Usefulness of basal and pilocarpine-stimulated salivary flow in primary Sjögren’s syndrome. Correlation with clinical, immunological and histological features

J. Rosas, M. Ramos-Casals, J. Ena, M. García-Carrasco, J. Verdu, R. Cervera, J. Font, O. Caballero, M. Ingelmo and E. Pascual

Rheumatology and Internal Medicine Units, Hospital of Vila-Joiosa, Alicante, Rheumatology Unit, University Hospital of Alicante, Nuclear Medicine Unit, University Hospital of San Juan, Alicante and Systemic Autoimmune Diseases Unit, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clinic, School of Medicine, University of Barcelona, Barcelona, Spain

Abstract

Objectives. To examine salivary function in patients with primary Sjögren’s syndrome (SS) by assessing unstimulated and stimulated flows using 5 mg of pilocarpine in a 5% solution, in order to define their clinical usefulness in the evaluation of xerostomia in patients with primary SS as well as to identify those factors related to the increase in salivary flow after pilocarpine stimulation.

Methods. We investigated the clinical and immunological characteristics of 60 consecutive patients with primary SS. All patients fulfilled four or more of the preliminary diagnostic European criteria for SS. We measured unstimulated (basal) salivary flow (BSF) in all patients. In patients with BSF less than 1.5 ml, stimulated salivary flows (SSF) were also measured after stimulation with an ophthalmic 5% pilocarpine solution (0.1 ml = 5 mg, administered sublingually). SSF was also measured after oral administration of 50 mg anetholetrithione (ANTT) in the same patients. These stimulated salivary flows were measured 1, 2 and 3 h after the stimulus.

Results. Of the 60 patients, 55 were women and five men, with a mean age at the SS onset of 61 yr (range 18–82 yr). The mean BSF for SS patients was 1.40 ± 0.17 ml. Fifty (83%) patients showed a BSF less than 1.5 ml. The stimulated salivary flow after 1 h was 3.23 ml in the pilocarpine group and 0.57 in the ANTT group (P < 0.001); after 2 h it was 1.32 ml in the pilocarpine group and 0.52 in the ANTT group (P = 0.02) and after 3 h it was 0.80 ml in the pilocarpine group and 0.41 in the ANTT group (P = 0.046). No clinical or immunological differences were found between SS patients with BSF more or less than 1.5 ml, although patients with a BSF less than 1.5 ml showed a parotid scintigraphy class III or IV more frequently (42 vs 0%, P = 0.01). SS patients with a pilocarpine SSF less than 1.5 ml had a longer duration of SS (73.3 vs 31.3 months, P = 0.03) and a higher prevalence of positive anti-Ro/SS-A (70 vs 36%, P = 0.038), anti-La/SS-B (65 vs 32%, P = 0.038), parotid scintigraphy class III–IV (79 vs 9%, P < 0.001) and positive salivary gland biopsy (90 vs 43%, P < 0.001).

Conclusion. The study of xerostomia using basal and pilocarpine SSF is simple to perform, acceptable to patients and needs no special equipment. We describe a significant increase in SSF using a solution of 5% pilocarpine in comparison with salivary flow obtained after stimulation with ANTT. Twenty-two of the 46 patients with low BSF had stimulated flows over 1.5 ml. These

Submitted 26 March 2001; revised version accepted 7 January 2002.

Correspondence to: J. Rosas, Hospital Marina Baixa, Unidad de Reumatología, Partida Galandú, no. 5, 03570 La Vila-Joiosa, Alicante, Spain.
Sjögren’s syndrome (SS) is an autoimmune disease that mainly affects the exocrine glands and usually presents as persistent dryness of the mouth and eyes due to functional impairment of the salivary and lacrimal glands [1]. In the absence of an associated systemic autoimmune disease, patients with this condition are classified as having primary SS. The histological hallmark is a focal lymphocytic infiltration of the exocrine glands, and the spectrum of the disease extends from an organ-specific autoimmune disease (autoimmune exocrinopathy) [2] to a systemic process with diverse extraglandular manifestations [3–9]. Due to this heterogeneity, attempts have been made to identify subsets of patients for whom the prediction of the course of primary SS in affected individuals would be more reliable [10–12].

