EXTENDED REPORT

Lamivudine is not effective in primary Sjögren's syndrome

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Background: Retroviral infection has been implicated in the pathogenesis of primary Sjögren's syndrome.
Objective: To examine the efficacy of the reverse transcriptase inhibitor lamivudine in patients with this syndrome.

Methods: 16 patients with primary Sjögren's syndrome were randomised to receive either lamivudine 150 mg twice daily or placebo for three months. Measures of lacrimal and salivary function, including minor salivary gland biopsies, were obtained before and after treatment.

Results: Treatment with lamivudine did not result in significant improvement in the primary outcome measure of unstimulated whole salivary flow or other secondary measures, including minor salivary gland biopsy focus scores.

Conclusion: Lamivudine is not effective in patients with primary Sjögren's syndrome, suggesting either that a retroviral aetiology is not present or that it may be important only in early disease.

Primary Sjögren's syndrome is a chronic inflammatory autoimmune disease affecting mainly salivary and lacrimal glands. It results in xerostomia and keratoconjunctivitis sicca. The following evidence suggests that primary Sjögren's syndrome may be antigen driven: there is accumulation of mononuclear cells around epithelial cells in salivary tissue that are HLA-DR⁺^{1–3}; the majority of infiltrating lymphocytes in the labial salivary glands are CD4⁺ T cells^{4–14}; and there is clonal T cell proliferation in salivary tissue.^{15–17}

Research suggests a role for retroviruses in the pathogenesis of Sjögren's syndrome. A Sjögren-like illness occurs in patients infected with human immunodeficiency virus (HIV)¹⁸ or HTLV-I.^{19–22} Thirty per cent of patients with primary Sjögren's syndrome produce antibodies to HIV p24 (gag) but not to other HIV proteins.^{23–24} In lymphoblastoid cells exposed to homogenates of salivary tissue from patients with Sjögren's syndrome, an intracisternal A-type retroviral particle antigenically related to HIV was identified.²⁵ Reverse transcriptase activity associated with anti-p24 positivity has also been detected in 30% of patients with Sjögren's syndrome.²⁴ Salivary glands also showed A-type like retroviral particles, suggesting the presence of an unknown retrovirus similar to HIV.²⁴ In minor labial salivary glands, the *tax* gene—but not the *gag*, *pol*, or *env* genes—of HTLV-I was identified in two of nine patients with Sjögren's syndrome but not in controls.²⁶ A sialadenitis similar to Sjögren's syndrome is seen in a HTLV-I-*tax* transgenic mouse.²⁷

Based on these data, Steinfeld undertook an open label study evaluating the efficacy zidovudine (AZT) in seven patients with primary Sjögren's syndrome.²⁸ Treatment resulted in improvement in both subjective and objective indices. We present our results of a placebo controlled, randomised, double blind study of lamivudine in primary Sjögren's syndrome.

METHODS

Study support

The study was funded by a private donation, the Arthritis Foundation of Northern California, Glaxo Wellcome, and the

Rosalind Russell Medical Center for Arthritis Research. Glaxo Wellcome also provided drug and placebo. None of the funding sources had influence on the study design, conduct, analysis, or manuscript preparation.

Subjects and samples

This was a single centre, randomised, double blind, and placebo controlled study in patients with primary Sjögren's syndrome. Subjects were recruited from the University of California, San Francisco (UCSF) affiliated rheumatology clinics and the private offices of rheumatologists throughout northern California. The protocol was approved by the UCSF Committee of Human Research and written informed consent was obtained from all patients. An investigational new drug application was filed with the Food and Drug Administration (IND No 37,158).

