reports by Shiozawa *et al.*,[29] who used a saliva collection method (Saxon test) that is similar to the stimulated whole saliva collection performed in the present study.

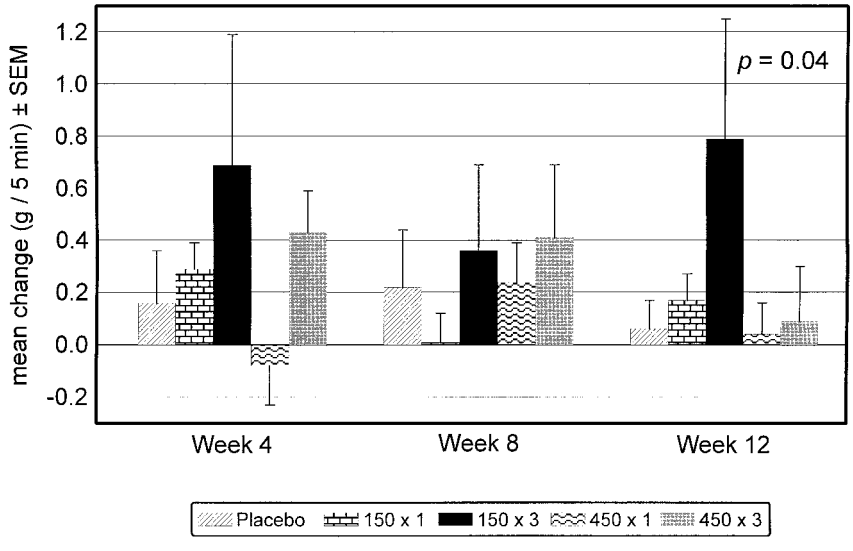

FIG. 3. Mean change (g/5 min ± sem) in stimulated whole saliva flow rates in all groups at weeks 4, 8, and 12. The 150 IU IFN TID group had a significant increase in output at week 12 ($p = 0.04$) compared to the placebo group.