Xerostomia, the subjective feeling of oral dryness, is the key feature in the diagnosis of primary SS. Despite this, xerostomia is a symptom that usually receives little attention, and may be considered trivial by both doctor and patient. For the diagnosis of xerostomia, it is therefore considered necessary to use some objective test [13–14]. Several methods have been proposed [15], such as measurement of the salivary flow rate, sialochemistry, sialography, scintigraphy and examination of a labial salivary gland biopsy. Some of these are either invasive (minor salivary gland biopsy and sialochemistry) or require special equipment (scintigraphy or magnetic resonance scanning), and may not be suitable for everyday use in a rheumatology clinic. The measurement of the salivary flow, with or without stimulation, is a simple method in the evaluation of xerostomia, that is acceptable to patients and needs no special equipment [16].

The purpose of this study was to examine salivary function in patients with primary SS by assessing unstimulated and stimulated flows using a 5% pilocarpine solution, in order to define their clinical usefulness in the evaluation of xerostomia in patients with primary SS, as well as to correlate these flows with the clinical, immunological and histological features of SS patients.

Methods

Patients

We investigated the clinical and immunological characteristics of 60 consecutive patients with primary SS. All patients fulfilled four or more of the preliminary diagnostic criteria for SS proposed by the European Community Study Group in 1993 [17] and underwent a complete history and physical examination. Diagnostic tests for SS were applied according to the recommendations of the European Community Study Group [17].

Measurement of salivary flows

All subjects had their whole unstimulated (basal) salivary flow (BSF) measured between 9:00 a.m. and 11:00 a.m., at least 1–2 h after the last food intake. The subject was asked to sit in a relaxed and upright position, and all saliva was allowed to drain into a beaker by drooling or gentle spitting with the patient instructed not to masticate, swallow or speak. Saliva was collected for a period of 15 min and then measured in a graduated syringe. Volumes of saliva ≤1.5 ml were considered abnormal [17].

Stimulated salivary flow (SSF) was measured in 46 of the 50 patients with BSF ≤1.5 ml, because four patients did not give consent to receiving oral pilocarpine after being informed of its potential side-effects. In a second visit, we measured the SSF after stimulation with 0.1 ml administered sublingually of an ophthalmic 5% pilocarpine solution (5 mg pilocarpine). In a third visit, SSF was measured after oral administration of one tablet of 50 mg anetholetirithione (ANTT). All stimulated salivary flows were measured 1, 2 and 3 h after the stimulus.

For each measurement, all patients completed a self-administered questionnaire that included a 100 mm visual analogue scale (VAS), which evaluated the perceived oral dryness, with responses ranging from extremely dry (0 mm) to no oral dryness (100 mm).

Salivary scintigraphy

Each subject received 5 mCi of sodium pertechnate (99mTc) intravenously. Sequential 5-min scintigrams were obtained for the first 30 min and then at 10-min intervals for the following 20 min. The different scintigraphic patterns were classified according to Schall et al. [18]. Briefly, class I is considered normal, with rapid uptake by the salivary glands within the first 10 min, a progressive increase in concentration, and prompt excretion into the oral cavity within 20–30 min. In the final, static status, the activity is higher in the mouth than in the glands. Class II denotes mild to moderate involvement. There is a relatively normal salivary dynamic, but reduced concentration or normal uptake with a delay in the sequence. Class III corresponds to severe involvement (marked delay in uptake and diminished concentration and excretion). Oral activity
may be detectable rarely, even in the static phase. Class IV indicates very severe involvement (complete absence of active concentration).

