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SALIVARY PH AFTER A GLUCOSE RINSE: EFFECTS OF A NEW SODIUM BICARBONATE MUCOADHESIVE SPRAY. A PRELIMINARY STUDY

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ABSTRACT

Aim of the work The aim of this research was to evaluate if sodium bicarbonate, applied on the oral mucosa through a new mucoadhesive spray, can counteract the drop of saliva pH after a glucose rinse and support the buffering capacity of saliva. The mucoadhesive spray contains sodium bicarbonate, xylitol and excipients.
Materials and methods A sample of 30 healthy adult subjects was selected. The measurement of the salivary pH was performed at day 1 in the lower fornix in correspondence of the lower molars. Each subject rinsed with 10 ml of a 10% glucose solution and then pH was monitored for 40 minutes. At day 2 the same experimental procedure was repeated, but the mucoadhesive spray was applied three times in correspondence of the buccal mucosa of the cheek.
Results The subjects showed individual variability in the pH values recorded. Without the mucoadhesive spray salivary pH resulted significantly lower (p 0.0016). The time span during which pH values were lower than 6 was significantly shortened when the product tested was applied on the oral mucosa (p 0.001). This effect was observed for all the 40 minutes during which pH was recorded.
Conclusion The mucoadhesive spray tested resulted able to contrast the drop of the salivary pH after a glucose rinse. The support of the buffering capacity of the saliva with this new approach could play an important role in the prevention of caries and dental erosion.

KEYWORDS salivary pH, buffering capacity, glucose, sodium bicarbonate, mucoadhesive spray.

INTRODUCTION

Dental caries is closely linked to diet, salivation and the presence of a bacterial biofilm on dental surfaces. Frequent consumption of carbohydrates and the consequent variation in pH generated by the fermentation of sugars by dental plaque bacteria lead to microbiological changes within the biofilm in which these bacteria are organized (1-3). The drop of pH creates an environment that helps the growth of acidophilic microorganisms, such as Streptococcus mutans and the Lactobacilli, which find the ideal conditions for promoting further pH drops and possible areas of demineralization of the dental enamel. Sucrose is considered the most cariogenic of sugars in the diet, being quickly metabolized by bacteria with production of acids, which produce a lowering effect on plaque pH.
Plaque pH therefore plays a fundamental role in balancing the biofilm flora on the tooth surfaces and a drop of pH represents a selective stimulus in favor of acidophilic bacteria which, if adequately nourished, can maintain persistent acidity in the environment of the biofilm itself. When foods and beverages are introduced into the oral cavity and a drop in pH occurs, this is followed by a rise towards more basic values due to the action of the saliva which works as a controlling buffer on the pH, which in healthy conditions allows the replacement of minerals that have been lost following an acid attack on the dental enamel (7, 8). Hence the buffering capacity of saliva plays a fundamental role in balancing the phenomena of demineralization and remineralization (9). The buffering capacity of saliva also demonstrates an inversely proportional correlation with caries prevalence and is mainly due to sodium bicarbonate in the actual saliva (10). Sodium hydrogen carbonate or sodium bicarbonate is a sodium salt of carbonic acid used as an antiacid in different pharmaceutical preparations. In the field of dentistry, the effect of sodium bicarbonate in the form of chewing gum, gel and tablets on oral pH has been studied and results confirm that it considerably supports the buffering capacity of saliva (11, 12).
Scientific literature also bears evidence of the relationship between the buffering capacity of saliva and dental erosion; an insufficient capacity to counteract the drop in pH was observed in subjects with tooth erosion, underlining the importance of supporting the buffering capacity in such cases (13-16). This capacity therefore seems to have a fundamental role in cases of erosion, even more than in cases of patients with caries (17). It has also been observed that patients suffering from xerostomia may present a reduced buffering capacity and therefore it may be assumed that this type of subjects could also benefit from a product improving the efficacy of salivary pH control systems (11,12,16).
Several authors also underline that there are still patient groups whose fluoride exposure seems to be insufficient to equilibrate the acidogenic effect of carbohydrates introduced with the diet (18, 19) and therefore new, more targeted prevention strategies need to be set up.
This study aimed to evaluate the behavior of salivary pH after a glucose rinse and the subsequent application of a new mucoadhesive spray (Cariex; Brux s.r.l, Cislago - VA). The product tested contains sodium bicarbonate, xylitol, polyols, hyaluronic acid and excipients.
The aim was to observe if applying sodium bicarbonate to saliva with the mucoadhesive spray can counteract the drop in pH and thereby support the buffering capacity of saliva, and if the product's adherence to the oral mucosa allows a continued action over a time long enough to prevent clinically relevant decreases in pH.