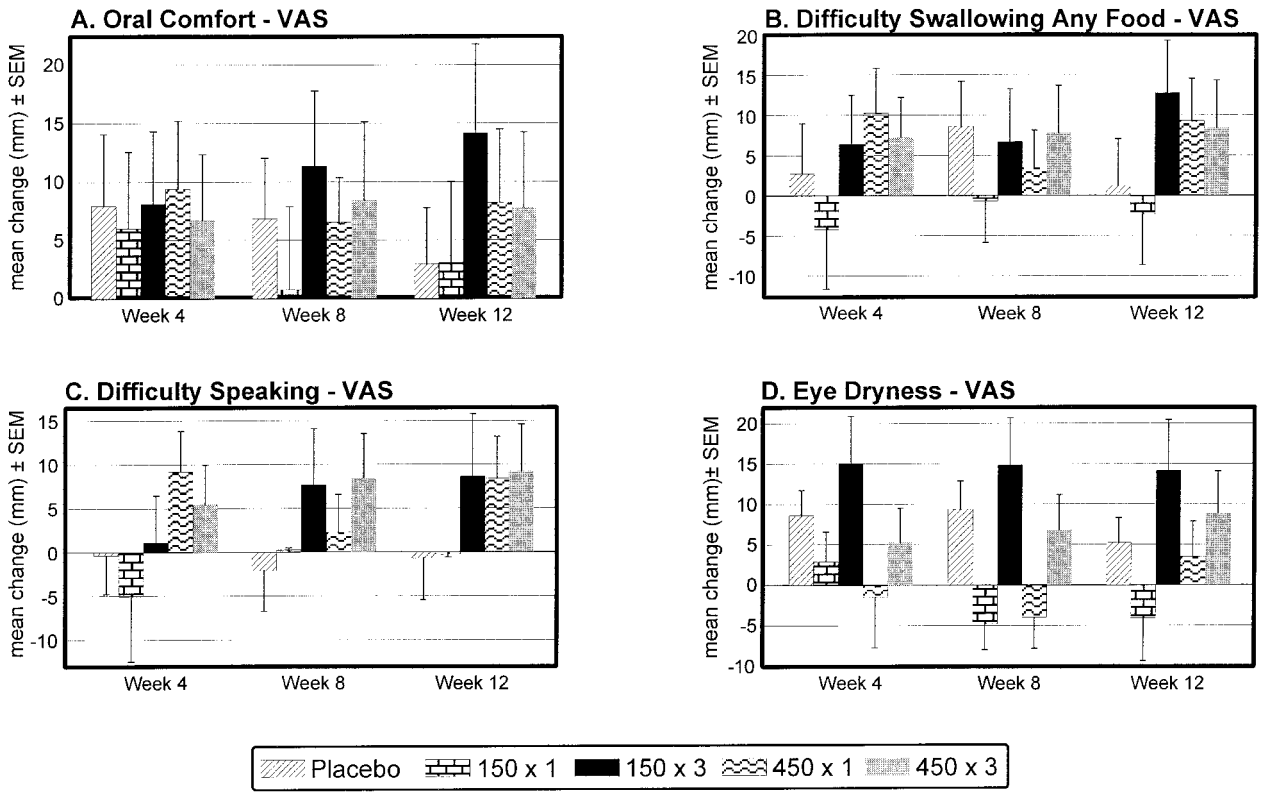

FIG. 4. Mean change (mm ± SEM) in responses to VAS measures in all groups at weeks 4, 8, and 12. **(A)** Oral comfort. **(B)** Difficulty swallowing any food. **(C)** Difficulty speaking. **(D)** Eye dryness. Positive numbers denote improvement in the symptom.

Additionally, the 150 IU TID dosage was suggestive of benefit for five of seven subjective measures of oral and ocular dryness. None of the other three dosages tested (150 IU QD, 450 IU QD, and 450 IU TID) showed the consistency of response observed for the 150 IU dose given TID. All doses of the IFN-α had good safety profiles. The overall safety analysis of IFN-α lozenges demonstrated no significant differences between the four dosages of IFN-α and placebo.

In the present study, none of the treatment groups achieved statistically significant improvement compared to placebo in the analysis of the components of the primary outcome measure. Analysis was complicated by the presence of one subject in the placebo group whose UWS response was found to exceed the range of responses experienced by other study subjects. Subsequent analyses using influential point methodology[35] determined this response to be a statistical outlier. *Post hoc* removal of this subject from the analysis of the primary outcome measure eliminated the sole responder in the placebo group. Comparison of the 150 IU IFN-α TID with the placebo group with the outlier removed resulted in a trend toward improvement in the 150 IU IFN-α TID group. Further analyses of UWS alone with the outlier removed suggested improvement in the 150 IU IFN-α TID group compared to the placebo group for this measure as well.

The finding of a significant increase in SWS output after 12 weeks of 150 IU mHu IFN-α TID suggests that there were effects at the level of the affected salivary gland. In Sjögren's syndrome, it is well recognized that a chronic inflammatory infiltrate and loss of acinar (fluid-producing) cells lead to a loss in secretory capabilities. The increase in stimulated output of saliva found with IFN treatment may be associated with histopathologic improvement. One previous study demonstrated that the amount of histologically normal salivary gland tissue was increased in 9 subjects with Sjögren's syndrome treated with 150 IU IFN-α TID for 6 months.[29] A second study[22] demonstrated histologic evidence of decreased salivary inflammation after parenteral IFN-α therapy in labial minor glands of subjects with Sjögren's syndrome. It is possible that IFN, through its immunomodulatory capabilities, may reduce the lymphocytic infiltration that characterizes the salivary glands affected by Sjögren's syndrome.[11,12] This may, in turn, explain the clinical finding of increased salivary output in the present study and previous investigations.[21,22,27,28]

The mechanism by which small amounts of orally administered IFN can exert biologic actions remains unclear. IFNs are not circulatory proteins[36] and they work physiologically in minute concentrations[37] in a localized fashion. IFN has been detected in nasal secretions of humans and animals, and is thought to serve as a marker of viral replication.[23] The fate of most nasal IFN is ingestion through swallowing, where it is thought to be inactivated rapidly by enzymatic activity within the intestines.

