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Dental Health

ZINC AND CHLORHEXIDINE MOUTHWASH

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Zn and CHX mouthwash effective against VSCs responsible for halitosis for up to 12 hours

Per S. Thrane[1], Grazyna Jonski[2], Alix Young[3] and Gunnar Rölla[2]

Abstract

Objectives: The objective of this study was to explore the duration of effectiveness of a mouthwash combining zinc and chlorhexidine on morning breath odour in subjects without periodontal disease.

Methods: Nineteen healthy volunteers (14 females and 5 males) participated in this study. Volatile sulphur compounds (VSC: H2S and CH3SH) were measured in mouth gas samples 12 hours after using a mouthwash (SB12®)*† combining 0.3% zinc (Zn) acetate and 0.025% chlorhexidine diacetate (CHX) or after water as a negative control. During each test period the participants refrained from oral hygiene, eating or drinking. VSC measurements were performed by gas chromatography, each subject serving as their own control. A cysteine challenge was also used.

Results: The results showed that the Zn + CHX mouthwash had a significant VSC-inhibiting effect compared to water even after 12 hours with a mean reduction of more than 70% (H2S: 73.55 ± 7.70%, $p < 0.05$, CH3SH: 74.03 ± 5.52%, $p < 0.05$).

Conclusions: A mouthwash containing low concentrations of Zn and CHX effectively inhibited oral VSC production for over 12 hours, both with and without cysteine challenge. This excellent duration of efficacy is likely to be due to a synergistic effect of Zn and CHX on VSC.

Key words: halitosis; mouthwash, VSC, H2S; CH3SH; chlorhexidine; zinc

*SB12® was kindly supplied free of charge by the producer Antula Healthcare, Stockholm, Sweden † SB12 is also known as MyPro12 in some European Markets

Introduction

Malodorous breath, or halitosis, affects many people occasionally, particularly in the form of unpleasant morning breath.[1,2] However, various oral conditions can lead to a more chronic, persistent halitosis. This is often treated or prevented by use of antibacterial mouthwashes. However, high concentrations of antibacterial agents, particularly chlorhexidine, in mouthwashes can cause unwanted side-effects including tooth discolouration, mucosal irritation and taste disturbance.[1,2,3] Unfortunately, these higher concentrations are usually necessary for effective control of halitosis.[1,2] A variety of different antibacterial mouthwash formulations has been developed in recent years, with the aim of obtaining efficacy against halitosis without unwanted side-effects.[1-11]

Volatile sulphur compounds (VSC), such as hydrogen sulphide (H_2S) and methyl mercaptan (CH_3SH), are reported to be responsible for 90% of the odour of halitosis, although other volatile compounds may also be involved.[12-15] The VSCs associated with halitosis may also be involved in the pathogenesis of periodontitis.[16-19] Methyl mercaptan has been shown to inhibit epithelial growth and proliferation, increase the degradation of collagen and inhibit the protein synthesis of fibroblasts.[20-23] This may contribute to the breakdown of periodontal tissue, creating a vicious circle resulting in increasing severity of both periodontal disease and halitosis.

Volatile sulphur compounds are responsible for 90% of the odour of halitosis

The current professional approach to this common problem is mainly mechanical, based on root scaling. Innovative new approaches to periodontal disease and its consequences, such as halitosis, are clearly needed.

The production of VSC can be studied by inducing halitosis in healthy individuals using a cysteine rinse, as in the method developed by Kleinberg and Codipilly.[2,24] This method, which measures the capacity of residual microbes to produce VSCs, is used by a number of investigators and avoids the various complicating factors associated with periodontal disease.[2] It involves administering a standardised quantity of cysteine, a non-volatile sulphur-containing substrate, into the oral cavity. This leads to the production of VSCs (mainly H_2S) by anaerobic bacteria, which are typically concentrated in deep crypts at the back of the tongue and in periodontal pockets.[11] The provision of cysteine, a known substrate, should activate any oral bacteria capable of producing VSCs so this method should be fully representative of bacterially-induced

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halitosis, even in healthy volunteers.[24] Use of this methodology also enables each test subject to serve as their own control, providing reliable results even with a limited number of subjects with large individual variations in VSC production capacity. Although oral malodour can be measured in various ways, gas chromatography (GC) is an objective, specific and sensitive method for measuring volatile sulphur compounds in gas samples taken directly from the mouth in a research setting.[8,16,25]

The objective of the present study on morning breath odour was to study the duration of action in reduction of VSCs of an aqueous solution combining zinc and chlorhexidine in low concentrations. The possible side effect of tooth discolouration was also examined in a 4-week follow up study.

