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Research Article

Effect of Saline Mouthwash on the Oral Flora

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Summary

Mouthwash is the process of soaking the teeth, gums and mucosa of the mouth with a solution. Its purpose is to improve oral hygiene and to treat certain oral pathologies. The aim of this work is to evaluate the in vitro effect of salt water mouthwashes on oral flora. We conducted a cross-over clinical trial in the Bacteriology Department of the Central Laboratory of the Yaounde General Hospital. This study lasted 6 months, from November 2019 to April 2020. During the course of this work, we carried out convenience sampling. Each participant in our study received the same intervention (mouthwash) at a specific time and the effect was observed at regular intervals (H1 - H5). A period of 3 days (wash-out period) was observed between the interventions (mouthwashes with different saline solutions: 0.9%; 2%; 5.8% and 23%) in order to avoid carry-over effects. Gram staining was performed before the mouthwashes and 7 hours after the mouthwashes to determine the bacterial species affected by the mouthwashes.

We recruited 10 participants from whom we took 240 saliva samples. The sex ratio was 1:1. Our results showed that 2 minutes after mouthwash all solutions decreased the oral flora (e.g., 5.8% saline reduced the initial flora by half). The activity of the 0.9% saline was limited to this interval, while the homemade saline (2%) had an antibacterial effect on the oral flora that lasted for 3 hours. However, as the sampling continued only the 5.8% and 23% concentrations reduced the oral flora significantly. The 5.8% salt concentration had an antibacterial action of 5 hours while the 23% salt concentration had an action of 7 hours. Furthermore, we noted a more marked activity on gram positive bacteria. The 5.8% saline has a long-lasting efficacy and is better tolerated by the participants. The homemade saline can be prescribed for short term indications as it has an antibacterial action that lasts for 3 hours. The 23% saline should be avoided because of its pungent and irritating taste.

Keywords: Mouthwash; Saline Solution; In Vitro Study; Oral Flora

Introduction

Mouthwash is the process of soaking the teeth, gums and mucous membranes of the mouth with a solution. The purpose of this procedure is to improve oral hygiene and to treat certain oral pathologies [1]. Salt water, various herbal infusions and an alkaline solution of sodium bicarbonate have long been used in oral hygiene. But, due to the antiseptic properties of new solutions such as Chlorhexidine, Povidone Iodine, etc., these natural solutions are used less frequently.[1] However, due to the side effects of these solutions, natural solutions such as saline mouthwash would seem to be more advantageous. Indeed, Kim et al. demonstrated that salt water mouthwash (at specific concentrations) could improve oral hygiene. He also showed that a salt water mouthwash would decrease xerostomia, halitosis and significantly decrease the bacterial mass of the oral cavity [2]. We conducted this study with the aim of comparing the in vitro effect of different concentrations of saline solution to that of 0.9% saline on the oral flora; ultimately, we want to find the best alternative with less risk in oral care.

Methodology

We conducted a cross-over clinical trial over a period of 6 months from November 2019 to April 2020. This study took place in the bacteriology department of the central laboratory of the Yaoundé General Hospital. We selected 10 participants from among the Dental medicine students of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1. Participants had to meet 6 criteria: be aged 20-25 years in apparent good health; have good oral health; not have taken any medication that could modify the oral flora; not wear dentures; not perform any oral hygiene act 12 hours before the start of the clinical trial and have had a scaling within the last 6 months. We excluded any participant who was suffering from a disease that could affect the oral flora, neglecting oral hygiene, unavailable during the study period; Also excluded were those who took medication that could affect the flora; who ate less than 1 hour before the start of the sampling; who ate during the sampling period. We then obtained ethical clearance from the institutional ethics and research committee of the Faculty

of Medicine and Biomedical Sciences of the University of Yaoundé 1 and research authorization from the Yaoundé General Hospital. The information leaflet was explained and the informed consent form given to the participants. Mouthwashes were free of charge and the results of our clinical trial were given and explained to participants.

In the procedure, each participant in our study performed a mouthwash between 9:00 - 9:30am with 10ml of solution for 30seconds. Effects were observed at regular intervals during the day including 2 minutes, 1 hour, 3 hours, 5 hours and 7 hours after mouthwash. A period of 3 days was observed between each mouthwash in order to ensure that the flora was reconstituted ad integrum. On the saliva samples, gram staining was performed before the mouthwash and 7 hours after the mouthwash in order to determine any changes in the bacterial flora. Saliva samples were analyzed for bacterial colony count (viable plate count method) [3]. Saliva samples consisted of 1ml of unstimulated saliva collected in a dry sterile tube. We used 4 different concentrations of saline for our study (these concentrations were chosen according to the classification of bacterial halotolerance) [4]. These were 0.9% saline (0.16M); 2% saline (home-made preparation: 5mg of salt in 250ml of water) (0.34M) [5]; 5.8% hypertonic saline (1M); 23% hypertonic saline (4M). The preparation of our solutions was carried out with conventional table salt and sterile distilled water. The salt used had the following concentration: Sodium chloride: 97% min; Iodine: 100ppm; Anti-caking agent E-536: 10ppm; Calcium: 25mg/kg max; Magnesium: 150mg/kg max; Sulphate: 400mg/kg max; Insoluble: 0.01% max; Moisture: 0.1% max. We determined the molar concentration of each solution before use using the following formulae: molar concentration = number of moles (mol)/volume (l) and mole = mass (g)/molar mass (mol/g).