Oral ingestion is not generally used as a route of administration for IFN because of this inactivation by digestive en-

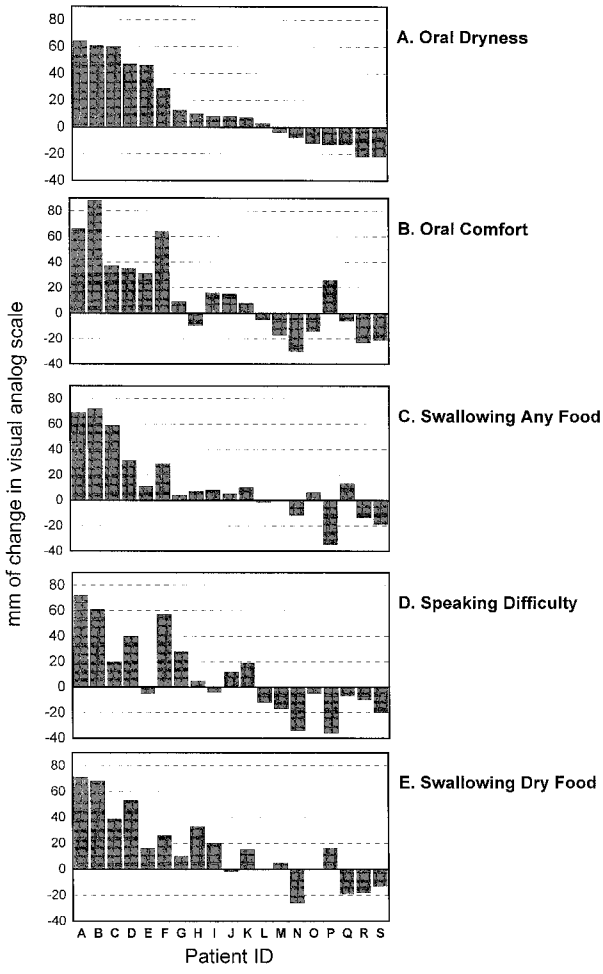

FIG. 5. Responses of individual subjects (n = 19) in the 150 IU IFN TID group to the oral symptom VAS measures at week 12. **(A)** Oral dryness. **(B)** Oral comfort. **(C)** Difficulty swallowing any food. **(D)** Difficulty speaking. **(E)** Difficulty swallowing dry food. Positive numbers denote improvement in the symptom.

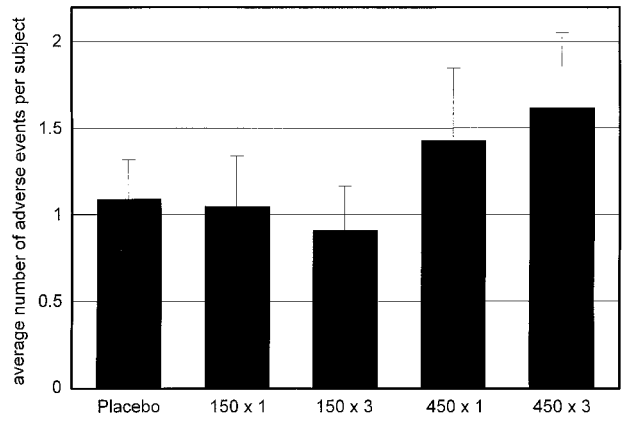

FIG. 6. Mean number of adverse events per subject in all groups for the 12-week study period.

zymes. Indeed, IFN is not detectable in the bloodstream after oral ingestion.[38] However, studies in several species, including humans,[39] suggest that IFN can trigger a systemic biological response in very low doses when administered via the oral cavity.[40]

Although direct evidence is not presently available, it is hypothesized that low-dose orally-administered IFN may bind to IFN receptor-bearing cells in the oral pharynx before being swallowed, which could presumably act as the initiator of a cascade of events resulting in increased immunologic activity.[40] Orally administered IFN has been shown to exert biologic activity *in vivo* in several clinical conditions[41,42] in addition to Sjögren's syndrome. Importantly, the oral route of drug administration is often preferable to the parental route due to a lower incidence of adverse medical events.

Interestingly, in the present study, 450 IU IFN TID did not demonstrate a consistently greater objective or subjective response than 150 IU IFN TID. This is consistent, however, with evidence from previous clinical studies that suggests that higher dosages of parenteral or lozenge-delivered IFN may not necessarily lead to a greater clinical response.[43–45] On the basis of the results of the present study, 150 IU IFN TID appears to be the more efficacious dose for the treatment of salivary dysfunction and xerostomia in subjects with primary Sjögren's syndrome. Furthermore, the results indicate that administration of IFN in lozenges three times daily is preferable to once-a-day dosing.

