



Comparative study of a stabilized 0.1 %chlorine dioxide with 0.2% chlorhexidine mouthrinse in inhibiting the formation of volatile sulphur compounds (VSC)

KEYWORDS

oral malodor, halimeter, VSCs, chlorine-di-oxide, chlorhexidine

Dr. Neetha J. Shetty

Associate Professor, Dept. of Periodontology, Manipal College of Dental Sciences, Manipal University Mangalore

Dr. David .K

Reader, Dept. of Periodontology, Manipal College of Dental Sciences, Manipal University , Mangalore

Dr. Kamala D. N

Research Co-ordinator, Group Pharmaceuticals Limited, Bangalore

Dr. Ramya Shenoy

Associate Professor, Department of Public Health Dentistry, Manipal College of Dental Sciences, Manipal University, Mangalore

ABSTRACT Background: Research has been done previously on the oxidizing property and the clinical efficacy of chlorine dioxide (ClO_2) on malodor. The aim of the present study was to compare the inhibitory effect of a mouthwash containing 0.1% ClO_2 with 0.2% Chlorhexidine mouthwash on morning oral malodor.

Materials and methods:

A randomized, double blind, crossover trial was conducted among 18 healthy male volunteers, who were divided into 2 groups. Group-1 subjects were instructed to rinse with the experimental mouthwash containing 0.1% ClO_2 and group-2 subjects were asked to rinse with 0.2% chlorhexidine, twice per day for 7 days. After a one week washout period, each group then used the opposite mouthwash for 7 days. At baseline and after 7 days, oral malodor was evaluated with halimeter which measures Volatile Sulphur Compounds (VSCs) in parts per billion. The clinical variables that were measured included the plaque and gingival indices.

Results and conclusion:

A decrease in the amount of VSCs was noted after a 7 day use of ClO_2 as well as with the use of chlorhexidine mouthwash, which were statistically significant, with greater significance after the use of ClO_2 . Also, a significant reduction in plaque scores was noted after the use of test mouthwash. The decrease in the amount of VSCs indicates a reduction in oral malodor.

Future research is needed to examine the long-term effects, as well as effects of ClO_2 on plaque accumulation and periodontal diseases in a well-defined sample of oral malodor patients and broader population samples.

Introduction

The etiology of halitosis is numerous, involving many intra- and extra-oral factors such as gingivitis, periodontitis, nasal inflammation, chronic sinusitis, diabetes mellitus, liver insufficiency, cirrhosis, uremia, lung carcinoma, trimethylaminuria and post nasal drip¹. Epidemiological researches have reported that around 87% of the bad breath cases have oral causes, whereas only 5-8% of the cases can be attributed to ear, nose and throat causes.²

Considering the relevance of the oral etiological aspects of oral malodor, recent studies have been designed to verify the real efficacy of some products such as toothpastes and mouthrinses containing antimicrobial agents that are claimed to produce a reduction on bad breath^{3, 4}. Not only have the antimicrobial capacity of these products, but also the oxidative biochemical effect on volatile sulphur compounds (VSCs) been investigated⁵.

The clinical use of mouthrinses containing chlorine dioxide has been reported to reduce oral malodor by the control of VSCs. Experimentally, the use of chlorine dioxide associated with chlorite anion has been shown to result in oxidative consumption of amino acids like cysteine and methionine, which are precursors of VSCs⁶. Thus, clinical use of this mouthrinse can be expected to reduce oral malodor by reducing concentrations of VSCs. Chlorine dioxide, a strong oxidizing agent, consumes oral substrates containing cysteine and methionine, thus preventing the production of VSCs. Since chlorine dioxide readily loses its activity, its stability

has been prolonged through a "stabilization" process, which converts chlorine dioxide to molecular chlorine dioxide at a low pH^{7, 8, 9}.

There is a lack, however, of controlled clinical trials conducted in order to prove the effectiveness of this formula. Thus, the aim of this study was to evaluate the inhibiting effect of a commercially available chlorine dioxide mouthwash (Fresh-clor) in comparison with 0.2% chlorhexidine mouthwash on VSC levels in a panel of healthy subjects.

