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The Effects of Pilocarpine Mouthwash on Vocal Quality

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Abstract: *Objective: to verify the effects of pilocarpine mouthwash on vocal acoustic parameters and salivation of healthy volunteers. Method: a clinical, randomized, placebo-controlled (saline) trial was conducted with 36 healthy individuals. Salivation was measured before and 15, 30, 45, 60 and 75 minutes after the administration of pilocarpine solutions (1% or 2%) or saline solution control. Blood pressure, heart rate, voice acoustic analysis, and assessment of symptoms resulting from the use of pilocarpine by visual analogue scale were measured before and at 75 minutes after treatment. Results: the increase in salivation was dosed (p = 0.021) and time-dependent. The 2% pilocarpine solution showed a significant difference in the salivation level of the volunteers who received saline at 60 and 75 minutes after the mouthwash (p = 0.001). Vocal evaluation was obtained with 22 individuals. Women did not present significant differences in the vocal acoustic parameters after pilocarpine use, while men presented a significant difference in the fundamental voice frequency after the use of pilocarpine solutions (p = 0.026). In addition, men presented a significant increase in absolute and relative Shimmer and in the amplitude variation coefficient (p = 045, p = 0.034 and p = 0.006, respectively) after the use of 1% pilocarpine. Conclusion: Our results show that topical treatment with pilocarpine via mouthwash increased salivation without significant adverse clinical effects. However, increased salivation caused voice alterations that could be explained by small saliva penetrations in the larynx, causing an incoordination in the vocal fold vibration cycle.*

Keywords:, *Pilocarpine, Salivation, Xerostomia, Vocal quality*

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I. Introduction

The voice is the main means of human communication. The vocal quality depends, among other factors, on the good hydration and lubrication of the vocal tract structures. Salivation is an important factor in preventing changes in these structures and avoiding trauma. Salivary imbalances can cause changes in quality of life and communication. Hyposalivation can be observed in patients with xerostomia[1], with the most common causes being Sjögren's Syndrome, the use of drugs that reduce salivary flow and irradiation in the head and neck region[2, 3]. Hyposalivation can cause unpleasant effects, such as decreased lubrication leading to difficulties in swallowing and speech, sleep disturbances, voice disorders, great discomfort, problems with gustatory loss, pH decrease, reduction of buffering capacity, changes in oral microflora and may increase the risk for caries and periodontal disease[4 - 12].

Changes in the viscosity of laryngeal secretions, often due to radiation, may alter vocal functions affecting the biomechanics of the upper airway, leading to changes in acoustic and aerodynamic properties of the vocal tract[13, 14]. Therefore, inadequate hydration and lubrication of the vocal folds impede the natural production of the voice, producing phonatory effort by changing the viscosity of the vocal folds tissue, interfering in the phonatory pressure threshold[15]. Drug stimulation of saliva can be achieved with peripheral administration of cholinergic agonists such as methacholine and pilocarpine[16].

Pilocarpine, orally administered, as most studies suggest [6 - 8, 17 - 19], has a large number of pharmacological effects and is accompanied by systemic side effects which may be very unpleasant. Previous studies have described the side effects of using pilocarpine mouthwash. In healthy volunteers, 2% pilocarpine mouthwash significantly increased salivation, with no secondary cardiovascular effects. However, in this work, the effects of pilocarpine on phonation have not been studied[20]. Therefore, the aim of the present study was to

verify the effects of the 1% and 2% solution of pilocarpine on the salivation of healthy volunteers and its possible interference on the acoustic vocal parameters of the individuals.

II. Materials And Methods

It is a randomized, double-blind, placebo-controlled study. The research was approved by the Research Ethics Committee under N. (148/03 and 088/05). Thirty six healthy volunteers participated in this study, aged between 19 and 40 years, being 17 men and 19 women, Medical undergraduate students of a University. All participants signed the free and informed consent document and were submitted to a confidential medical questionnaire and physical examination (including a complete otorhinolaryngological examination and a speech and language assessment). Exclusion criteria were vocal or laryngeal changes, bronchial asthma, cardiovascular diseases, recent drug use (1 to 5 days, including tobacco), pregnancy (suspected or confirmed), liver, renal or cardiac disease, peptic ulcer, hyperthyroidism, epilepsy, Parkinson's disease, human immunodeficiency virus (HIV) infection or induction of salivation by chemical or mechanical means (eating, drinking, toothbrushing, chewing gum) within the 90 minutes preceding the experiment.

