EFFECT OF TWO MOUTHWASHES ON SALIVARY PH

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ABSTRACT

To analyze the effect of two mouthwashes on salivary pH and correlate it with age, buffer capacity and saliva flow rate in healthy volunteers, a crossover phase IV clinical study involving three age-based groups was designed. Two commercial mouthwashes (MW), Cool Mint Listerine® (MWa) and Periobacter® (MWb) were used. The unstimulated saliva of each individual was first characterized by measuring flow rate, pH, and buffer capacity. Salivary pH was evaluated before rinsing with a given MW, immediately after rinsing, 5 minutes later, and then every 10 min (at 15, 25, 35 min) until the baseline pH was recovered. Paired t-test, ANOVA with a randomized block design, and Pearson correlation tests were used. Averages were 0.63 mL/min, 7.06, and 0.87 for flow rate, pH, and buffer capacity, respectively. An immediate significant increase in salivary pH was observed after rinsing, reaching average values of 7.24 (MWb) and 7.30 (MWa), which declined to an almost stable value 15 minutes. The great increase in salivary pH, after MW use shows that saliva is a dynamic system, and that the organism is capable of responding to a stimulus with changes in its composition. It is thus evident that pH of the external agent alone is not a good indicator for its erosive potential because biological systems tend to neutralize it. The results of this study enhance the importance of in vivo measurements and reinforce the concept of the protective action of saliva.

Key words: *saliva; mouthwashes; tooth erosion; tooth wear*

EFECTO DE DOS COLUTORIOS SOBRE EL PH SALIVAL

RESUMEN

Se diseñó un estudio clínico cruzado fase IV, con tres grupos etarios de adultos voluntarios sanos, para analizar el efecto de dos colutorios sobre el pH salival y relacionarlo con la edad la capacidad buffer y el flujo salival. Se utilizaron dos marcas comerciales de colutorios (MW), ListerineCoolMint® (MWa) y Periobacter® (MWb). Primero se caracterizó la saliva sin estimular de cada individuo, se le midió el volumen minuto, el pH y la capacidad buffer. El pH salival se evaluó antes del buche con cada MW, inmediatamente después del enjuague bucal, 5 minutos después y luego cada 10 minutos (a los 15, 25, 35 min) hasta que el pH inicial se recuperó. Para el análisis estadístico de los datos se utilizaron: ANOVA en bloque, test t apareado y el test de correlación de Pearson. Al caracterizar la saliva, se obtuvieron los siguientes valores promedio: 0.63 mL/min, 7.06 y 0.87 de volumen minuto,pH, y capacidad buffer. Luego del enjuague se observó un incremento inmediato y significativo del pH salival alcanzando valores de 7.24 (MWb) y 7.30 (MWa) para descender a un valor estable luego de 15 minutos. El importante incremento del pH salival luego del uso del colutorio, muestra que la saliva es un sistema dinámico y que el organismo es capaz de responder a estímulos con cambios en su composición. Se hace evidente que el pH del agente externo, no es un buen indicador de su potencial erosivo sobre los elementos dentarios ya que los sistemas biológicos tienden a neutralizarlo. Los presentes resultados ponen de manifiesto la importancia de las mediciones en vivo y refuerzan el concepto de la función protectora de la saliva.

Palabras clave: *saliva; colutorios; erosión dental.*

INTRODUCTION

The strong association between alcohol consumption and the development of oral cancer (OC) has been extensively reported in the literature [1-4]. In this context, as some mouthwashes (MW) contain significant amounts of ethanol, the possible relationship to the risk of OC has also been a source of study and controversy for decades [5,6]. This was until in 2012 a quantitative meta-analysis of epidemiologic studies revealed quite consistently that there is no such association with **oral cancer** [7].

Not only alcohol consumption but also soft drinks and commercial fruit juices have been associated with adverse effects on oral health, including dental erosion (DE), i.e., loss of dental structure by acid dissolution that does not involve bacteria but has multi-factorial etiology which includes acidic substances from dietary, environmental and gastric sources [8-16]. Over recent years, its prevalence seems to have increased, and it is considered a significant oral disease in Europe and the Middle East. It has been reported that the amount of acidic substances, exposure time to these agents and salivary buffer capacity could be related to DE. As some mouthwashes are acidic solutions, the possible harmful effects on caries and DE have also been extensively studied [17-21]. Nevertheless, most of these studies are performed *in vitro*, and little is known about the changes induced by acid products *in vivo*, where the type of drink and the method of drinking may have strong influence and should be considered [22-25].