Material and methods

Test solution

The mouthwash tested in this clinical experiment contained a combination of low concentrations of zinc ions (0.3%) and chlorhexidine (0.025%)(Zn + CHX). This has previously been shown to be a most effective formula for inhibiting VSCs.[28,33]

Test subjects

Nineteen healthy volunteers (14 females and 5 males, mean age 31 yr, range: 22-79 yr) recruited at a Norwegian Dental Faculty participated in the study. They had no conflicting medical history or medication and participated with informed consent. All received an explanation of the protocol, which had previously been approved by The National Committees for Research Ethics in Norway.

Clinical protocol

The test period spanned 5 days (Figure 1). At 9 pm on Day 1 each test subject was asked to rinse their mouth with 5 ml water and refrain from any tooth brushing, eating or drinking until base-line control VSC levels were recorded the next day at the clinic at approximately 9 am. Then, after a 2-day break, the subjects rinsed their mouth at 9 pm on day 4 with 5 ml of the test rinse (Zn + CHX) and again refrained from oral hygiene measures or consumption of food or drink until VSC measurements were recorded at approximately 9 am on Day 5. When arriving at the clinic in the morning of days 2 and 5 the test subjects were asked to keep their mouth closed for 90 seconds (s), after which VSC levels in mouth gas samples were measured in a standardised way as described below. Immediately thereafter, the participants rinsed with a standard amount of cysteine solution. A second VSC analysis followed.

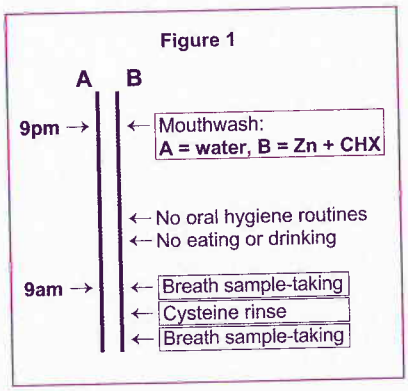

Figure 1: Experimental design. Timelines (A and B) showing the order in which the sequence of mouthwash, cysteine challenge rinsing and taking of breath samples occurred.

The difference between the VSC values obtained 12 hours (h) after rinsing with water and after rinsing with Zn + CHX was considered to be an effect of the test rinse. The two test periods were identical except for the nature of the mouthwash, so that the participants served as their own controls.

Rinsing with Zn + CHX significantly reduced the amounts of both H_2S and CH_3SH in all test subjects

Cysteine rinsing

The Kleinberg and Codipilly cysteine challenge model was used for inducing oral malodour in the subjects.[24] Test subjects rinsed for 30s with 5 ml of a 6 mM solution of L-cysteine (Sigma Chemical Co., St Louis, MO) at pH 7.2. Immediately following rinsing and expectoration of the rinse, subjects kept their mouth closed for 90s before mouth gas samples were taken for analysis. Cysteine challenge is a measure of the potential capacity of oral bacteria to produce VSCs.

VSC-analysis

Mouth gas samples were aspirated directly into a 6-ml sample loop connected to the injector of a gas chromatograph (GC-14B gas chromatograph, Shimadzu, Japan) using a mouthpiece as previously described.[7] A Teflon column (366 x 0.32 cm, packed with 5% polyphenol ether -0.05% phosphoric acid on 40/60 mesh Chromosorb T) was used with the following specifications: temperature 70°C, nitrogen gas flow 32 ml min^{-1}, hydrogen gas flow rate 125 ml min^{-1} and air flow rate 43 ml min^{-1}, together with a flame photometric detector.[16]

By using a 6ml sample loop, we were able to measure CH_3SH as well as H_2S directly in mouth gas with and without cysteine challenge after 12 hours.

Discolouration follow-up

Ten of the original study participants were followed up in order to see if longer-term daily use of the test rinse resulted in tooth discolouration. The ten subjects used the test rinse, 10 ml for 1 minute twice a day for 4 weeks. Clinical photos were taken of the subjects' teeth, both prior to and at the end of the 4-week follow-up period. The subjects did not receive any professional tooth cleaning prior to the start of this experiment. Tooth colour was assessed using the Vita scale.

Statistical analysis

The concentrations of H_2S and CH_3SH in breath samples were registered and calculated as AUC (area under chromatogram curve) by the GC software (EZStart v. 7.2.1 SP1, Shimadzu Scientific Instruments, Inc). The results for rinsing with water (control) and with the test rinse were compared, both with and without cysteine challenge. Comparisons were performed as a two related-samples test (Wilcoxon Signed Ranks Test) for both H_2S and CH_3SH levels. The differences between raw data from the base-line control measurements and measurements after the test rinse were calculated as percent reduction of oral H_2S and CH_3SH formation for each of the test subjects. The statistical package SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for all analyses.