Subjects were required to be at more than 18 years of age, with primary Sjögren's syndrome diagnosed by the new American–European Consensus Group criteria.²⁹ The enrolment period was from 1 September 2000 to 1 February 2003. Demographic details are given in table 1. As the study began before publication of the new diagnostic criteria, initial study patients satisfied the older San Diego criteria. Later, the inclusion criteria were changed to incorporate the recommendations of the American–European Consensus Group. All previously enrolled patients met these new criteria. A methodological change in the new diagnostic criteria specified that the unstimulated whole salivary (UWS) flow measurement period should be 15 minutes. However, as patients entering the study before the publication of the new criteria were studied using a UWS measurement time of five minutes, the investigators decided to maintain this procedure to maintain consistency of the data.

All patients had a stimulated whole saliva flow of at least 0.04 ml/min. There was a required 30 day washout period of cytotoxic drugs, decongestants, over the counter antihistamines, glucocorticoids, tricyclic antidepressants, mucolytic

Abbreviations: UWS, unstimulated whole salivary (flow); VAS, visual analogue scale

Table 1 Baseline demographic variables

Patient	Control	SE	Lamivudine	SE
Female	100%		86%	
Age (years)	52.6	2.4	46.9	1.6
Disease duration (months)	43.1	7.4	37.8	6.2
<i>Drugs</i>				
NSAIDs	29%		43%	
Hydroxychloroquine	14%		29%	
<i>Salivary gland histology</i>				
Focus score (foci/4 mm ²)	6.0	0.6	1.8	0.2
<i>Other</i>				
No of eye drops/day	27.7	8.2	25.6	5.8
Unstimulated whole saliva (g/5 min)	0.0367	0.0064	0.0963	0.0123
<i>Laboratory tests</i>				
IgM RF titre (U/ml)	831.4	172.2	191.7	28.9
ANA titre	411.4	31.9	462.9	43.2
SSA antibody positive	71%		43%	
SSB antibody positive	43%		43%	
CRP (mg/l)	1.2	0.2	0.8	0.1
ESR (mm/h)	38.4	4.7	36.1	3.8
IgG (672–1760 mg/dl)	1724.0	84.6	1814.3	77.5
IgM (39–333 mg/dl)	184.4	14.2	147.1	12.9
IgA (89–581 mg/dl)	346.7	24.9	370.4	8.9

Values are mean and standard error.
ANA, anti-nuclear antibody; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; NSAID, non-steroidal anti-inflammatory drug; RF, rheumatoid factor.

agents, cholorectics, and secretagogues. The visual analogue scale (VAS) for oral dryness was required to be ≥ 25 mm (that is, suggesting at least mild xerostomia) at baseline. Patients were excluded for the following reasons:

- history of another autoimmune disease;
- pregnancy;
- treatment with chemotherapy or radiotherapy;
- history of HIV-1, HTLV-I, hepatitis (B or C) or active Epstein–Barr infection;
- malignancy within 5 years;
- other significant medical conditions affecting salivary or lacrimal function.

Patients were not permitted to undergo dental or medical procedures that could increase salivation or lacrimation during the course of the trial.

Study drug and interventions

Lamivudine is a synthetic nucleoside reverse transcriptase inhibitor that inhibits replication of human retroviruses by interfering with viral RNA directed DNA polymerase (reverse transcriptase). Lamivudine is used in combination with other antiretroviral agents in the treatment of HIV infection, and is approved for the treatment of hepatitis B. The study drug, supplied as tablets containing either 150 mg of lamivudine or placebo, was given orally twice daily.

Procedures

Patients were randomly assigned to receive either lamivudine 150 mg twice daily or placebo for 12 weeks. Randomisation occurred on a one to one basis, and the sequence followed a permuted block design (blocks of two and four). Random numbers generated by a commercial spreadsheet program assigned treatment within each block.