Subjects responded to a self-response questionnaire on tobacco and alcohol, and completed a visual analogue scale (VAS) of pre-drug effects that consisted of 11 elements to identify the intensity of symptoms of nervousness, tremors, sweating, facial flush, gastrointestinal discomfort, lacrimation, salivation, palpitations, nausea, chest discomfort and blurred vision, in this order of evaluation. The subject indicated a point on a 10 cm line delineated by words expressing the highest and the lowest intensity of each symptom. Blood pressure and heart rate were measured using a blood pressure monitor (Omron, Vernon Hill, IL, USA). Finally, individuals' voice data were collected with a digital recorder (Power Pack DVR-2850) and the recorder was within 4 cm of the subject's mouth. Subjects were instructed to stand with arms along the body and utter the vowel / a / as long as possible, without using expiratory reserve air, at usual speed, loudness, and pitch. After this step, the data collection on salivation and the use of pilocarpine solutions were started. Subjects were randomized to receive one of the following solutions: saline, 1% pilocarpine or 2% pilocarpine. The collections were always held in the same quiet room. Several researchers were trained to perform all texts. First, volunteers were asked to swallow the saliva that was in their oral cavity and then place a pre-weighed cotton (4 cm x 1 cm) under the tongue and hold it in that position, with their mouth closed, for 1 minute. Then the cotton was removed and weighed. Individuals received the vial containing the solution with which they should rinse without too much force for one minute without swallowing. After this period, they should expel the drug, being careful to spit the whole volume after this time. From that moment, the saliva was collected at 15, 30, 45, 60 and 75 minutes after the mouthwash using the same procedure described before the mouthwash. The collections were performed at 15, 30, 45, 60 and 75 minutes after the mouthwash. The saliva samples were identified and weighed on a digital scale whose accuracy was predetermined to be 0.0001. At 75 minutes, blood pressure and heart rate were measured again. In addition, individuals were instructed to respond to the same analogue scale used at the beginning of the experiment and were asked to estimate the concentration of the solution administered, and their voices were recorded in the same manner as at the beginning of the experiment.

With the sample of the sustained emission of the vowel / a /, acoustic analysis of the voices was performed, with the removal of the initial and final unstable portions, standardizing the acoustic signals with not less than 3 seconds. The extraction of acoustic measurements was performed through the Multi-Dimensional Voice Program Advanced (MDVPA, Kay Pentax®).

Statistical analyzes were performed using the SPSS v.12.0 statistical program. For the analysis of the treatments effect with solutions of 1% and 2% pilocarpine and saline solution on salivation, mean arterial pressure and heart rate, the two-way analysis of variance of repeated measures (ANOVA, two-way-RM) was used, considering the parameters of time and concentration of pilocarpine. Fisher's exact test was used to establish a comparison of the number of individuals who correctly estimated the received solution. The results of visual analogue effects scale were analyzed by the ANOVA - MR - 2 ways test, considering the treatment parameters with solutions of 1 and 2% pilocarpine and saline solution and time before and after treatment. Linear regression was performed to determine the dose response relationship of salivary flow after 1% or 2% pilocarpine mouthwash and saline solution. The analysis of gender interference on voice acoustic parameters was performed using the three-way ANOVA-RM test, considering the time, pilocarpine concentration and gender parameters. The post-hoc comparison was made with the least significant difference test. Statistical significance was defined as $p < 0.05$.

III. Results

The characteristics of the study group are presented in Table 1. The amount of basal salivation flow did not differ in relation to gender, with an average of 0.59 g±42 in the female sex, and 0.76 g±0.50 in the male sex. Similarly, gender did not affect treatment results with 1% or 2% pilocarpine or physiologic solution.

Table 1. Baseline parameters of healthy volunteers participating in the study.