Laboratory studies
Immunological tests included antinuclear antibodies (ANA) (indirect immunofluorescence using mouse liver/ kidney/stomach as substrate), precipitating antibodies to the extractable nuclear antigens Ro/SS-A and La/SS-B [ELISA (enzyme-linked immunosorbent assay)] and rheumatoid factor (RF) (latex fixation and Waaler–Rose tests). Complement factors (C3 and C4) were estimated by nephelometry (BNA nephelometer; Behring, Deerfield, IL, USA). Serum cryoglobulins were measured after centrifugation. Blood samples were obtained and kept at 37°C for 30 s before separation. Serum was prepared by centrifuging at 37°C for 10 min at 2500 r.p.m. Fresh, centrifuged serum was incubated at 4°C for 7 days after collection, and examined for cryoprecipitation.

Statistical analysis
The χ2 test and Fisher’s exact test were used to analyse qualitative differences. To compare quantitative parameters, Student’s t-test was used for large samples of similar variance, and the non-parametric Mann–Whitney U-test was used for small samples. Values of quantitative variables are expressed as mean ± standard error of the mean (s.e.m.). Statistical significance was established at P < 0.05. The odds ratio (OR) was calculated to assess the risk of appearance of each variable, with a confidence interval (CI) of 95%. When several variables appeared to have statistical significance in the univariate analysis, an unconditional logistic regression analysis was performed by multivariate analysis in order to rule out possible confounding variables. Statistical analysis was performed with the SPSS program (SPSS, Chicago, IL, USA).

Results
SS features
Of the 60 patients, 55 were women and five men, with a mean age at SS onset of 61 yr (range 18–82 yr). All patients showed xerostomia, 45 (75%) xerophthalmia and six (10%) parotidomegaly. Fifty-two (87%) were classified in two groups according to their response to their BSF. Flow rates lower than 1.5 ml/15 min were considered as ‘low’ and those higher than 1.5 ml/15 min as ‘normal’ flows. No clinical or immunological differences were found between SS patients with BSF more or less than 1.5 ml, although those patients with a BSF less than 1.5 ml showed parotid scintigraphy class III or IV more frequently (42% vs 0%, P = 0.01).

Comparison between SS patients according to basilar salivary flow
SS patients were classified in two groups according to their BSF. Flow rates lower than 1.5 ml/15 min were considered as ‘low’ and rates above 1.5 ml/15 min as ‘normal’ flows. No clinical or immunological differences were found between SS patients with BSF more or less than 1.5 ml, although those patients with a BSF less than 1.5 ml showed parotid scintigraphy class III or IV more frequently (42% vs 0%, P = 0.01).

Comparison between SS patients according to pilocarpine-stimulated salivary flow
The 46 SS patients with BSF less than 1.5 ml in whom the pilocarpine-stimulation test was performed were classified in two groups according to their response to the pilocarpine stimulus. Flow rates after pilocarpine stimulus less than 1.5 ml/15 min were considered as ‘low’ and rates above 1.5 ml/15 min as normal (Table 2). The SS patients with SSF less than 1.5 ml showed a longer duration of SS (73.3 ± 14.4 vs 31.3 ± 10.7 months, P = 0.03) and a higher prevalence of positive anti-Ro/SS-A (70 vs 36%, P = 0.038, OR 3.50, CI 0.91–14.50), anti-La/SS-B (65 vs 32%, P = 0.038, OR 3.57, CI 0.91–14.50), parotid scintigraphy class III or IV (79 vs 9%, P < 0.001, OR 38.0, CI 5.55–392.82) and positive salivary gland biopsy (90 vs 43%, P < 0.001, OR 13.50, CI 2.75–73.41) in the univariate analysis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Basal Salivary Flow</th>
<th>Stimulated Salivary Flow</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocarpine</td>
<td>3.23 ± 0.57 ml</td>
<td>0.57 ± 0.09 ml</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANTT</td>
<td>1.32 ± 0.24 ml</td>
<td>0.52 ± 0.24 ml</td>
<td>0.022</td>
</tr>
<tr>
<td>3 h</td>
<td>0.80 ± 0.15 ml</td>
<td>0.41 ± 0.10 ml</td>
<td>0.046</td>
</tr>
<tr>
<td>Baseline VAS</td>
<td>17.36 ± 3.14 mm</td>
<td>15.38 ± 2.94 mm</td>
<td></td>
</tr>
<tr>
<td>Final VAS</td>
<td>50.50 ± 5.91 mm</td>
<td>22.42 ± 3.25 mm</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
although only parotid scintigraphy class III or IV ($P < 0.001$) and positive salivary gland biopsy ($P = 0.003$) were significant independent variables in the multivariate analysis (Table 2).