The primary outcome measure was UWS flow. Secondary outcomes included assessment of labial minor salivary gland focus score, stimulated parotid flow, tear production by way of an unanaesthetised Schirmer test (with open eyes), and the patient's perception of subjective variables related to the

sensation of dry eyes and dry mouth and other symptoms associated with primary Sjögren's syndrome. Evaluation of salivary function was measured by collection of unstimulated whole and stimulated parotid salivary flow at baseline, weeks 4 and 12, and follow up (eight weeks after termination of treatment). Whole and parotid saliva samples were collected separately, each over five minutes. The methods of saliva collection have been reported previously.^{30–33} Evaluation of lacrimal function at baseline and at weeks 4 and 12 was carried out by the same clinician (JPW) using the unanaesthetised Schirmer tear test, rose bengal staining of corneal conjunctival epithelium, and tear break-up time test. Evaluation of lymphocytic infiltration of the labial minor salivary glands was undertaken by focus score analysis at baseline and week 12. The biopsy sample was obtained by the Daniels' technique.³⁴ The samples were embedded in paraffin and sections cut through the middle of each gland. The severity of focal lymphocytic sialadenitis were graded according to a modified focus score procedure.³⁵ A focus is an aggregate of 50 or more mononuclear cells, and the focus score is reported as the number of such aggregates per 4 mm² of gland area. At least 20 mm² of histological material from each patient was examined.

At each visit, subjects were instructed to provide a rating for 18 items using a 100 mm VAS (table 2; VAS1 to VAS18). Oral and physical examinations were carried out at each visit.

Assays

At baseline, patients were tested for HIV, HTLV I and II, hepatitis B and C, antinuclear antibodies, SS-A and SS-B antibodies, rheumatoid factor, creatinine kinase, thyroid stimulating hormone, complete blood count, chemistry panel, creatinine, liver function tests, amylase, albumin, immunoglobulin levels, urinalysis, erythrocyte sedimentation rate (ESR), and serum C reactive protein. Certain haematological and serum profiles were repeated at weeks 4 and 12.

Statistical analysis

Treatment comparisons were undertaken using the Mann–Whitney test or Fisher's exact test. Associations of age and

Table 2 Changes from baseline to week 12

Salivary/lacrimal flow	Control mean	SE	Lamivudine mean	SE
Unstimulated whole (g/5 min)	0.0456	0.0154	0.0279	0.0103
Stimulated whole (g/5 min)	0.4377	0.1068	0.0179	0.0439
Right parotid (g/5 min)	-0.2599	0.0846	-0.0413	0.0075
Left parotid (g/5 min)	-0.0021	0.0403	-0.0430	0.0322
Right Schirmer (mm/5 min)	-2.9	0.3	-0.8	0.5
Left Schirmer (mm/5 min)	-0.1	0.7	-1.0	0.7
Right TBUT (s)	-0.9	0.8	-0.2	0.7
Left TBUT (s)	-1.9	0.7	-0.5	0.4
Right RB staining	-0.4	0.1	0.0	0.0
Left RB staining	-0.3	0.1	0.0	0.0
Right fluorescein	-0.3	0.2	-0.7	0.1
Left fluorescein	-0.2	0.2	-0.7	0.1
Salivary gland histology				
Focus score (foci/4 mm ²)	-1.8	0.6	0.3	0.3
Visual analogue scales*				
VAS 1	-27.0	3.6	4.6	3.9
VAS 2	-13.0	4.6	2.1	2.7
VAS 3	-9.6	1.4	-2.1	1.7
VAS 4	-0.6	2.6	-0.4	3.7
VAS 5	-2.1	3.1	3.7	4.0
VAS 6	-15.7	3.3	-18.6	2.3
VAS 7	-11.1	3.7	-11.9	3.0
VAS 8	-6.1	3.0	-3.7	2.0
VAS 9	-8.3	3.0	-4.9	2.1
VAS 10	-6.3	3.8	-4.6	3.6
VAS 11	-4.4	3.9	-16.3	5.7
VAS 12	-5.4	4.5	2.0	9.2
VAS 13	-10.0	5.1	4.4	3.1
VAS 14	-2.3	3.4	2.6	2.7
VAS 15	-4.9	2.9	-1.7	1.8
VAS 16	-4.6	2.4	-9.5	3.3
VAS 17	-10.1	2.6	5.7	1.1
VAS 18	5.6	4.0	3.7	1.4
No of eye drops/day	-1.7	0.6	2.6	1.4
Laboratory tests				
CRP (mg/l)	0.2	0.2	0.5	0.2
ESR (mm/h)	-7.6	2.2	6.0	1.4
IgG (mg/dl)	-84.0	25.0	113.3	24.1
IgM (mg/dl)	-5.7	1.4	12.8	3.0
IgA (mg/dl)	-16.4	3.4	22.7	5.4

