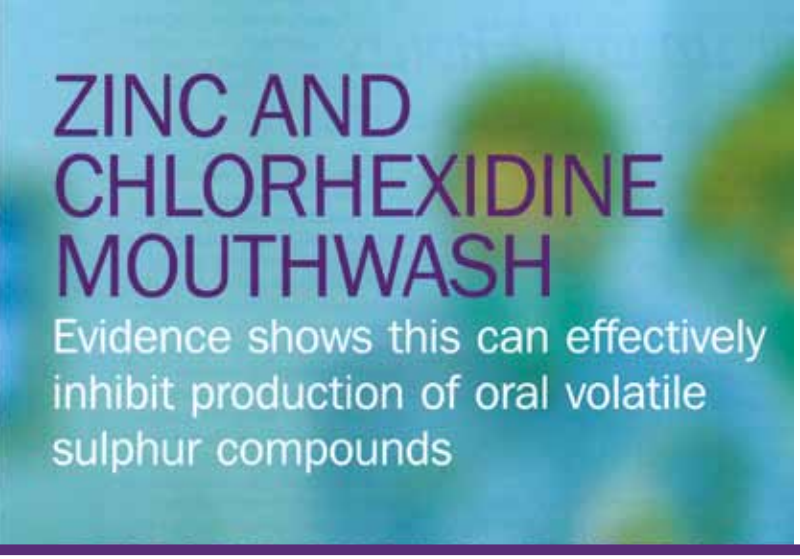

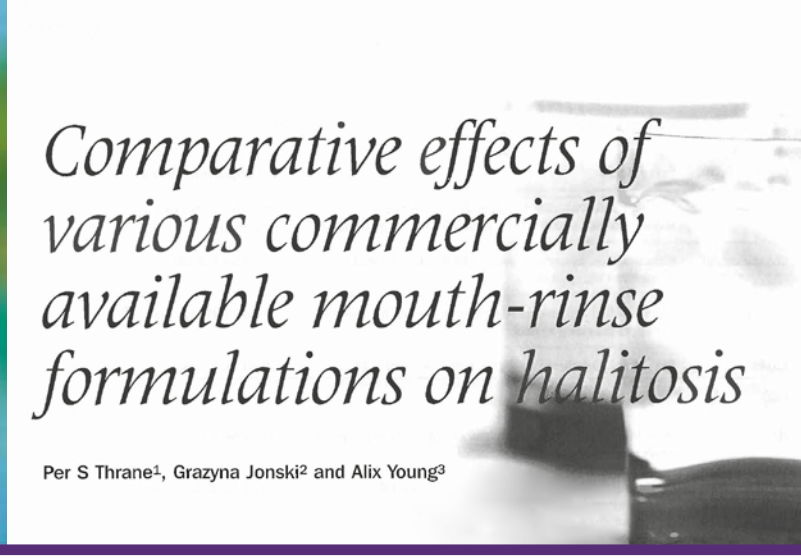

Dålig andedräkt
– Information och kliniska studier

ORIGINAL ARTICLE

Comparative effects of various commercially available mouthrinse formulations on oral malodour

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A New Mouthrinse Combining Zinc and Chlorhexidine in Low Concentrations Provides Superior Efficacy Against Halitosis Compared to Existing Formulations: A Double-Blind Clinical Study

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Dålig andedräkt (halitosis)

Dålig andedräkt bildas i 9 av 10 fall av bakterier i munhålan. Bakterierna i munnen finns runt tänder och i tandköttsfickor men också i gropar på tungans bakre del. Alla människor har dessa bakterier i munnen och därför kan alla också få dålig andedräkt. Bakterierna bryter ner matrester till illaluktande gasformiga svavelföreningar, som man sedan uppfattar som dålig andedräkt.

Svavelföreningarna kallas för VSC (Volatile Sulfur Compounds) vilket står för flyktiga, reaktiva svavelföreningar. VSC består av gaserna vätesulfid H_2S, metylmerkaptan CH_3SH och dimetylsulfid (CH3)2SH. Metylmerkaptan är den komponent av VSC som luktar mest illa även i mycket små mängder. Det är alltså metylmerkaptan man vill eliminera i första hand.

Zinkacetat

Zinkacetat är den zinkform som bekämpar VSC bäst[1]. I synnerhet så binder zink vätesulfid. Zinkjoner i en lösning interagerar med svavel och bildar olösliga sulfider som inte luktar. Zink i sig själv eliminerar vätesulfid men verkar inte lika effektivt på metylmerkaptan och dimetylsulfid. Därför behövs klorhexidin.