MATERIALS AND METHODS

Selecting the test group

A sample of 30 adults was selected from patients of the Dental Clinic at the University of Insubria. The selected subjects were in good oral health (free from caries, periodontitis, or lesions of the oral mucosa) and with negative medical history for any type of systemic diseaseThe patients then underwent a dental examination and completed a medical history questionnaire, an informed consent was obtained for the collection of personal information and the characteristics of the research based examination. Each patient that was examined was given an identification code (a letter and a number) in compliance with the Italian Data Protection Code (No. 675 of 31/12/1996) and subsequent amendments. The procedures performed complied with the ethical principles proposed by the committee for human experimentation and the 1975 Declaration of Helsinki.

pH analysis

Salivary pH was analyzed with a portable dual-channel pH meter (pH-day 2; Menfis bioMedica s.r.l.) that is used for 24-hour esophageal recordings. It has a disposable antimony probe and has been used in other salivary pH studies (20). The device continuously records the pH value with a sampling period from 1 to 6 seconds, a resolution of 0.1 pH and a range of 0.1 to 14 pH. Before every examination the probe is first calibrated with a pH 1 and pH 7 buffer solution according to the instructions provided by the manufacturer. The cable connecting the probe to pH meter is fine and flexible as it has been designed for nasogastric use, and its use is comfortable in the oral cavity, even for long monitoring sessions, by ensuring that the cable protrudes from the corner of the mouth without caus-

ing any changes to salivary homeostasis.
All readings were taken in the morning: subjects refrained from eating, drinking and performing oral hygiene procedures for at least two hours before each experimental session.
At day 1 salivary pH was recorded in the lower fornix in correspondence with the first molars. The subjects had rinsed with 10 ml of 10% glucose solution and the pH was monitored for 40 minutes. The same experimental procedure was repeated at day 2 and the mucoadhesive spray was applied three times in correspondence with the buccal mucosa of the cheek.

Statistical analysis

The means and medians of the pH values obtained with the relevant standard deviations in the subjects in the two tests were processed. The time span during which pH values were less than 6 was calculated as was the corresponding percentage for the 40 minutes during which the pH was monitored. The results were compared with the Wilcoxon-Mann-Whitney test to evaluate the significance of the differences that were encountered.

RESULTS

The experimental procedure was performed without any complications, and none of the patients tested complained of side effects. When asked if the application of the product caused a feeling of an unpleasant, bland or pleasant taste, 100% of the subjects re-