During the administration of pilocarpine solution, there was no change in vital signs (pulse rate, rhythm, blood pressure) or ECG in any of the subjects. Only two cases of adverse side-effects were observed in the pilocarpine-treated SS patients: both patients showed sweating and nausea of mild intensity after pilocarpine administration. In the ANTT-treated group, two patients also showed mild nausea.

**Discussion**

This study attempted to define the clinical usefulness, from a practical standpoint, of basal and pilocarpine-stimulated salivary flows in patients with primary SS. Collection of unstimulated whole salivary flow is simple to carry out and has been shown to be closely linked to salivary hypofunction [19–21], and recently Speight et al. [16] demonstrated that a low salivary flow (less than 0.1 ml/min) had a positive predictive value of 81% for the diagnosis of SS. We have described a BSF lower than 1.5 ml in 83% of patients with primary SS, and we have found a good correlation with parotid scintigraphic results. No patient with normal BSF ($>1.5$ ml) showed a pathological result in parotid scintigraphy (class III or IV). However, almost 50% of SS patients with low BSF had parotid scintigraphy class III or IV. It is well known that BSF may depend on the age of the individual [22–24], the time of day of examination [25], the use of drugs [26] and different medical and psychiatric conditions [27]. Despite these influences, many groups of investigators working with SS still use this test as confirmatory of xerostomia [28–33], and it is included in the European criteria for SS. Our results confirm its usefulness in diagnosing SS. We think that the measurement of BSF is a simple and useful test for the diagnosis of xerostomia in primary SS, and shows a good correlation with other tests that require special equipment, such as parotid scintigraphy.

In contrast to unstimulated flow, pilocarpine-stimulated flow measurements appear to be useful in the assessment and prognosis of patients. Stimulated parotid flow correlates with both the focus scores and the Tarpley scores of the labial minor salivary gland biopsies [34]. We describe a significant increase in stimulated salivary flows, using 5 mg in a 5% solution of pilocarpine, in comparison with the salivary flow obtained with the ANTT stimulus. This increase remains significant after 1, 2 and 3 h of the pilocarpine stimulus, which is confirmed by the subjective assessment of the patient, measured by the VAS score. Only 22 of the 46 patients with low BSF obtained a stimulated flow over 1.5 ml. Interestingly, these ‘responder’ patients showed a shorter duration of evolution of sicca syndrome, a lower frequency of positive immunological markers and milder grades of scintigraphic patterns and lymphocytic infiltrates in salivary biopsies. This subset of patients probably maintain a residual capacity in their salivary glands, as opposed to the 24 non-responder patients, who had a longer duration of sicca syndrome evolution with more severe involvement of the salivary glands. In our ‘responder’ patients, pilocarpine was able to stimulate this residual function, with an important improvement in salivary flow that resulted in a global symptomatic improvement of xerostomia, with a subjective amelioration in perceived oral moisture and lubrication.