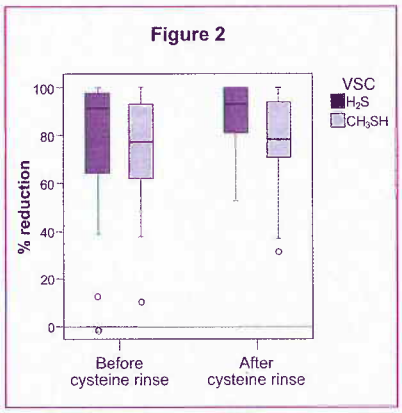

Figure 2: Boxplot-and-whisker plot of the results showing the mean percent reduction of VSC formation in mouth gas samples obtained 12 hours after the mouthwash with Zn + CHX. The lines within the boxes indicate the medians. Top and bottom boundaries of each box show 75th and 25th percentiles, respectively. Whiskers indicate the maximum/minimum points. An "o" indicates an outlier.

Results

Rinsing with Zn + CHX significantly reduced ($p<0.05$) the amounts of both H_2S and CH_3SH in all test subjects after 12 hours, in comparison with rinsing with water (H2S: 73.55% ± 7.7%, CH_3SH: 74.03% ± 5.52 %). The baseline VSC levels obtained showed significant individual variation. H_2S and CH_3SH levels were also analyzed at the 12 hour time point following cysteine challenge in all test subjects in both test periods. VSC levels after the cysteine rinse were significantly reduced ($p<0.05$) after Zn + CHX compared to water (H2S: 86.96% ± 3.83%, CH_3SH: 77.88% ± 4.61%) with less individual variation. The results are summarised in Tables 1 and 2 and Figure 2.

Discolouration follow-up

No tooth discolouration or other unwanted side effects (mucosal lesions or taste disturbance) were observed in any of the participants in the initial overnight study. Over the four weeks of the follow-up study, there was no change from baseline on the Vita scale for tooth colour in any of the 10 subjects using the test rinse daily, as confirmed by clinical photographs.

Discussion

Close correlations have been observed between assessments of halitosis by organoleptic methods, by use of a Halimeter® and by objective measurements of VSC level by GC.[12,14,16,34,35] This demonstrates the relevance of VSC measurements in the assessment of oral malodour. Moreover, both the total VSC levels and the H_2S/CH_3SH ratio have been shown to contribute to the quality of halitosis.[16] Thus, GC measurement of VSCs can be considered as valid and of direct significance for oral malodour. Experimental data, furthermore, clearly demonstrate that both zinc and chlorhexidine inhibit halitosis as well as VSC formation.[8] Zinc ions (Zn++ in aqueous solutions) interact with the sulphur in the substrate or in precursors of VSC oxidising sulphydryl groups to form insoluble sulphides.[8,21] In addition, zinc ions directly inhibit thiol proteinase activity related to VSC production.[4]

The combination of zinc and chlorhexidine has previously been shown to have a greater anti-VSC effect than that of either zinc or chlorhexidine alone and a prolonged VSC-inhibitory effect - over a 9 hour period.[11] The present study was designed to assess the efficacy over a longer period of 12 hours. Assessing efficacy overnight is considered to be a stringent test since the levels of VSC are generally at a maximum on awakening (morning breath) and tend to be lower and more variable during the day.

In fact, levels of VSC have a diurnal variation, rising overnight and peaking on first waking. Saliva is a key element in reducing VSCs, secondary to washing out the bacteria in the oral cavity. During the night, salivary production is greatly reduced, with a consequent increase in both the number of residual microbes and their metabolic rate.[1,2] The metabolic activities of these bacteria on tongue biofilms, plaque and other substrates in turn produce increasing levels of VSCs throughout the night, resulting in morning bad breath. Morning oral hygiene will decrease VSC levels which then start to rise until the person eats or drinks.[4] Contrary to general expectation, eating and drinking either reduce the levels of VSCs or have no short term effect.[37] Levels of VSC slowly rise between meals but are

Table 1

Mouthrinse 12 hours before sample taking	Prior to cysteine rinse			Following cysteine rinse		
	H_2S	Percent reduction vs. control		H_2S	Percent reduction vs. control	
Agent	*AUC*	*%*	*± SE*	*AUC*	*%*	*± SE*
H_2O (control)	429342.21			10311456.00		
Zn + CHX	37611.97	73.55	7.70	903125.73	86.96	3.83
Wilcoxon Signed Ranks Test	$p < 0.05$			$p < 0.05$		

Table 1: Mean raw data, calculated % reduction and statistical significance showing the 12 hour-long lasting effect of Zn + CHX rinse on oral H_2S production. AUC = Area under chromatogram curve, SE = Standard Error, H_2S = Hydrogen sulphide.