Data analysis

The data were recorded with Microsoft Excel. Differences between groups were determined by Student's paired-sample t-test; the repeated measures analysis of variance allowed us to compare the means obtained by paired samples. Probability values of $P < 0.05$ were considered statistically significant. However, the adjustment of the p-value with the Bonferoni correction was used due to the multiple statistics that were performed. We used SPSS 26.0 software to perform these different statistical tests.

Results

In this study we had 10 patients from whom we took a total of 240 saliva samples (24 samples per patient). The age of the patients ranged from 22 to 25 years, with an average of 23.6 years±0.96 in girls and 22.6 years±0.80 in boys. The bacterial load of our patients before mouthwash (H0) was different from each other as revealed in Table 1. Furthermore, this table shows the effect of the different mouthwashes on the oral flora at all different intervals H1 - H5. The action of the solutions is different but the differences are not statistically significant. The results of Gram-stained smears (Table 2) show a flora composition of 60-70% gram positive bacteria and 30-40% gram negative bacteria at H0. On analysis of the smears at H5 we observed a much greater reduction of gram-positive bacteria after mouthwash with the following solutions: saline 5.8% and saline 23%. During our clinical trial we collected the subjective assessments of the participants towards the different mouthwashes. This shows that:

Table 1: Effects of different saline mouthwashes on oral flora with time

	Mouthwash			
Time	0,9% saline	Homemade saline 2%	5,8% saline	23% saline
H0	56,5 ± 62,8	36,1 ± 12,7	36,2 ± 10,8	66,9 ± 105,5
H1	25,9 ± 15,4	26,9 ± 9,2	23,2 ± 15,6	33,4 ± 26,7
H2	86,1 ± 116	58,2 ± 92,8	32,1 ± 14,3	26,8 ± 11,7
H3	118,6 ± 129,1	35,6 ± 16,7	34,7 ± 10,7	34,2 ± 15,1
H4	42,7 ± 22,9	36,5 ± 9,7	27,7 ± 11,9	31,1 ± 9,1
H5	63,03 ± 70,6	35,7 ± 13,4	37,8 ± 13,9	40,2 ± 9,8
P value	0,097	0,438	0,003	0,317

All values above are multiplied by 10^7 and expressed in CFU/ml
H0: sampling before mouthwash; H1: sampling 2 minutes after mouthwash; H2: sampling 1 hour after mouthwash; H3: sampling 3 hours after mouthwash; H4: sampling 5 hours after mouthwash; H5: 7 hours after mouthwash

Table 2: Results of Gram-stained smears at H0 and H5

Time	Stains	0.9% saline	Home made saline 2%	5,8% saline	23% saline
H0	G+	69 ± 3,2	63 ± 4,8	64 ± 5,2	68 ± 9,2
	G-	31±6,8	37±5,2	36±4,8	32±0,8
H5	G+	66 ± 5,2	64 ± 5,2	53 ± 4,8	49 ± 8,8
	G-	34±4,8	36±4,8	47±5,2	51±1,2
P		0.081	0.726	<0,001	<0,001

In black: gram positive bacteria; in blue: gram negative bacteria. (Result in %)

H0: sampling before mouthwash; H5: 7 hours after mouthwash

a) All participants experienced a salty taste after the 0.9% saline and homemade saline mouthwashes. They described this taste as normal and usual.

b) Half of the participants reported that the 5.8% saline was very salty but still tolerable. While the rest of the participants found its taste equivalent to the solutions mentioned above.

c) The 23% saline was not tolerated by any of the participants during our clinical trial. All noted its pungent, aggressive and irritating taste which took time to subside.

Discussion

Mouthwashes are frequently prescribed by dentists. Some so-called 'comfort mouthwashes' are even available in supermarkets. All these commercial or medical solutions have progressively replaced an old solution that had excellent results, namely the saline mouthwash [2,6,7]. Indeed, the use of salt water mouthwash is mainly based on empirical considerations, which are based on the fact that a solution with a high salt content can have antibacterial properties. Often referred to as a "grandmother's solution", it is simple to make and its constituents are affordable, namely drinking water and table salt. The price of a kilogram of table salt is much cheaper than the price of a bottle of chlorhexidine, hydrogen peroxide or povidone iodine. In addition to its low toxicity, it would be advisable to return to this solution, which has been proven in the past [1,6]. In view of the scarcity of scientific data on this subject, we undertook this study to investigate the antibacterial effect of different concentrations of salt water on the oral flora. For this analysis we used different saline solutions: 0.9%, home-made saline (2%), 5.8% and 23%. These solutions were chosen according to the halotolerance classification of bacteria described by Vreeland [8]. Each solution corresponded to a class of bacteria in the halotolerance classification. The effect of these different solutions was compared to the effect of 0.9% saline.