In a general context of *in vivo* studies concerning the influence of MW on oral health, we have performed the present research with the aim of analyzing the effect of two mouthwashes on salivary pH and correlating it with age, buffer capacity and flow rate in healthy adult volunteers. Both the change in pH value after rising and the time taken to return to the physiological condition were considered.

MATERIALS AND METHODS

A crossover, phase IV clinical study involving three age-based volunteer groups was performed. The volunteers were organized according to age range as follows: Group 1: 21 to 30 years, Group 2: 31 to 40 years, and Group 3: 51 to 60 years. Group 3 was included to consider people who not only use MW but also begin to undergo changes in some physicochemical properties and in the flow of saliva [26-28]. Diabetic patients, alcohol users and subjects who had hyposalivation or were under antibiotic, anti-inflammatory or anticholinergic therapy were excluded. Approval was obtained from the Ethics Committee for Scientific Research on Human Beings of the Hospital Nacional de Clínicas, Córdoba, Argentina (N°1150) and signed informed consent from all the volunteers before the beginning of the project.

Salivary pH was determined with an Orion 3 Star pH-meter. Buffer capacity (the amount of acid or base of a given concentration to be added to a solution to change the pH by one unit) was measured as the difference between pH values before and after the addition of 1.0 mL of a 5 mM HCl solution to 1.0 mL of saliva and expressed as $\beta = \delta$ mL / δ pH [29].

Two commercial MWs, available in Córdoba, Argentina, were used: Cool Mint Listerine® Antiseptic containing essential oils (pH= 4.35) (MWa), and Periobacter® a 0.12% w/v clorhexidine rinse (pH 5.30) (MWb); drinking water (pH 7.56 -7.80) served as a control (MWc).

Individual saliva characterization

Before evaluating the effect of the MWs, the saliva of each individual was characterized. All volunteers were instructed not to eat at least 8 hours before the test, but were allowed to brush their teeth at home. The use of MW was not allowed between the different stages of the test. A sample of unstimulated saliva for the analysis was obtained by spitting directly into a graduated sterile tube and the time taken to collect it was recorded. Then pH and buffer capacity were immediately measured.

Study design (mouthwash effect)

To analyze the effect of the MW on the salivary pH, all the volunteers were examined once a week for 3 consecutive weeks (cycle 1) as shown in Fig. 1. After these three weeks, there was one week without treatment, considered as the washout period. Then the test cycle was repeated, changing the order of the MW (cycle 2).

Each cycle consisted of the following steps:

- On each test day at 9:00 am, after breakfast and regular home brushing, four samples of unstimulated whole saliva were collected from each individual, at ten-minute intervals, by subjects salivating directly into a sterile tube. The pH in each tube was immediately measured and the mean and standard deviation of the samples from each subject were calculated; this value was considered as baseline of each volunteer (step 0 in the fig. 1).
- The subjects then rinsed their mouth for 30 seconds with 10mL of the pre-determined mouthwash and 1mL of saliva was collected in the following steps: immediately after the rinse (step1), after 5 minutes (step 2), and every 10 minutes until the baseline pH was recovered (steps 3, 4 or 5), and a final sample 15 minutes after the baseline pH was recovered or at the most, 50 minutes after the test started, if the initial pH value was not recovered in step 5 (step 6) (Fig 1).
- The same protocol was repeated with tap water and with the other MW in the second and the third weeks respectively as shown in Fig 1. The use of mouthwashes was not allowed in the days between the different stages of the test.

Statistical analysis

As the data followed a Gaussian distribution, parametric tests were used for the statistical analysis. Paired t-test and ANOVA with a randomized block design were used to evaluate the results. The subjects were considered as blocks to reduce the biological differences among them. The Pearson correlation test was also used. Statistical analysis was done using InfoStat / Professional version 1.1 (School of Agronomic Science at the National University of Cordoba) statistical software packages.

RESULTS

Saliva characterization

Table 1 shows the average values of flow rate (mL/min), pH and buffer capacity for each group of volunteers.