The observation that pilocarpine stimulates salivary flow in patients with primary SS is not a novel one. Mandel and Wotman [35] reported in 1976 that

---

**Table 2. Epidemiological, clinical and immunological features of patients with primary SS according to salivary flow after pilocarpine stimulation (more or less than 1.5 ml)**

<table>
<thead>
<tr>
<th></th>
<th>SSF $&gt;1.5$ ml $n = 22$</th>
<th>SSF $&lt;1.5$ ml $n = 24$</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female)</td>
<td>20 (91%)</td>
<td>23 (96%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age onset (yr); mean ± S.E.M.</td>
<td>63.1 ± 13.7</td>
<td>58.8 ± 13.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Evolution (months); mean ± S.E.M.</td>
<td>31.3 ± 10.7</td>
<td>73.3 ± 14.4</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>Xerostomia</td>
<td>22 (100%)</td>
<td>24 (100%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xerophthalmia</td>
<td>15 (68%)</td>
<td>20 (83%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Parotidomegaly</td>
<td>1 (5%)</td>
<td>5 (21%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Articular involvement</td>
<td>9 (41%)</td>
<td>10 (42%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>1 (5%)</td>
<td>4 (17%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>6 (27%)</td>
<td>6 (25%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>3 (14%)</td>
<td>5 (21%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tubular nephropathy</td>
<td>3 (14%)</td>
<td>8 (33%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>2 (9%)</td>
<td>1 (4%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>1 (5%)</td>
<td>2 (8%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ANA</td>
<td>16 (73%)</td>
<td>21 (90%)</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>Ro/SS-A antibodies</td>
<td>8 (36%)</td>
<td>16 (70%)</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>La/SS-B antibodies</td>
<td>7 (32%)</td>
<td>15 (65%)</td>
<td>0.03</td>
<td>–</td>
</tr>
<tr>
<td>RF</td>
<td>11 (50%)</td>
<td>7 (29%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive Schirmer’s test</td>
<td>18 (82%)</td>
<td>21 (88%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Parotid scintigraphy III or IV</td>
<td>2 (9%)</td>
<td>19 (79%)</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Positive salivary gland biopsy</td>
<td>6 (43%)</td>
<td>18 (90%)</td>
<td>$&lt;0.001$</td>
<td>0.003</td>
</tr>
</tbody>
</table>
pilocarpine was known to stimulate lachrymal, salivary, gastric, intestinal, respiratory and pancreatic secretions. Recently, several reports have described the use of pilocarpine in various doses and routes of administration [36–39]. In our study, pilocarpine was used as a liquid ophthalmic solution, as opposed to the tablet form first used by Rhodus and Schuh [40]. Interestingly, in our study the adverse side-effects appeared to be negligible: no patient showed any type of cardiovascular or respiratory disease, and only two (3%) showed minor side-effects. This figure is significantly lower than that observed in patients receiving the compound by the oral route, in whom the incidence of withdrawal related to important side-effects ranged from 2 to 9%. With appropriate patient selection, pilocarpine in solution form is easily administered and seems to be effective in treating the oral dryness that accompanies salivary gland hypofunction. However, pilocarpine has limitations to its usefulness in treating dry mouth. We found that those individuals who failed to respond to pilocarpine stimulus were those with a longer duration of sicca syndrome. We hypothesize that the response of salivary glands to pilocarpine requires residual functional salivary gland tissue [41]. The existence of this residual functional capacity can be defined by a good response to pilocarpine SSF, and pilocarpine SSF could identify those patients capable of responding to pilocarpine in different doses and routes of administration. In addition, this test identifies a subset of SS patients, non-responders to pilocarpine, with irreversible damage of the salivary glands. This subset of SS patients probably have a higher risk of local pathology, such as caries and oral candidiasis, requiring specialized oral care.

In conclusion, this study has demonstrated that the stimulated flow rate of the major salivary glands, when determined under standardized conditions, can be a useful measure of salivary gland inflammation in primary SS and an aid in diagnosis. The method described in this study is simple to perform, acceptable to patients and needs no special equipment. In a similar way to Schirmer’s test, it can be performed easily in a busy clinic and the two tests together provide an immediate means of assessing the oral and ocular components of SS.

References