	Monitoring salivary pH for 40 minutes after a glucose rinse without the application of the mucoadhesive spray				Monitoring salivary pH for 40 minutes after a glucose rinse and the application of the mucoadhesive spray			
	Media	Median	Time span at pH<6		Media	Median	Time span at pH<6	
			min	%			min	%
1	6,1	6,1	11,3	28,3	6,8	6,6	0,8	2,0
2	6,4	6,2	12,8	32,1	7,1	7,0	0,0	0,0
3	6,2	6,1	12,0	29,0	7,4	7,4	0,0	0,0
4	6,2	6,1	4,2	10,5	6,7	6,6	0,0	0,0
5	6,8	6,9	0,2	0,6	6,8	6,9	0,0	0,0
6	6,7	6,6	0,1	0,2	7,2	7,1	0,1	0,2
7	6,7	6,6	0,3	0,7	7,0	7,0	0,1	0,1
8	6,9	6,7	0,1	0,1	6,9	6,8	0,0	0,0
9	7,0	7,1	0,8	2,1	7,1	7,0	0,1	0,1
10	6,3	6,4	3,3	8,2	6,4	6,4	2,0	5,0
11	6,3	6,2	5,2	12,8	7,1	7,0	0,0	0,0
12	6,1	6,0	11,0	27,3	6,3	6,2	6,1	15,1
13	6,4	6,4	4,7	11,8	6,5	6,4	2,0	4,9
14	5,9	5,9	21,8	53,2	6,2	6,0	11,5	28,7
15	5,7	5,7	26,2	65,3	6,4	6,5	0,6	1,5
16	6,3	6,2	13,7	34,0	6,7	6,5	0,3	0,8
17	6,8	6,9	0,1	0,3	7,2	7,2	0,1	0,3
18	6,8	6,7	0,4	1,0	7,0	6,9	0,0	0,0
19	5,8	5,7	24,7	61,7	6,7	6,5	0,0	0,0
20	7,5	7,5	0,2	0,4	7,9	7,9	0,2	0,4
21	7,1	7,0	0,5	1,2	7,2	7,2	0,2	0,3
22	6,8	6,6	0,5	0,1	6,8	6,6	0,0	0,0
23	6,5	6,5	0,2	0,4	6,8	6,6	0,0	0,0
24	7,2	7,2	0,0	0,0	7,3	7,4	0,0	0,0
25	6,8	6,7	0,4	1,0	6,9	6,6	0,1	0,3
26	7,1	7,1	0,0	0,0	7,1	6,9	0,0	0,0
27	5,9	5,9	21,2	55,2	6,8	6,5	0,0	0,0
28	6,3	6,0	11,5	29,0	6,4	6,4	10,9	27,7
29	6,3	6,0	9,6	24,4	6,5	6,5	0,3	0,8
30	6,5	6,5	2,4	6,2	7,3	7,2	0,0	0,0
media	6,5	6,5	6,6	16,6	6,9	6,8	1,2	2,9

TAB. 1 *Mean, median, time and percentage of time span with salivary pH at <6 during 40 minutes of monitoring with and without the application of the mucoadhesive spray in the 30 test subjects.*

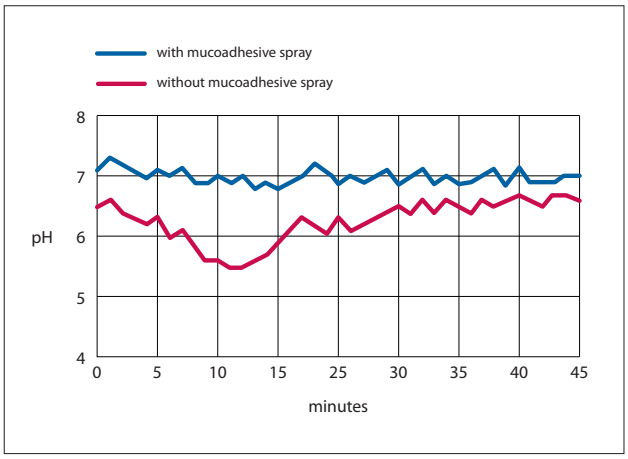

FIG. 1 *Progression of the means of the salivary pH with and without the sodium bicarbonate mucoadhesive spray during 40 minutes of monitoring.*