No statistically significant difference was observed in flow rate and pH among the three groups; general averages of 0.63 ± 0.23 mL/min with values ranging from 0.2 to 1.0 and of 7.06 ± 0.25 within a range of 6.45-7.69 for these variables were calculated respectively. On the other hand buffer capacity was significantly higher (p= 0.024) in the 31- to 40-year-old group, where the mean value was 1.01.

Effect of mouthwashes on the salivary pH value

Table 2 shows the average baseline pH values (step 0), the corresponding standard deviations and age of the volunteers before rinsing with the MW. The pH values differed significantly from the values obtained when the saliva of each individual was characterized, as the paired test t indicated (p<<0.05).

The Pearson test showed an interesting correlation between baseline and chacterization values r=0.63, p<<0.05 (Fig. 2).

The results of the experiment performed to analyze the influence of MW used are shown in Fig. 3. After rinsing, there was an immediate significant increase in the salivary pH of all individuals. This rise was immediate and pH reached maximum average values of 7.24 (MWb) and 7.30 (MWa). The values declined after step 2 to 7.11 and 7.15, and to an almost stable value after step 3.

Block ANOVA showed that the increase produced by both MWs was significant (p<0.05) in all groups, and no significant difference was found between MWa and MWb. Fifty nine per cent (59%) of the subjects who used MWa and fifty one per cent (51%) of those who used MWb recovered their original pH values 15 minutes after rinsing.

Fig. 3 also shows that when water was used as MW, the salivary pH increased slightly and then decreased to the initial value.

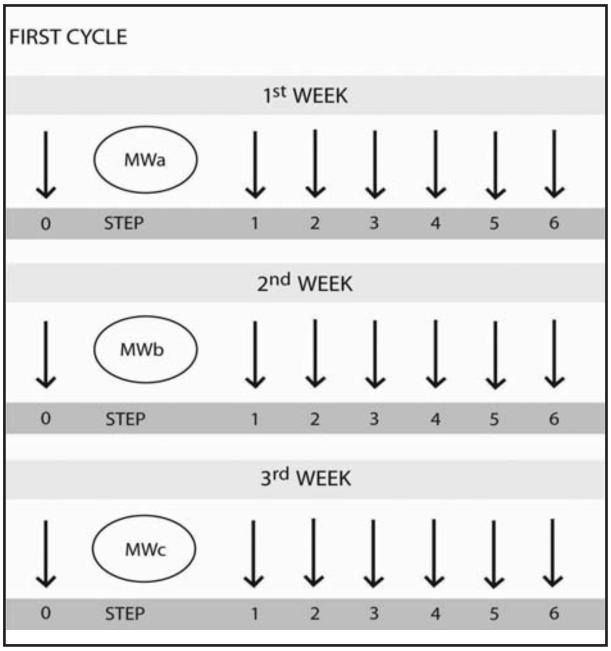

Fig.1: Study design.First cycle of the procedure for evaluating the mouthwash effect on the salivary pH. The arrows indicate the times at which samples were collected. See text for details.

Table 1: Saliva characterization of the three groups.

Age group	n	mL/ min mean ± SD	pH mean ± SD	buffer capacity mean ± SD
G1 (21-30)	10	0.67 ± 0.25	6.99 ± 0.31	0.79 ± 0.27
G2 (31-40)	10	0.59 ± 0.27	7.07 ± 0.16	*1.01 ± 0.30
G3 (51-60)	10	0.62 ± 0.18	7.12 ± 0.26	0.82 ± 0.28

*(p<0.05)

Table 2: Average baseline pH* (step 0) and age of the volunteers in the three groups.

Volunteer	G1 Age	G1 pH	G2 Age	G2 pH	G3 Age	G3 pH
1	23	6.94±0.10	40	7.14±0.24	51	6.75±0.16
2	28	6.78±0.09	36	6.79±0.03	54	7.18±0.27
3	30	7.22±0.14	39	7.04±0.17	52	6.49±0.19
4	27	7.12±0.07	39	6.79±0.08	57	6.57±0.41
5	22	6.89±0.10	31	7.13±0.07	51	7.19±0.17
6	29	7.15±0.09	40	6.62±0.03	60	6.81±0,25
7	30	6.55±0.18	32	6.98±0.07	51	6.91±0.16
8	24	6.72±0.32	33	6.56±0.24	53	6.84±0.16
9	23	6.61±0.20	33	6.88±0.19	59	6.59±0.27
10	22	6.87±0.07	32	7.16±0.11	54	6.75±0.14