The safety analysis results suggest that low-dose use of IFN in lozenges is safe and without clinically significant untoward effects. Parenteral IFN treatment is a well-accepted therapeutic modality for several medical conditions.[20] However, it is accompanied by severe and medically serious adverse side effects,[23–26] including a flu-like syndrome, central nervous system depression, and myelosuppression. Detailed examination of the safety analyses of all subjects receiving active treatment in the present investigation did not reveal any of these untoward events or serious side effects leading to withdrawal from the study. Low-dose administration of IFN-α by lozenges appears safe and well tolerated.

In conclusion, lozenges containing 150 IU mHu IFN-α given three times daily for 12 weeks to subjects with primary Sjögren's syndrome led to a significant increase in SWS output and subjective improvement in measures of oral and ocular dryness. The IFN doses administered in this study appeared safe and were not associated with the significant side effects seen with high-dose parenteral IFN treatment. Additional clinical trials are required to confirm the safety and effectiveness of low-dose lozenges of IFN for the treatment of salivary gland dysfunction and xerostomia in subjects with primary Sjögren's syndrome.

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REFERENCES

1. FOX, R.I., HOWELL, F.V., BONE, R.C., and MICHELSON, P. (1984). Primary Sjögren's syndrome: clinical and immunopathologic features. Sem. Arthritis Rheum. **14,** 77–105.
2. FOX, R.I. (1996). Clinical features, pathogenesis, and treatment of Sjögren's syndrome. Curr. Opin. Rheum. **8,** 438–445.
3. FOX, R.I. (1997). Sjögren's syndrome. Controversies and progress. Clin. Lab. Med. **17,** 431–444.
4. DANIELS, T.E. (1996). Sjögren's syndrome: clinical spectrum and current diagnostic controversies. Adv. Dent. Res. **10,** 3–8.
5. STRICKLAND, R.W., TESAR, J.T., BERNE, B.H., HOBBS, B.R., LEWIS, D.M., and WELTON, R.C. (1987). The frequency of sicca syndrome in an elderly female population. J. Rheum. **14,** 766–771.
6. DROSOS, A.A., ANDONOPOULOS, A.P., COSTOPOULOS, J.S., PAPADIMITRIOU, C.S., and MOUTSOPOULOS, H.M. (1988). Prevalence of primary Sjögren's syndrome in an elderly population. Br. J. Rheum. **27,** 123–127.
7. FRITZLER, M.J., PAULS, J.D., KINSELLA, T.D., and BOWEN, T.J. (1985). Antinuclear, anticytoplasmic, and anti-Sjögren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. Clin. Immunol. Immunopathol. **36,** 120–128.
8. FOX, P.C. (1997). Management of dry mouth. Dent. Clin. N. Amer. **41,** 863–876.
9. FOX, R.I., and MARUYAMA, T. (1997). Pathogenesis and treatment of Sjögren's syndrome. Curr. Opin. Rheum. **9,** 393–9.
10. DANIELS, T.E., and FOX, P.C. (1992). Salivary and oral components of Sjögren's syndrome. Rheum. Dis. Clin. N. Am. **18,** 571–589.
11. FOX, R.I. (1996). Sjögren's syndrome: immunobiology of exocrine gland dysfunction. Adv. Dent. Res. **10,** 35–40.
12. FOX, P.C., and SPEIGHT, P.M. (1996). Current concepts of autoimmune exocrinopathy: immunologic mechanisms in the salivary pathology of Sjögren's syndrome. Crit. Rev. Oral Biol. Med. **7,** 144–158.
13. ATKINSON, J.C., and FOX, P.C. (1993). Sjögren's syndrome: oral and dental considerations. J. Amer. Dent. Assoc. **124,** 74–86.
14. WU, A.J., and FOX, P.C. (1994). Sjögren's syndrome. Sem. Dermatol. **13,** 138–143.
15. FRIEDLAENDER, M.H. (1992). Ocular manifestations of Sjögren's syndrome: keratoconjunctivi tis sicca. Rheum. Dis. Clin. N. Am. **18,** 591–608.
16. WITHERSPOON, L.R. (1984). The interferons—clinical applications and new technologies. J. Nucl. Med. **25,** 817–821.
17. STEWART, W.E. II. (1980). Interferon nomenclature recommendations. J. Infect. Dis. **142,** 643.
18. DJEU, J.Y., STOCKS, N., ZOON, K., STANTON, G.J., TIMONEN, T., and HERBERMAN, R.B. (1982). Positive self regulation of cytotoxicity in human natural killer cells by production of interferon upon exposure to influenza and herpes viruses. J. Exper. Med. **156,** 1222–1234.