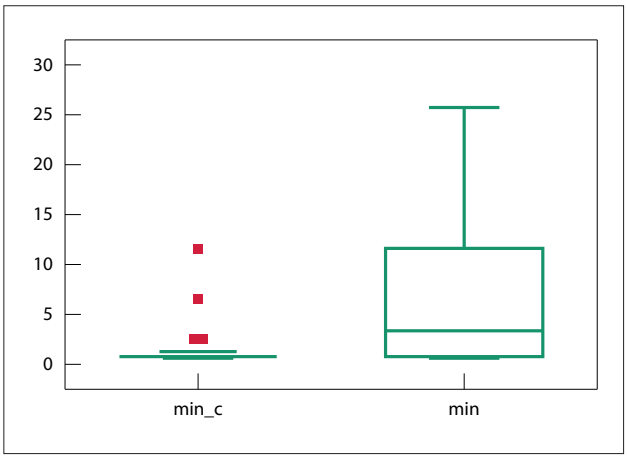

FIG. 2 *Distribution of the mean values of the time spans during which the pH was <6 with (min_c) and without the tested product (min).*

Mean pH without mucoadhesive spray	Mean pH with mucoadhesive spray	Significance
6,5	6,9	P=0,0016

TAB. 2 *Comparison of mean pH values obtained during 40 minutes of monitoring with and without the tested product.*

pH<6 without mucoadhesive spray	pH<6 with mucoadhesive spray	Significance
6,6 minuti	1,2 minuti	P=0,0001

TAB. 3 *Comparison of mean time spans, expressed in minutes, during which pH<6, in the 40 minutes of monitoring, with and without the tested product.*

plied that it was pleasant. After each time the pH was monitored, the pH meter software produced a graph showing the pH values, and processed the mean values recorded, the time span with values of pH<6 and the percentage of time in minutes, of the 40 tested, in which the pH had values of <6.
The results revealed unstable pH values, with continual fluctuations compared with the basal values. The data recorded during the 40 minutes of monitoring the salivary pH with and without the application of the mucoadhesive spray are reported in the summary table (tab. 1). A certain amount of individual variability was detected in the pH values recorded, and the initial values actually range between 5.9 and 7.5. The upwards or downwards pH variations showed different ranges for each subject, even though results were used that calculated as a mean minute by minute over the 40 monitored minutes, it was possible to obtain a graph of the progression of the mean salivary pH of all the subjects analyzed with and without the tested product (fig. 1). Tables 2 and 3 show the data relating to the comparison between the mean data with and without the application of the tested product. In Figure 2 we can observe the distribution of the means of the time spans during which pH was lower than 6 during the 40 minutes of monitoring the pH.

DISCUSSION

Salivary secretion presents a circadian rhythm which appears to be influenced by the antidiuretic hormone; it is known that the salivary flow rate decreases considerably during sleep (21). For this reason it was decided to conduct the tests during the morning and all subjects were tested after fasting for least 2 hours, in order to make the starting point as uniform as possible before stimulation with the glucose rinse.
The thin, flexible cable that connected the active point of the probe to the pH meter was positioned so that it exited the corner of the mouth. This allowed the salivary values of the pH to be continually recorded with minimal disturbance to the homeostasis and the salivary clearance of the oral cavity. Other authors have underlined the importance of respecting salivary clearance when studying pH, showing how the pH tends to drop and become more acidic when a surface in the mouth becomes dehydrated (22-24).
The individual variability encountered in the salivary pH values is a phenomenon that is already recognized in literature. The pH of the oral cavity shows mean basal values between 6.0 and 7.4 with no significant difference between the two sexes but with considerable differences with regard to the sample sites (25).