*mean ± SD

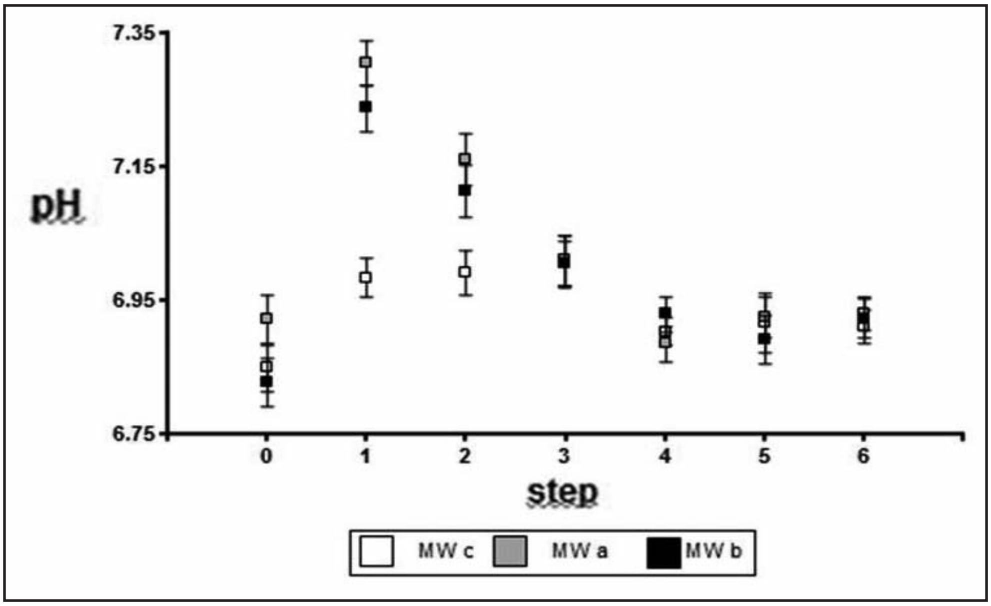

Fig.2: Salivary pH vs. time needed to recover the baseline pH after rinsing with different MWs: ■ MWa, ■ MWb, □ MWc.

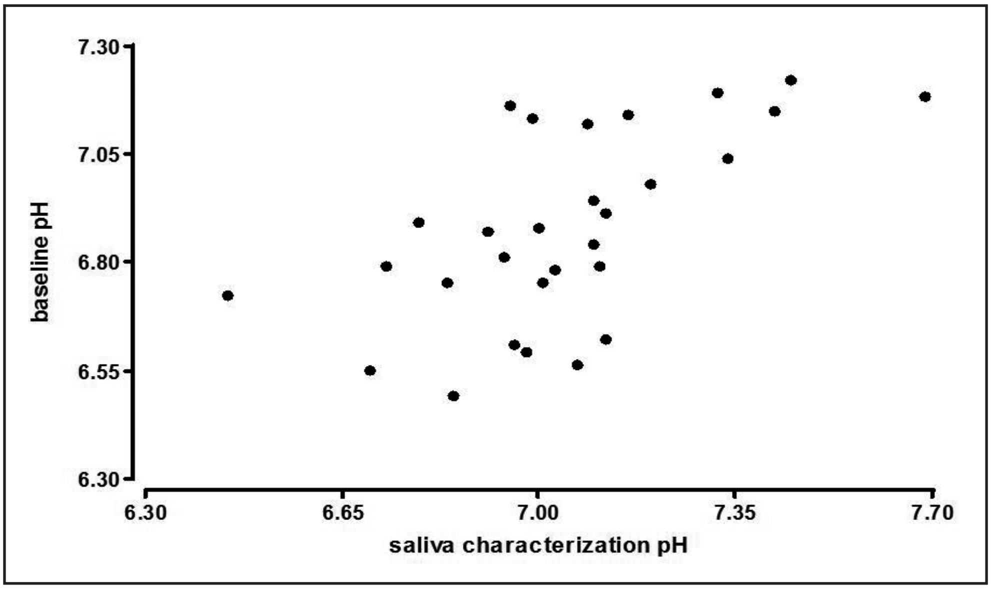

Fig.3: Correlation between pH values corresponding to baseline and saliva characterization.