19. Systemic Interferons. (1992). *United States Pharmacopeia Dispensing Information*, 12th ed. Rockville, MD: US Pharmacopeia Convention, pp. 1587–1592.
20. PFEFFER, L.M., DINARELLO, C.A., HERBERMAN, R.B., WILLIAMS, B.R., BORDEN, E.C., BORDENS, R., WALTER, M.R., NAGABHUSHAN, T.L., TROTTA, P.P., and PESTKA, S. (1998). Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. Cancer Res. **58,** 2489–2499.
21. SHIOZAWA, S., MORIMOTO, I., TANAKA, Y., and SHIOZAWA, K. (1993). A preliminary study on the interferon-alpha treatment for xerostomia of Sjögren's syndrome. Br. J. Rheum. **32,** 52–54.
22. FERRACCIOLI, G.F., SALAFFI, F., DE VITA, S., CASATTA, L., AVELLINI, C., CAROTTI, M., BELTRAMI, C.A., CERVINI, C., and BARTOLI, E. (1996). Interferon alpha-2 (IFN α2) increases lacrimal and salivary function in Sjögren's syndrome patients. Preliminary results of an open pilot trial versus OH-chloroquine. Clin. Exp. Rheum. **14,** 367–371.
23. SCOTT, G.M., WALLACE, J., TYRRELL, D.A., CANTELL, K., SECHER, D.S., and STEWART, W.E. II (1982). Interim report on studies on "toxic" effects of human leucocyte-derived interferon-alpha (HuIFN-α). J. Interferon Res. **2,** 127–130.
24. SCOTT, G.M. (1983). The toxic effects of interferon in man. Interferon **5,** 85–114.
25. QUESADA, J.R., TALPAZ, M., RIOS, A., KURZROCK, R., and GUTTERMAN, J.U. (1986). Clinical toxicity of interferons in cancer patients: a review. J. Clin. Oncol. **4,** 234–43.
26. DEYTON, L.R., WALKER, R.E., KOVACS, J.A., HERPIN, B., PARKER, M., MASUR, H., FAUCI, A.S., and LANE, H.C. (1989). Reversible cardiac dysfunction associated with interferon alfa therapy in AIDS patients with Kaposi's sarcoma. N. Engl. J. Med. **321,** 1246–1249.
27. SHIOZAWA, S., KUROKI, Y., YOSHIHARA, R., CHIHARA, K., HIRATA, M., TANAKA, T., and SHIOZAWA, K. (1993). Increasing effect of oral interferon alpha in saliva secretion of Sjögren's syndrome patients. Ann. Meeting Japan Rheum. Assoc. Abstract W-12–10.
28. SHIOZAWA, K., TANAKA, Y., YOSHIHARA, R., HIRATA, M., KABEBUMA, S., and SHIOZAWA, S. (1994). Effect of orally administered interferon alpha on saliva production in Sjögren's syndrome. Ann. Meeting Japan Rheum. Assoc. Abstract F586.
29. SHIOZAWA, S., TANAKA, Y., and SHIOZAWA, K. (1998). Single-blinded controlled trial of low-dose oral IFN-alpha for the treatment of xerostomia in patients with Sjögren's syndrome. J. Interferon Cytokine Res. **18,** 255–262.
30. VITALI, C., BOMBARDIERI, S., MOUTSOPOULOS, H.M., BALESTRIERI, G., BENCIVELLI, W., BERNSTEIN, R.M., BJERRUM, K.B., BRAGA, S., COLL, J., DE VITA, S., *et al.* (1993). Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum. **36,** 340–347.
31. VITALI, C., BOMBARDIERI, S., MOUTSOPOULOS, H.M., COLL, J., GERLI, R., HATRON, P.Y., KATER, L., KONTTINEN, Y.T., MANTHORPE, R., MEYER, O., MOSCA, M., OSTUNI, P., PELLERITO, R.A., PENNEC, Y., PORTER, S.R., RICHARDS, A., SAUVEZIE, B., SCHIODT, M., SCIUTO, M., SHOENFELD, Y., SKOPOULI, F.N., SMOLEN, J.S., SOROMENHO, F., TISHLER, M., WATTIAUX, M.J., *et al.* (1996). Assessment of the European classification criteria for Sjögren's syndrome in a series of clinically defined cases: results of a prospective multicentre study. The European Study Group on Diagnostic Criteria for Sjögren's Syndrome. Ann. Rheum. Dis. **55,** 116–121.
32. NAVAZESH, M. (1993). Methods for collecting saliva. Ann. NY Acad. Sci. **694,** 72–77.
33. MULLIGAN, R., NAVAZESH, M., and WOOD, G.J. (1995). A pilot study comparing three salivary collection methods in an adult population with salivary gland hypofunction. Spec. Care. Dent. **15,** 154–157.
34. SUGIMOTO, K., and YUEN, S. (1981). Interferon and preparations containing interferon. U.S. Patent No. 4,276,282.
35. NETER, J., WASSERMAN, W., and KUTNER, M.H. (1990). *Applied Linear Statistical Models*, Boston: Irwin.