		Dose			
	Total	**SF 0.9% Nº = 8**	**1% Nº = 16**	**2% Nº = 12**	**P**
Sex					0,823
Male	17	3	8	6	
Female	19	5	8	6	
Age	23.2±6.3 (19-40 years old)	24.5±6.4 (20-40 years old)	22.6±8.0 (19-33 years old)	23.2±3.5 (20-33 years old)	0.800
Salivation					
Female (19)	0.59±0.42	0.86 ± 0.60	0.38 ± 0.24	0.67 ± 0.32	0.112
male (17)	0.76±0.50	1.09 ± 0.68	0.64 ± 0.42	0.75 ± 0.52	0.447
mean	0.67±0.46	0.95 ± 0.60	0.51 ± 0.36	0.71 ± 0.42	0.083
Mean blood pressure	94.64 ± 9.13	94.19 ± 9.29	95.74 ± 9.86	93.48 ± 8.62	0.81
Mean heart rate	81.17±12.53	80.38± 1.72	81.88 ± 13.58	80.75 ± 12.62	0.956

Data reported as mean ± DP.

However, the analysis of the amount of salivation measured at different time intervals revealed that treatment with pilocarpine solution significantly increased the salivation of healthy volunteers being time-dependent ($F_{5.165}$ = 5.543, p = 0.001) with significant interaction between time and treatments ($F_{10.165}$ = 2,573, p = 0.021). The salivary increase was detected at 60 and 75 minutes after administration of the solution, as shown in Figure 1.

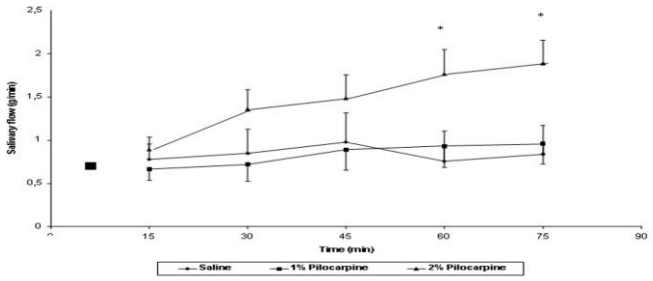

Figure 1. Salivation after a saline, or pilocarpine (1% or 2%) mouthwash (means ± DP).

The linear regression analysis on the amount of salivation after the use of 2% pilocarpine solution demonstrated a significant correlation (Y = 0.664 + 0,533 dose; r = 0.488, p <0.001). The analysis of data referring to measurements of mean arterial pressure and heart rate showed no modifications of these parameters by treatments with 1% and 2% pilocarpine (p = 0.235 and p = 0.0952) respectively. Data on tobacco and alcohol were not statistically significant. The presence of possible adverse effects as a consequence of the treatments was evaluated by visual analogue scale results (Table 2).

Table 2. Adverse effects reported by healthy volunteers after with a pilocarpine solution using visual analogical scale.

Symptom	**DOSE**			
	SF	**1%**	**2%**	
Nervousness	2.67± 3.54	0.80 ± 1.26	2.12 ± 2.62	0.417
Tremor	2.10 ± 3.45	1.00 ± 1.39	1.56 ± 2.08	0.388
Sweating	2.73 ± 3.05	0.76 ± 1.18	3.05 ± 3.18 *	0.017
Facial flushing	2.43 ± 3.59	0.90 ± 1.48	2.30 ± 3.17	0.323
Abdominal distress	1.71 ±2.42	1.08 ± 2.05	2.48 ± 3.74	0.296
Lacrimation	1.55 ± 1.88	0.59 ± 0.87	1.69 ± 2.73	0.715
Sialorreha	4.96 ± 2.95	6.25 ±2.55	6.78 ± 3.06	0.920
Palpitations	1.24 ± 2.36	1.16 ± 2.02	2.27 ± 3.13	0.621
Nausea	0.96 ± 1.75	1.08 ± 1.65	2.42 ± 4.02	0.176
Thoracic distress	1.91 ± 3.17	1.13 ± 2.41	1.75 ± 3.23	0.679
Blurred Vision	0.52 ± 0.82	0.47 ± 0.66	1.96 ± 3.45	0.072

Data reported as mean ± DP.

Analysis of these responses revealed that the 2% pilocarpine solution determined a significant increase in the perception of sweating when compared to the other treatments ($F_{2.33} = 4.634$; $p = 0.017$). The perception of increased salivation measured by the same scale showed a significant difference with an increase in time-dependent perception ($p = 0.035$) and in relation to treatment with 2% solution ($p = 0.025$) without interaction between time and treatment parameters. The 2% pilocarpine solution determined a higher degree of correct perception (75%) in relation to the administered treatments, compared to solutions of 1% pilocarpine (61.5%) and saline solution (66.7%), although they did not statistically deferred.