The response of each patient was independent of the MW used and of the order in which it was used. There was also no difference in the response between the cycles. No correlation was found between the increase in pH produced by the MW and the variables analyzed, i.e., age, buffer capacity and salivary flow.
Pearson's correlation test also showed that there was no relationship between saliva buffering capacity and the time it took to recover the baseline pH for either of the MWs.
As negative control, 1mL of saliva and 1mL of MW were mixed and, as expected, a decrease in the pH value was observed.

DISCUSSION

The importance of saliva in the maintenance of good oral health is illustrated by xerostomic patients who often rapidly develop rampant caries along with atrophic and erosive mucosal lesions, inflammation and infection [30-32]. It should be expected that saliva also plays an important role in dental erosion, whose prevalence has increased in recent years and is currently considered to be a significant oral disease and a focus of increasing interest both in clinical dentistry and in research.
As this process has been strongly associated to acidity of drinks, and assuming that pH is the critical

threshold for dental hard tissue dissolution, testing of erosive potential on human teeth makes sense with substances having pH values below 5.5 [33]. However, it should be noted that pH alone is not a good indicator for the erosive potential of any substance; the degree of saturation of the solution with respect to dental hard tissue and consistency of the agent (duration of the contact with the tooth surface) play an important part in the erosion process. Demineralization depends, among other things, on host factors such as the fluoride concentration of the hard tissues, pellicle and plaque formation, and also calcium fluoride precipitation on the surfaces of the teeth (acting as a fluoride reservoir) [34-36].

Not only drinks but also the long-term use of MW has been associated to DE. In an *in vitro* study, Addy et al. observed that the dentine smear layer was affected by long-term use of some MWs, especially if used in conjunction with mechanical toothbrushing[18]. Zero reported that low pH oral care products might have erosive potential if used frequently [19]. In an *in-vitro* study Pretty et al. showed that Listerine®Antiseptic caused erosion compared to the negative control, although this was only significant after 14 hours of continuous use [21]. This undesired side effect was attributed to the low pH value of the MW.

Unfortunately, most studies have been performed *in-vitro* or *ex-vivo,* leading to results of limited value, considering that in addition to the host factors mentioned above, saliva plays an important role in DE. Salivary flow rate, composition and buffer capacity could influence this process and cannot be simulated by in vitro experimentation [33,36,37]. This is why we decided to carry out the present study *in vivo* to analyze the behavior of saliva of a host under the action of mouthwashes, which are widely used, often long-term.

The results of saliva characterization together with the intra- and inter-individual variations show that the acid-base properties of saliva depend to a great extent on the host and the conditions under which the sample was collected. The differences in pH values before and after breakfast, as well as the correlation between these values, support this conclusion. Small variations in salivary flow and buffer capacity could be expected, considering that all the volunteers were healthy persons who were screened using strict inclusion criteria. Thus, the very similar response in all cases after MW action is not surprising.

The great increase in salivary pH after using MW shows that saliva is a dynamic system, and that the organism is capable of responding to a stimulus with changes in its composition. We found that this rise in pH was unrelated to saliva baseline pH, salivary flow rate, buffer capacity or subject age. In agreement with our observations, other authors have suggested that stimulation of salivary flow provides an increase in calcium and phosphate concentrations as well as the alkaline environment necessary for remineralization[38]. They also observed an increase in the buffer capacity and bicarbonate content in comparison to unstimulated saliva. Acidic mouth rinses may trigger the same mechanism, stimulating salivary flow and producing a rise in pH. Finally our results on the one hand, enhance the importance of *in vivo* measurements, and on the other, reinforce the concept of the protective action of saliva.

CONCLUSIONS

The results of this study provide further evidence that the pH of the external agent alone is not a good indicator for its erosive potential because biological systems tend to neutralize sudden changes in pH generated by these agents. As demineralization depends strongly on a combination of host factors, the importance of clinical research becomes even more evident. Further observational *in vivo* studies are needed to elucidate the prevalence of DE and its association to the consumption of foods, juices, and oral care products, as well as to gastrointestinal disorders.

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