36. BOCCI, V. (1982). Catabolism of interferons. Surv. Immunologic Res. **1,** 137–143.
37. KISHIDA, T., KITA, M., YAMAJI, M., and IMANISHI, J. (1987). Interferon producing capacity in patients with liver disease, diabetes mellitus, chronic diseases and malignant diseases. In: *Biology of the Interferon System*. K. Cantell and H. Schelleken (eds.) Boston: Martinus Nijhoff Publishers, pp. 435–441.
38. CANTELL, K., and PYHALA, L. (1973). Circulating interferon in rabbits after administration of human interferon by different routes. J. Gen. Virol. **20,** 97–104.
39. FUJIOKA, N., AKAZAWA, R., SAKAMOTO, K., OHASHI, K., and KURIMOTO, M. (1995). Potential application of human interferon-alpha in microbial infections of the oral cavity. J. Interferon Cytokine Res. **15,** 1047–1051.
40. BEILHARZ, M.W., McDONALD, W., WATSON, M.W., HENG, J., McGEACHIE, J., and LAWSON, C.M. (1997). Low-dose oral type I interferons reduce early virus replication of murine cytomegalovirus in vivo. J. Interferon Cytokine Res. **17,** 625–630.
41. HUTCHINSON, V.A., ANGENEND, J.L., MOK, W.L., CUMMINS, J.M., and RICHARDS, A.B. (1990). Chronic recurrent aphthous stomatitis: oral treatment with low-dose interferon alpha. Mol. Biother. **2,** 160–164.
42. BROD, S.A., and KHAN, M. (1996). Oral administration of IFN-alpha is superior to subcutaneous administration of IFN-alpha in the suppression of chronic relapsing experimental autoimmune encephalomyelitis. J. Autoimmunity **9,** 11–20.
43. CUMMINS, J.M., TOMPKINS, M.B., OLSEN, R.G., TOMPKINS, W.A., and LEWIS, M.G. (1988). Oral use of human alpha interferon in cats. J. Biol. Resp. Mod. **7,** 513–523.
44. CUMMINS, J.M., HUTCHESON, D.P., CUMMINS, M.J., GEORGIADES, J.A., and RICHARDS, A.B. (1993). Oral therapy with human interferon alpha in calves experimentally injected with infectious bovine rhinotracheitis virus. Arch. Immunol. Therap. Exp. **41,** 193–197.
45. MOORE, B.R., KRAKOWKA, S., CUMMINS, J.M., and ROBERTSON, J.T. (1996). Changes in airway inflammatory cell populations in standardbred racehorses after interferon-alpha administration. Vet. Immunol. Immunopathol. **49,** 347–358.

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