VAS1-18: oral dryness, oral comfort, difficulty swallowing dry food without additional liquids, difficulty swallowing any food without additional liquids, difficulty in speaking without drinking liquids, eye dryness, eye burning, eye grittiness, eye foreign body sensation, energy level, arthralgias, myalgias, appetite, dry cough, nasal dryness, vaginal dryness, skin dryness, and quality of sleep.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; RB, Rose-Bengal; TBUT, tear break up time.

disease duration with outcome measures were examined using Spearman correlations. An overall longitudinal analysis was carried out using a mixed model with fixed effects for time and treatment and random subject effect. The model included a time by treatment interaction and time was treated as a linear effect, with separate slopes estimated for each treatment group. Paired comparisons of time points within treatment groups were made using Wilcoxon signed ranks tests. Variables that were not normally distributed (for example, parotid and fluorescein) were dichotomised to zero or more than zero and analysed using general estimating equations. The model effects were the same as in the mixed model, but without the random subject effect.

RESULTS

In all, 210 patients were screened. Twenty two met eligibility criteria, and 17 were enrolled in the study (three declined participation). Three patients were dropped from the study after randomisation for protocol violations (sarcoidosis, malignancy, and non-compliance). Fourteen patients completed the study.

Baseline characteristics

Most of the patients were female (93%) (table 1). The lamivudine patients had significantly higher baseline UWS ($p = 0.0213$, table 2) and lower minor salivary gland biopsy

focus scores ($p = 0.0087$). The placebo patients had a greater degree of oral dryness as assessed by VAS 1 ($p = 0.0298$). There were no other significant differences at baseline.

Baseline variables correlations

There were no significant correlations of age with baseline variables. Disease duration was positively correlated with fluorescein ratings ($r = 0.69$, $p = 0.0123$) and negatively with Schirmer I measurements (left: $r = -0.55$, $p = 0.0405$; right: $r = -0.69$, $p = 0.0066$) and tear break up times (left: $r = -0.63$, $p = 0.0154$; right: $r = -0.51$, $p = 0.0608$).

Longitudinal analyses

There were no significant changes in any salivary or lacrimal flow measurements or any VAS questions. No saliva collection values showed significant changes. Although focus scores decreased by 1.8 in the lamivudine group and increased by 0.3 in the placebo group, these changes were not significant.

Several laboratory measurements showed significant differences in time trends. The slope for the white blood count was -0.013 cells/l per week compared to 0.039 cells/l for placebo ($p = 0.0280$). Blood urea nitrogen increased at a rate of 0.17 mg/dl per week for lamivudine patients compared with a -0.018 mg/dl decrease for placebo ($p = 0.0370$). Similarly, IgG increased at a rate of 4.46 mg/dl per week for

lamivudine compared with a decrease of -6.47 mg/dl for placebo ($p = 0.0059$).