Materials and methods**Patient Population:**

Eighteen periodontally healthy male subjects between 18 – 35 years of age were included in the study. Females, subjects with known medical disorders, smokers, subjects on antibiotic therapy and those who were on antimicrobial therapy for the last 3 months, were excluded from the study. Females were excluded as menstrual cycle and the hormonal changes that follow could affect oral malodor on the crossover design with one week washout.¹⁰

The subjects received verbal and written information about the study and signed consent forms to participate. An oral examination was conducted to assess oral status of the subjects prior to the study. At least 20 teeth that do not present gingival probing depths greater than 3 mm and gingival indices and plaque indices equal to 1 in more than 10% of the sites (considering 0 = no plaque and 1 = plaque present).

Subjects were asked to refrain from using commercial mouthwash, antibacterial tooth paste, tongue brush and dental floss.

All dental examinations were conducted by the one trained examiner for all subjects, both for baseline and for follow-up examinations.

Study design

This study is a randomized, controlled clinical trial of 18 volunteers divided into two crossover groups, performed in two experimental periods of 7 days. A 7-day washout interval was established between the treatment periods.

Test and control products

Test samples were commercial mouthrinse samples (Fresh-Clor) containing stabilized chlorine dioxide 0.1 %. Control samples consisted of 0.2 % chlorhexidine mouthrinse.

Experimental phase

Baseline data on dental plaque (PI¹¹) and gingival (GI¹¹) indices were recorded in order to exclude volunteers with periodontal disease, following the exclusion criteria. Group-1 Subjects were instructed to rinse 10 ml of the experimental mouthwash containing 0.1% chlorine di oxide for 30 seconds twice per day (after waking up and before sleeping) for 7 days and those in group-2, were asked to rinse with 10 ml of 0.2% chlorhexidine. During the washout interval, a control dentifrice was used with a new toothbrush to avoid any carry-over effect. In the second phase, after a week washout period, each group then used the opposite mouthwash for 7 days.

Morning breath evaluation

At the beginning and at the end of all experimental periods, VSC concentrations was recorded using a portable industrial sulphide monitor (Halimeter), using the technique established by Rosenberg *et al.*^{12,13} The data were recorded before rinsing at 9 a.m. (day 1), and twelve hours after the last rinse (day 7). The measurements were repeated three times for each subject. Before the morning measurements (9 a.m.) on day 1 and 7, the volunteers were refrained from toothbrushing, drinking, eating, gargling and using scented cosmetic products.¹⁴

Oral status assessments

Clinical assessments of Plaque Index and Gingival Index were performed on four sites (Buccal, lingual, mesial, distal) of the six key teeth (FDI tooth number 16, 12, 24, 36, 32, 44). Each of the sites was given a score from 0-3 depending on the severity of the gingival condition. All measurements were performed by the same examiner.

RESULTS

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS16.5). Means and standard deviations of the clinical indices were calculated, following which oral malodor scores between two mouthwashes were compared by the student 't' test. The difference between before and after rinsing scores at baseline and after 7 days were analyzed by student paired 't' test.

All 18 subjects completed the study. The oral status of subjects was as follows (mean \pm S.D.): mean number of mean periodontal sulcus depth, 2.0 ± 0.5 mm. There was no statistically significant difference in the oral conditions of the subjects in the two groups at the beginning of the study.

Subjects perceptions of the mouthwashes

Over the 7-day period, either the test or control mouthwash was used on 14 occasions.

The interval from last rinsing with the experimental mouthwash and the control mouthwash to assessment on subjects malodor was 9.00 ± 2.00 hours. There was no statistically

significant difference between the time intervals and examination with the two mouthwashes. Twelve subjects reported a "fresh breath feeling" after rinsing with the experimental mouthwash. On the other hand, only four subjects reported the same feeling with the control mouthwash. Thirteen subjects perceived they had a "reduced bad breath" after rinsing with the experimental mouthwash and three subjects reported the same feeling with the control mouthwash. With the experimental mouthwash, three subjects reported problems such as "does not foam and that of unpleasant taste". With the control mouthwash, most of the subjects reported of having an unpleasant taste.