The data reported in the present studysupport these observations, and the monitoring of the pH began immediately after rinsing with the glucose solution and the initial values varied between 5.9 and 7.5 without the application of the tested product. This result can be interpreted by considering the different basal pH values between one subject and another and the different individual responses to the glucose challenge in relation to the acidophilic bacterial count and the buffering capacity of the subject's saliva (2). An increase in salivary pH values was observed when the buccal mucosa of the cheek was sprayed three times with the mucoadhesive spray (tables 2 and 3); this was noticeable in mean values over 40 minutes ($p=0.0016$) and also in the time spans at $pH<6$ ($p=0.0001$). These are clinically relevant variations as demineralization of the dental enamel occurs when the pH value nears 5.7. It is actually reported that the critical pH of dental enamel does not have a fixed value and varies for each individual also in relation to the different ions in the saliva, such as the calcium and phosphate ions which make up the hydroxyapatite of dental enamel (26). Fluoride transforms hydroxyapatite in fluorapatite which, being less soluble than hydroxyapatite, has the effect of reducing the critical pH. However we know that despite exposure to fluoride there are still large parts of the population of the Western world in which this is not enough to equilibrate the acidogenic challenge of diet (18, 19). Phenomena such as dental erosion and xerostomia are becoming markedly more prevalent (17). Furthermore, recent epidemiological research in Italy highlighted a prevalence of about 44% of children with experience of caries at the age of 12 years (27). So much work still needs to be done in order to effectively prevent these diseases of the oral cavity. Supporting the buffering capacity of saliva represents an approach that has still not been sufficiently studied. For example, Anderson et al. (8) and Igarashi et al. (11) have highlighted the value of adding sodium bicarbonate to saliva by means of chewing gum with the aim of controlling the drop in plaque pH after administering glucose. The results of our study agree with the data reported by the above mentioned authors. The mucoadhesive spray tested in this study kept the pH above the values considered to be harmful for dental enamel for the entire 40 minutes in which the pH was monitored. The mean percentage of time in which the pH values were below 6 changed from 16.6% to 2.9% when the mucoadhesive spray was applied (tab. 1, fig. 2). Therefore it appears that the mucoadhesive effect permits a gradual release of sodium bicarbonate and a prolonged increase in the buffering capacity of saliva. Further studies, which are already in progress, should confirm whether the obtained increase in salivary pH actually translates into a benefit with regard to the amount of hard tissue lost by dental enamel when attacked by acid. It should also be emphasized that the sample in this study was made up of young adults free from caries and dental erosion.

We may assume that the beneficial effect of supporting the buffering capacity of saliva could be even more noticeable in patients affected by these diseases.

The convenience of the mucoadhesive spray means that it is a very versatile tool, which the patient can apply at different times of the day for his/her personal use after consuming snacks, beverages or foods; returning pH values to levels in which the phenomena of demineralization have been controlled before carrying out mechanical oral hygiene practices could be particularly useful. An acid attack exerts stress on dental enamel and a certain amount of mineral tissue is expended to neutralize the acidity of the plaque. This observation agrees with the findings of Wiegand et al. (28) who upheld the value of waiting for an acid attack on dental enamel to finish before brushing teeth to minimize erosion. Observations about pH have also been made with regard to dentine hypersensitivity; therapeutic solutions have been proposed that have achieved effective results based on the increase of pH with dentifrices, solutions and gels containing arginine, a strongly basic amino acid (29). We may therefore assume that the product tested in this study could have a beneficial effect for dentine hypersensitivity; research is currently in progress to verify these observations.

Therefore there are real grounds for regarding sodium bicarbonate as an effective active ingredient when managing the prevention of caries even on a large scale; to this end it could be used in chewing gum or in sweets sold in supermarkets (30).

CONCLUSION

Supporting the buffering capacity of saliva represents an approach to the prevention of caries and dental erosion and to the treatment of xerostomia and dentine hypersensitivity that has still not been sufficiently studied. The product tested by us in this preliminary study significantly increased the salivary pH after a glucose rinse, and took it above the threshold values for dental enamel demineralization. We therefore believe it can be used as a preventive tool that will be useful for reducing the amount of mineral tissue lost following acid attacks on tooth surfaces. Further studies are currently in progress to verify the effects of the product on salivary pH and dental enamel in patients affected by with caries and dental erosion.

CONFLICTS OF INTEREST

None of the authors have financial conflicts of interest; this is an independent study and has not been supported by sources of financing.

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