DISCUSSION

The underlying aetiological events leading to primary Sjögren's syndrome remain unknown. In contrast to the results of a pilot open label study using zidovudine,²⁸ we were unable to show that lamivudine was effective in treating this condition, though we employed multiple measures of salivary and lacrimal function, eye staining, labial minor salivary gland focus scores, and VAS scores. The results of the earlier pilot study must be interpreted with caution owing to the significant placebo effect,³⁶ especially when the outcomes are soft.³⁷ This bias was present in other apparently positive open label drug studies involving hydroxychloroquine,³⁸⁻⁴² ciclosporine,⁴³⁻⁴⁴ glucocorticoids,⁴⁵⁻⁴⁶ and infliximab⁴⁷⁻⁴⁸ in primary Sjögren's syndrome, the results of which were not validated by later placebo controlled studies. Additionally, differences in study design or inclusion criteria might account for the disparate results. The zidovudine study patients had to fulfil the European Community²⁹ and the San Diego⁴⁹ classification criteria for primary Sjögren's syndrome. The patients in our study had to fulfil the new American-European Consensus Group criteria.⁵⁰ Based on different diagnostic criteria, some of the patients in the open study might not have qualified for this study. Also, patients in the zidovudine study were required to have evidence of active disease based on complaints of ocular and oral dryness, extraglandular symptoms, and the presence of an active inflammatory process, such as a raised ESR or hypergammaglobulinaemia.²⁸ Not all of these were required in the lamivudine study and no patient has extraglandular features. Finally, the zidovudine patients had disease duration of less than one year, while the average disease duration in the lamivudine patients was 38 months. The lack of change in sicca symptoms and signs in the lamivudine study could reflect irreversible damage in glandular tissue in longstanding disease.

Pharmacokinetic profiles are unlikely to explain the different results, as lamivudine would be expected to be more effective. Lamivudine and zidovudine both inhibit reverse transcriptase; however, lamivudine has better bioavailability, higher potency, and a longer half life.⁵¹

There were small numbers of subjects in the study, and thus it might not have been powered to detect subtle differences. Our initial power calculation was based on the magnitude of change seen in the zidovudine study. In retrospect, this calculation should have included allowance for the relatively insensitive outcome measures, as studies of pilocarpine and cefelimine required much larger numbers.⁵²⁻⁵⁴

Unfortunately, validated activity indices and outcome measures are not available for trials of primary Sjögren's syndrome.⁵⁵⁻⁵⁷ Existing outcome measures such as salivary and lacrimal flow have significant variability even under well controlled conditions. Additionally, in the Canadian dry eye epidemiology study, 25% of surveyed patients reported dry eye symptoms, yet a lack of correlation between symptoms and objective measures, such as salivary and tear flow, was reported.^{9 58-60}

Our study is one of the few to evaluate changes in minor labial salivary gland histology before and after treatment in a placebo controlled fashion. Although focus scores decreased in the lamivudine group and increased in the placebo group, the difference was not significant. The lack of effect could have reflected lower disease activity in the lamivudine group at baseline, as assessed by focus score, compared with the placebo group. A second possibility is that the study was not powered to show a significant difference. A third possibility involves the difficulty in using a histological outcome measure. Although biopsy of the exocrine glands shows

inflammatory lymphocytic aggregates and, in later disease, acinar destruction, the correlation between the degree of inflammation or damage on biopsy and the degree of functional impairment of the glands leading to symptoms and signs of dry eyes and mouth is modest.⁶¹⁻⁶² Consequently, measurement of glandular function by flow rates cannot be used to distinguish between the pathophysiological processes of inflammatory activity, acinar dysfunction, or acinar cell loss in the gland, any or all of which could be responsible for the functional impairment.⁵⁷ Salivary gland biopsies are also subject to significant sampling bias.⁶³

Lamivudine treatment did not result in improvement in 36 item short form health assessment questionnaire (SF-36) components. However, in another study, the SF-36 was unable to detect significant differences between subjects with primary Sjögren's syndrome and those with xerostomia, despite large differences in the unstimulated salivary flow rate between the two groups.⁶⁴ Given the poor sensitivity of the SF-36, it may not be surprising that the instrument was unable to detect a difference.

This study does not provide evidence that inhibiting reverse transcriptase is effective in primary Sjögren's syndrome. Future research might examine a treatment in early disease and could be done in conjunction with retroviral testing on minor salivary gland biopsies and saliva.

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