General Characteristics of the Population

Our population consisted of young oral health students with little age difference due to their ease of understanding the study procedure, accessibility and similarity of oral flora [9,10]. The differences in flora between individuals are comparable to the results obtained in the work of Addy and Smith who worked at the University of Cambridge on students and administrative staff and the work of Shapiro et al who worked at the University of Bergen on administrative staff [9-11].

Effects of Mouthwash on Oral Flora

The results of our study showed that 2 minutes after mouthwash all solutions reduced the oral flora (e.g., 5.8% saline reduced the initial flora by half). Two reasons could explain this reduction: the first would be related to the physiological hyper-salivation that follows the mouthwash. This serous saliva, mainly from the parotid region, would dilute the oral flora and thus reduce the number of bacteria counted. The second explanation would be the immediate antiseptic effect of mouthwash on bacteria: saline solutions can cause cells to dry up and then fatally lose their cell structure and function. This bacterial cell depletion is especially observable in light halophiles, as they have very little capacity to adapt to a saline environment.

The 0.9% saline solution has a limited action in this range because it is isotonic for mucous membranes and bacteria. This result is similar to the work of Shapiro and Smith [10,11]. However, as sampling continued only two substances reduced oral flora significantly (saline 5.8%, and saline 23%). The first: the 5.8% saline reduced oral flora for 5 hours while the effect of the 23% saline was 7 hours. The effect of the 2% saline solution was 3 hours. These results suggest that these mouthwashes have a long-lasting effect on the oral flora. This is probably due to: The long-time taken by the salivary hypersecretion to dilute these high salt concentrations. Thus, the strong osmotic pressure exerted by a high Na Cl level is maintained. This will have a lasting effect on the growth and survival of the bacteria.

The long-lasting action of 5.8% and 23% saline could be due to their adhesion to the dental plaque. Indeed, the patients included in our study had not performed any oral hygiene act 12 hours before the beginning of the mouthwashes. This is sufficient time for plaque to form (30 minutes) [12]. It is likely that the 5.8% and 23% salts adhere to the glycoprotein matrix (exogenous acquired film) and thus destroy the plaque bacteria. It is also likely that the non-removal of salt-rich bacterial cell debris perpetuates the action of salt on plaque. Given that the concentration of Na+ in saliva increases with salivary flow to the point of reaching plasma

levels, it is likely that this concentration of Na+ combined with that obtained after salt water mouthwash increases the alkalinity of the medium and thus inhibits bacterial growth [13]. When comparing the action of these saline solutions with that of other mouthwashes described in the literature, it can be seen that these mouthwashes have a greater or equal effect (for example : Povidone iodine has an effect that lasts 1 hour according to Addy et al[9]; Triclosan and Hexeditin have an antibacterial effect that lasts 3 hours according to Jenkins et al, Shapiro et al and Tartaglia et al[11,14,15]; Acidified sodium chlorite and sodium lauryl sulphate have an effect that lasts 7 hours according to Yates et al and Jenkins et al[15,16] etc.) .)

Bacterial modification after mouthwash

After analysis of the results, we can observe that the solution that had the most effect on the oral flora was the 23% saline solution. This reduced the oral flora by half during the whole experiment (7 hours). However, the participants were bothered by its pungent, irritating and disgusting taste compared to homemade saline (2%) and saline 5.8%. This reaction can be explained by the hyperstimulation of the ionophores of the taste cells. Indeed, the Na+ cation is responsible for the salty taste; therefore, the mouthwash with 23% saline will cause an excessive stimulus discharge because sodium ion receptors are ubiquitous on the tongue [17]. These assessments, although subjective, lead to the conclusion that it would be advisable to turn to 5.8% saline, which is better tolerated and has a 5-hour antibacterial effect. However, for short term indications (3 hours), home-made saline can be used or prescribed. Examination of the Gram-stained smears showed us a flora composition of 60-70% gram positive bacteria and 30-40% gram negative bacteria at H0. On analysis of the smears at H7 we observed a much greater reduction of gram-positive bacteria after mouthwash of the following solutions: saline 5.8% and saline 23%. This observation would indicate that the 5.8% and 23% saline would be much more active on gram positive bacteria.

Conclusion

The 5.8% saline has a long-lasting efficacy and is better tolerated by the participants. The homemade saline can be prescribed for short-term indications as it has an antibacterial action that lasts for 3 hours. The 23% saline should be avoided because of its pungent and irritating taste.

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