Klorhexidin

Klorhexidin bryter ned svavelgasmolekylerna så att zink lättare kommer åt att reagera med svavel så att olösliga sulfider bildas. Vad gäller metylmerkaptan så är svavel så hårt bundet i denna gas att zink i sig själv inte kommer åt att binda svavlet. Men eftersom klorhexidin spjälkar upp metylmerkaptan och dimetylsulfid, medverkar tillförsel av klorhexidin till att dessa två gaser spjälkas upp så att zinket kan bilda olösliga sulfider som inte luktar.

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1 Thrane PS, Young A, Jonski G, Rölla G. A new mouthrinse combining zink and chlorhexidine in low concentrations provide superior efficacy against halitosis compared to existing formulations: a double blind clinical study. J Clin Dent 2007; 18 (3): 82-87.

SB12 - dokumenterad effekt mot dålig andedräkt i 12 timmar[2]

SB12 är ett munvårdande medel som motverkar dålig andedräkt. SB12 har testats i flera vetenskapliga studier som finns att studera närmare i detta kompendium. SB12 har studerats på testpersoner både med och utan dokumenterad halitosis (dålig andedräkt).

SB12 neutraliserar och hämmar uppkomsten av de 3 svavelgaser som orsakar dålig andedräkt:

- Metylmerkaptan
- Dimetylsulfid
- Vätesulfid

För att motverka dålig andedräkt är det viktigt att samtliga 3 svavelgaser elimineras i utandningsluften.

SB12 munvårdande medel

- Patenterad sammansättning av zinkacetat (0,3%) och klorhexidin diacetat (0,025%) i låga koncentrationer
- Motverkar dålig andedräkt i 12 timmar genom att neutralisera och hämma de svavelgaser som orsakar dålig andedräkt.
- Studier visar att låg koncentration zinkacetat och låg halt klorhexidin diacetat ger en långvarig effekt[2]
- Tillverkas i Sverige
- Innehåller fluor

2 Thrane et al. Zn and CHX mouthwash effective against VSCs responsible for halitosis for up to 12 hours. Dental Health (2009)

Översikt kliniska studier SB12

Studie		Titel	Resultat
Rölla	2003	Combined effects of zion ions and cationic antibacterial agents*	Zink och klorhexidin verkar synergistiskt mot dålig andedräkt
Thrane	2007	A New Mouthrinse Combining Zn and CHX in Low Concentrations *	SB12 är överlägsen 5 konkurrerande produkter
Thrane	2009	Zn and CHX mouthwash effective against VSCs responsible for*	SB12 motverkar dålig andedräkt i minst 12 timmar
Thrane	2010	Comparative effects of various commercially available mouth-rinse*	SB12 är överlägsen 7 konkurrerande produkter
Greenman	2011	Comparative effects of various commercially available mouth*	SB12 är överlägsen 4 konkurrerande produkter
Erovic-Ademovski et al	2011	Comparison of different treat-ment modalities for oral halitosis	SB12 är överlägsen placebo hos patienter med dokumenterad halitosis

International Dental Journal (2003) 53, 237-242

Combined effect of zinc ions and cationic antibacterial agents on intraoral volatile sulphur compounds (VSC)

A. Young, G. Jonski and G. Rölla
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Objective: Volatile sulphur compounds (VSC) are major components of oral malodour. As both zinc ions and cationic antibacterial agents inhibit the formation of oral VSC, this study aimed to determine whether these agents combined have synergistic anti-VSC actions. **Methods:** Baseline oral VSC measurements of mouth air from 10 volunteers following cysteine rinsing (6mM, pH 7.2) were obtained using gas chromatography (GC). Subjects rinsed for 1 min with 10ml of the test solutions, 0.3% zinc acetate (Zn), 0.025% chlorhexidine (CHX), 0.025% cetyl pyridinium (CPC), and the combinations Zn+CHX and Zn+CPC. Cysteine rinses were repeated at 1h, 2h and 3h and VSC measurements recorded. Three subjects rinsed with the Zn+CHX combination and fasted for 9h, undergoing cysteine rinses and VSC measurements at 3h intervals. 10µl of the test solutions were also added to 1ml aliquots of human whole saliva (n=8). Following incubation at 37°C for 24h VSC levels in the saliva headspace were measured by GC. Inhibition of VSC formation and the fractional inhibitory index indicating synergy were calculated. **Results:** Zn+CHX mouthrinse had a synergistic anti-VSC effect, and was effective for at least 9h. Zn+CPC mouthrinse was less effective. Both combinations showed a synergistic inhibiting effect *in-vitro*. **Conclusions:** Synergy between Zn and the antibacterial agents confirms different mechanisms of operation.

Key words: Volatile sulphur compounds, oral malodour, zinc ions, cationic antibacterial agents, periodontal disease

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It is well established that the majority of cases of oral malodour are caused by Gram negative, anaerobic bacteria located in the crypts at the back of the tongue, or in periodontal pockets[1–3]. These bacteria are proteolytic and produce volatile sulphur compounds (VSC) by catabolisation of organic substrates, in particular cysteine[4]. The VSC have an unpleasant odour even in extremely low concentrations, and are the major component of oral malodour[1,5,6]. The main VSC are hydrogen sulphide and methyl mercaptan, but small amounts of dimethyl sulphide may also be present[1].