Oral status evaluation

The results of the PI, GI at baseline and after 7 days is shown in the Table-1. With the test mouthwash used for 7 days, a statistically significant inhibition in plaque accumulation was evident compared with before rinsing ($p < 0.05$). With the control mouthwash used for 7 days on the other hand, no statistically significant inhibition was observed compared with before rinsing. Further, the mean score of the testgroup was significantly lower than the mean plaque score of the control group after 7 days. Although the mean score of GI reduced with the experimental mouthwash used for 7 days, there was no statistically significant difference compared with the GI value before rinsing.

Oral malodor assessments

Oral malodor assessments using halimeter is listed in table-1 and also show the same general trends. There was no statistically significant difference between the test group and the control group at baseline. Statistically significant improvements in reducing oral malodor occurred in the experimental group with ClO₂ mouthwash used for 7 days, compared with baseline scores ($p < 0.01$). The chlorhexidine mouthwash used for 7 days, on the other hand, also showed statistically significant difference in oral malodor compared with baseline scores, but when compared to the experimental group, it was not statistically significant.

DISCUSSION

VSCs have been shown to result from the bacterial putrefaction of proteins with sulfur-containing amino acids. Bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Treponema denticola*, and several species of other oral bacteria associated with gingivitis or periodontitis are known to produce large amounts of VSCs, which are malodorous. Periodontal disease causes high concentrations of VSCs in mouth air. The concentrations of methylmercaptan (CH₃SH) are significantly higher in patients with periodontal disease than those in orally healthy individuals¹⁶. Although the current study was conducted with orally healthy subjects, the results suggest that a mouthwash containing ClO₂ might reduce bacterial load and lower oral malodor in patients with periodontal disease.

In this randomized clinical trial, two mouthwashes were compared; one with ClO₂ and one without ClO₂, to investigate their effect on oral malodor. The results of this study demonstrate that rinsing with a mouthwash containing ClO₂, used over a 7-day period, was effective in reducing morning oral malodor and plaque scores in healthy subjects.

Chlorine dioxide (ClO₂) is a stable free radical which is readily soluble in water and can remain intact for considerable periods of time¹⁷. Previous studies have suggested that ClO₂ and ClO₂⁻ are chemically reactive oxidants with powerful reducing capacity on VSCs. Lynch *et al.* reported that reaction of L-cystein, a thiol compound which contribute substantially towards oral malodor¹⁸ with ClO₂, which contained 0.10% (w/v) ClO₂, the same as the experimental mouthwash used in this study.

Fusobacterium nucleatum (Fn) is considered a 'bridge-organism' that facilitates colonization of other periodontal

malodorous bacteria.^{19, 20} Moreover it is reported that Fn is an important bacterium in the development of complex dental plaque biofilms.²¹ Therefore the results of this study suggested that the reducing effects on morning oral malodor and plaque accumulation, was partially caused by reducing the counts of F. n. In this study, we found a significant effect on plaque accumulation using the ClO₂ mouthwash over a 7-day period, but which did not translate into a significant inhibitory effect on gingivitis.

Frascella tested the effectiveness of a ClO₂-containing mouthwash at different points of time for a total of 96 hours after rinsing.¹⁷ The results showed a significant improvement in oral malodor scores when the tested mouthwash was compared to a chlorhexidine control. The mean VSC concentration in the test group maintained its effective level at 9 hours after rinsing. In the present study, the interval from the last rinsing (before sleeping) with the experimental mouthwash to the assessment of subjects oral malodor was an average 9 (range 6.8 to 10) hours. We found that rinsing with ClO₂ dramatically reduced the halitosis on the morning of the assessment day. However after the one week washout period, the VSCs level returned to those at the baseline. It is suggested that residual ClO₂ remaining in the saliva or oral cavity may have reduced VSC level for at least about 9 hours. Further research should define the maximum effective time on VSC reduction and that trials should be conducted over longer time periods, 2-4 weeks or longer. Recently, a number of over-the-counter mouthwashes have been used in the treatment of oral malodor. Some of them merely mask malodor. The optimal mouthwash to treat oral malodor would be an antiseptic agent with proven long-lasting efficacy for reduction of oral malodor and VSC concentrations, with no or few side effects.