The sample of the voice data counted on only 22 individuals, 14 men and 8 women. Other data were lost due to technical problems**.** Regarding the voices, women did not present modifications in the acoustic parameters after the treatments.

However, men showed a significant reduction in baseline frequency following treatment with 1% pilocarpine (averages of 118.17Hz±18.14 111.04Hz±19.55 pre- and post-treatments, respectively; $p = 0.026$). Men also presented significant modifications in other three acoustic parameters of the voice: ShdB (absolute Shimmer), Shim (Shimmer percentile or relative) and vAm (amplitude variation coefficient), considered as disturbance measures of amplitude. ShdB ($F_{2,11} = 4.174$, $p= 0.045$) and Shim ($F_{2,11} = 4.691$, $p = 0.034$) were statistically significant after treatment with 1% pilocarpine. And vAm ($F_{2,11} = 8.399$, $p = 0.006$) was significant after the 1% pilocarpine dose, with significant interaction between time and treatments ($F_{2,11} = 4,681$, $p = 0,034$). These results are presented in Table 3.

Table 3. Acoustic parameter changes in males following treatment. Bold represents a significant difference from control

	TIME		**DOSES**			
		SF	**1%**	**2%**	**F**	**P**
Fundamental	Before	138.57Hz±33.74	118.17Hz±18.14	125.75Hz±29.52		**0.026**
Frequency	After	125.18Hz±-19.71	**111.04Hz±19.55**	127.39Hz±32.33		
	F time: (2,11)=6,637					
ShdB	Before	0.242±0.155	0.752±0.443	0.598±0.162	(2,11)=4,174	**0.045**
	After	0.225±0.152	**0.923±0.349**	0.587±0.163		
Shim	Before	2.678±1.677	8.092±4.544	6.499±1.598	(2,11)=4,691	**0.034**
	After	2.346±1.454	**9.888±3.351**	6.391±1.555		
Vam	Before	10.356±10,187	29.560±8.516	29.805±8.485	(2,11)=8,399	**0.006**
	After	12.004±3.455	**41.888±12.335**	25.888±3.733		

P Interaction: 0.0; F interaction: (2,11)=4,681

IV. Discussion

The present study demonstrates that the treatment of healthy volunteers with solutions of 1% or 2% pilocarpine administered in the form of mouthwash results in a significant increase in salivation. However, as the mean salivary amount was significantly higher for the 2% concentration in relation to 1%, it can be inferred that the increase in the salivary amount is dose-dependent, which confirms the data in the literature[7, 19, 21].

Saliva increase with 2% pilocarpine solution occurred after 60 and 75 minutes. These data coincide with those found in a similar study at these concentrations, where it was found that the concentration of 2% was more efficient in increasing salivation[20]. In addition to the objective of increasing salivary flow, the perception of increased salivation by volunteers was more intense, although not statistically significant, in the group receiving 2% pilocarpine solution when compared with 1% and placebo groups.

The perception about increased salivation became evident with the results of the analogue scale and the degree correctly attributed to the treatment previously received. Data that also coincide with a study in which the majority of individuals correctly pointed out the solution received[20]. Previous studies with oral pilocarpine have shown that a minimal increase in salivation volume promotes significant subjective improvement in symptoms[7, 8, 19]. In the present study, the only statistically significant adverse effect perceived by the subjects was increased sweating, related to the use of 2% pilocarpine. Sweating was also the most frequent adverse effect in several studies using the oral administration of pilocarpine [6, 7, 22-25]. The other items within the adverse effects scale were of low intensity and without statistical significance, corroborating the results of previous studies [6, 10, 19, 25]. This suggests that, at low doses, pilocarpine affects the stimulation of saliva with mild adverse effects[20]. Similarly, other parameters, such as heart rate and blood pressure did not change after pilocarpine mouthwash at both concentrations, and this result coincides with other clinical studies[20].