Table 2

Mouthrinse 12 hours before sample taking	Prior to cysteine rinse			Following cysteine rinse		
	CH_3SH	Percent reduction vs. control		CH_3SH	Percent reduction vs. control	
Agent	*AUC*	*%*	*± SE*	*AUC*	*%*	*± SE*
H_2O (control)	1560492.50			100211.05		
Zn + CHX	5451.00	74.03	5.52	14460.00	77.88	4.61
Wilcoxon Signed Ranks Test	$p < 0.05$			$p < 0.05$		

Table 2: Mean raw data, calculated % reduction and statistical significance showing the 12 hour-long lasting effect of Zn + CHX rinse on oral CH_3SH production. AUC = Area under chromatogram curve, SE = Standard Error, CH_3SH = Methyl mercaptan

unlikely to reach the overnight level.

A study conducted overnight should therefore give a more accurate and consistent assessment of the effects of a mouthwash on breath odour over a 12-hour period than a daytime study in which eating or drinking may complicate the interpretation of the results.

The results of this overnight study confirmed a more than 70% reduction in the VSC levels 12 hours after having rinsed with the test combination compared with rinsing only with water. The authors have also performed a small pilot study over an even longer time period. The Zn + CHX test solution continued to exert its effect from 12 hours to 16 hours after rinsing, reducing both H_2S and CH_3SH levels vs. water (unpublished results).

Chlorhexidine is known for its prolonged retention in the mouth and is considered to be the most efficient plaque inhibiting agent available at present.[26,27,29,36] However, the concentration (0.2%) of this antibacterial agent used in many commercial formulations is usually much higher than the 0.025% concentration used in this study. The higher concentration of chlorhexidine has been associated with local side effects.[3,36,38] No side effects have so far been observed after using the low 0.025% chlorhexidine concentration in combination with 0.3% zinc acetate solution. This may be due to a specific and synergistic mode of action, as suggested in a previous study and also described in more detail below.[33]

The retentive properties of chlorhexidine appear to be conserved even when it is given in low concentration. Zinc has also been shown to be retained in the mouth for 2-3 hrs but this cannot account for the > 12-hour effect of the combination.[2,6,7] The reduced nocturnal saliva flow may have allowed a more prolonged retention of the test rinse and its subsequent preventive effect against morning breath odour. The significant synergistic effect of the two agents in such low concentrations suggests that the mode of action may be different from that of zinc and chlorhexidine used alone.[8, 36, 39]

Chlorhexidine and related antibacterial agents are strong denaturating agents which can split disulphide bonds.[38] Since oral bacteria mainly contain desulphydrases, splitting of disulphide bonds would be beneficial.[11] A new hypothesis is that when zinc and chlorhexidine are used in combination against halitosis, there is a two step mechanism specifically directed against VSC production.

Firstly, chlorhexidine splits the disulphide bonds (-SH). Subsequently, zinc ions bind to the released sulphur (S-), resulting in insoluble and non-odorous zinc-sulphides that are partly spat out with the rinse and partly swallowed.[8,11] In this way, the sulphur gases (VSCs) causing bad breath are transformed to virtually insoluble non-odorous sulphides that are removed from the mouth. The splitting of disulphide bonds could, moreover, provide an explanation for the long-lasting effect of this mouthwash. In the current study, both H_2S and CH_3SH values were obtained directly from mouth gas using the GC. A combination of long test period (12 hours) and more sensitive equipment (GC) allowed us to measure oral VSC production both with and without cysteine pre-challenge. CH_3SH, although less abundant than H_2S, has been shown to have a more intense odour, as well as being more directly implicated in the pathogenesis of periodontitis. Obtaining reliable CH_3SH values thus seemed particularly important.

Conclusions

A mouthwash containing a combination of zinc and chlorhexidine in low concentrations is a very efficient inhibitor of intra-oral VSC formation and so greatly reduces the problem of morning breath odour. This efficacy lasts for 12 hours or more with no apparent side-effects. A new hypothesis suggests that this might be due to a synergistic effect of zinc and chlorhexidine acting directly on bacterial VSC production capacity via a two-step mechanism. The low concentrations of the active ingredients involved suggest that the antibacterial effect of the rinse is probably less important with regard to preventing breath malodour. Further studies are needed to substantiate this hypothesis.

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