Chlorhexidine-containing mouthwashes inhibit formation of VSCs and are effective oral antiseptics with antiplaque and antigingivitis effects.²² But in our study, there was not much difference in the gingival index. Although CHX is considered the most effective oral antiseptic agent, Grgan et al reported using 0.2% alcohol-free CHX mouthrinse for 1 week caused more irritation to oral mucosa, greater burning sensation, and increased altered taste perception compared to the placebo rinse [14]. Listerine ® (Johnson and Johnson, New Jersey, USA), a mouthwash containing essential oils, may also have antiplaque and antigingivitis activity.²³ However, due to its high alcohol concentration taste sensation is reduced and it can cause oral pain.²⁴ Zinc ions are known to inhibit oral malodor but again, it had a taste problem.²⁵ Triclosan and cetylpyridium chloride (CPC) are antimicrobial agents widely used as antiseptic agents.²⁶ However, their clinical reduction of VSCs is still yet to be proved.

Sodium chlorite (NaClO₂), equivalent to ClO₂, is a non-toxic substance approved by the U.S. Food and Drug Administration (FDA) as an antimicrobial agent.²⁷ We found that ClO₂ not only was effective at reducing oral malodor, but also none of the volunteers complained about tongue stimulation or discoloring with the 0.10% ClO₂ (0.16% NaClO₂) mouthwash.

For some subjects, the taste and smell of this mouthwash were disagreeable. This may be resolved in new formulations which masks these problems.

A substantial proportion of healthy people complain of "morning bad breath". A proliferation of oral bacteria during sleep is responsible for the release of offending gases, most of which are VSCs. Healthy individuals who suffer from bad breath most often use mouthwashes containing several masking or antimicrobial agents. Therefore, recent papers have pointed out the relevance of comparative studies to verify the efficacy of the mouthwashes on "morning bad breath" in healthy subjects.^{28, 29} Most of the former studies on mouthwash used healthy subjects who never complained about oral malodor, often lacked an adequate control and were known effective mouthwashes containing CHX.^{30, 31} Additional research should also be conducted in broader population samples, also including females. Nonetheless, in this explorative study, the oral malodor score was improved using the ClO₂ mouthwash. Therefore, the mouthwash clearly demonstrated an anti-malodor effect on morning breath, potentially without any measurable side effects in healthy subjects.

CONCLUSION

In this study, the results showed that a mouthwash containing ClO₂ improved morning bad breath measured with the halimeter in healthy subjects. Also, ClO₂ mouthwash used over a 7- day period was effective in reducing plaque accumulation. However, future studies are needed to examine more long-term effects of the mouthwash in halitosis patients and broader population samples.

Table 1 - Intergroup and Intragroup comparison of mean value of Plaque Index, Gingival Index, VSC'S

	Group	Baseline value	7 days after	t value	P value
Plaque Index	Group I	0.69±0.32	0.32±0.21	5.24	0.000*
	Group II	0.53±0.28	0.45±0.29	1.08	0.295
		P=0.13	P=0.04*		
Gingival Index	Group I	0.55±0.27	0.55±0.27	0.53	0.601
	Group II	0.66±0.33	0.53±0.41	1.30	0.210
		P=0.28.	P=0.35		
VSC's	Group I	193.72±85.76	138.72±80.82	4.07	0.000*
	Group II	207.78±96.71	141.89±98.67	3.27	0.004*
		P=0.64	P=0.05*		

*p<0.001

Conflict of interest: Nil

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