A pilocarpine is a parasympathomimetic, non-selective muscaine agonist, has β-adrenergic properties, and stimulates as exocrine glands, among them salivary glands, reducing xerostomia in this field[26]. Muscarinic receptors M1 and M3 are located in the salivary glands and can also be found in other organs which causes the appearance of adverse effects27 such as sweating, also identified in this study.

In previous studies using chronic oral administration of pilocarpine for more than 4 weeks, there was no significant change in vital signs such as blood pressure and heart rate on the electrocardiogram or

biochemical and hematological exams[7, 19]. The absence of any statistically significant changes in signs and symptoms in this study can be attributed to the topical form of pilocarpine administration that would provide a lower serum concentration of this drug compared to the oral route.

The results of the present study show that there were no statistically significant differences in any of the vocal acoustic parameters after the mouthwash, for women. However, men presented a statistically significant difference after the administration of 1% pilocarpine treatment in the following acoustic parameters: fundamental frequency, ShdB, Shim and vAm. The fundamental frequency showed a reduction from 118.7 Hz to 111.04 Hz after mouthwash with 1% pilocarpine, although it was within acceptable limits for male voices (80Hz to 150Hz). The fundamental frequency reflects the biomechanical characteristics of the vocal folds (laryngeal structures and muscular forces of tension and stiffness) in their interaction with infraglottic pressure[28].

The fact that the male subjects present this type of alteration in the fundamental frequency suggests that, probably, there was an increase in the lubrication provoking salivary penetration in the larynx region, being able to influence the velocity of the opening and closing cycles of the vocal folds. Although the values are within acceptable limits, the difference between the solutions effect indicates that, probably, there was a greater vibratory irregularity between cycles following the use of 1% pilocarpine. These results suggest that increased secretion in the mucous membrane of the vocal folds may have altered the vibratory pattern that converges with the literature[29].

This study reports that changes in frequency measurements are more likely to result from mechanical changes in the vocal folds covering and in the mucus covering them, generating surface tension and causing adhesion with non-linear vocal vibration from the source.

ShdB, Shim and vAm are considered measures of short-term disturbance of amplitude or Shimmer, being auditively perceived as vocal noise. These acoustic measures show the variability of the amplitude of the vocal sample and they can be associated with noise (roughness, breathiness or hoarseness), mainly breathiness. The data of this acoustic parameter shows that, probably, the increase of the mucus has caused this disorganization in the passage of the air that comes from the lungs and the closing of the vocal folds (one cycle releases little air during the closing of the vocal folds, the next one releases a little more), increasing noise, but still within the parameters considered normal. However, vAm is a measure of long-range amplitude variation. It is audibly perceived and corresponds to the amplitude variation along the emission. During swallowing, the larynx should close, rise and advance. However, it is possible that the laryngeal closure during swallowing is not hermetic at this time. This may justify the possibility that, in a certain way, we all present micro-aspirations during swallowing of liquids and saliva. This assumption is in line with a study that states that laryngeal penetration is a normal phenomenon.

In this study with healthy individuals of different ages, even with material penetration, the individuals did not present sensory-motor responses, perhaps because the penetration was not so intense[23]. In other words, the volume of penetration was not sufficient to cause coughing or mucus. In this way, it can be inferred that, with increased salivation, some droplets (not enough to cause coughing or mucus) may have penetrated the larynx, humidifying the endolarynx and being deposited on the vocal folds, possibly provoking cycle-to-cycle incoordination and along the emission with the use of 1% pilocarpine solution, being possible to be perceived only in the objective analysis of the voice. The accumulation of saliva in the region of vocal folds that disrupted these parameters did not exceed normal levels and was not auditively noticed, probably because subjects were healthy young individuals. However, further studies with pilocarpine mouthwashes are needed to verify if these effects are repeated in patients with xerostomia and to assess the impact on their quality of life.

V. Conclusion

The results of this work show that the use of topical pilocarpine in the form of mouthwashes is effective in producing increased salivation without serious adverse clinical effects. In this study, disturbances of the vibratory cycles show that there was some modification in the glottic level, probably due to the increase of secretion, thus demonstrating the effectiveness of pilocarpine on the mucus of the vocal cords. The fact that some individuals have quickly adapted to the voice condition without disturbance also shows that it is possible to use this solution to increase the external hydration of the vocal folds, without risk of instability generated by the increase of mucus on the vocal folds of the individuals.

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