It is also well established that VSC are able to penetrate the tissues in periodontal pockets[7], that they have a direct deleterious effect on the synthesis of proteins in gingival fibroblasts[8], and that for these reasons VSC may be important in the aetiology of periodontal disease[7–10].

It is furthermore known that certain metal ions, in particular zinc, can be used to reduce or inhibit oral malodour, and that along with other metal ions, zinc inhibits the formation of VSC[8,11–13]. There may be several mechanisms involved:

- The zinc ions (in aqueous solutions or as dissolvable tablets) interact with the sulphur in the

substrate or in precursors of VSC, forming insoluble sulphides, since zinc has an affinity for sulphur and oxidises sulphydryl groups[7]

- Heavy metal ions such as zinc directly inhibit thiol proteinase activity related to VSC production[11].

Certain antibacterial agents such as chlorhexidine or cetyl pyridinium may also inhibit oral malodour and VSC formation[14,15]. In beagle dogs, zinc ions have been shown to enhance the plaque-inhibitory effects of cetyl pyridinium chloride[16]. A similar enhancement of the effect of mouthwashing with chlorhexidine was shown with zinc in humans[17]. If zinc ions and cationic antibacterial agents operate by different mechanisms with regard to oral VSC inhibition, it is conceivable that the combination of these agents may also provide an enhanced or synergistic anti-VSC effect. The aim of the present study was to examine this concept. The hypothesis to be tested was thus that zinc and a cationic antibacterial agent have synergistic effects when combined in aqueous solution and used as a mouthrinse to inhibit oral VSC formation.

Materials and methods

Test solutions

The solutions tested included 0.3 per cent zinc acetate 2-hydrate (Zn) (Reidel-deHaën, Germany), 0.025 per cent chlorhexidine diacetate monohydrate (CHX) (Fluka Chemie, Switzerland), 0.025 per cent cetyl pyridinium chloride monohydrate (CPC) (Sigma-Aldrich, Germany) and the combinations: 0.3 per cent zinc acetate + 0.025 per cent chlorhexidine (Zn+CHX), and 0.3 per cent zinc acetate + 0.025 per cent cetylpyridinium chloride (Zn+CPC). All solutions were made with de-ionised water.

Mouth rinse experiments

Collection of samples and VSC analysis of mouth air

Test subjects consisted of ten volunteers (4 males, 6 females, aged 30 to 72 years) recruited from the staff at the Dental Faculty, University of Oslo. The volunteers did not complain of oral malodour and had no obvious medical history that could relate in any way to oral malodour. All test subjects took part in the experiments with informed consent, after having received an explanation of the protocol approved by an ethics committee. On test days, the subjects were instructed to refrain from their normal oral hygiene routine following breakfast and present at the clinical research laboratory at 9.00am. The cysteine challenge model according to Kleinberg and Codipilly[18] was used for inducing oral malodour in the subjects. This involved the subjects rinsing for 30s with 5ml of 6mM L-cysteine solution (pH 7.2) (Sigma Chemicals, USA). Subjects then kept their mouth closed for 1 min 30s, after which mouth air samples were taken (baseline/control measurements).

Mouth air samples were aspirated using a 10ml syringe connected to the outlet of the auto-injector, and analysed for VSC directly in a gas chromatograph as described below. Immediately after this procedure, the subjects rinsed for 1 min with 10ml of one of the test solutions. Cysteine rinsing and mouth air analyses were repeated at 1h, 2h and 3h after rinsing with the respective solutions. Although the study was not double blind, the different test solutions were given at random to the test subjects on different days without the subjects knowing which mouth rinses they were using.

In a second mouth rinse experiment, three healthy persons (one female aged 41 years and two males aged 45 and 73 years) were used as test subjects to examine the long-term effect of a single mouth rinse containing 0.3 per cent zinc acetate and 0.025 per cent chlorhexidine acetate. The cysteine challenge model was used as described above. Subjects rinsed for 1 min with 10ml of the test solution. Cysteine rinsing and mouth air analyses were repeated at 3h, 6h and 9h. Subjects did not eat or drink during the entire test period. Due to the length of fasting, the number of test subjects was limited.

Gas chromatography

The VSC analysis system included a GC-14B gas chromatograph (Shimadzu, Japan) equipped with a flame photometric detector, a 12-ft x 1/8 inch Teflon column packed with 5 per cent polyphenyl ether-0.05 per cent phosphoric acid on 40/60 mesh Chromosorb T, and an auto-injection system with a 3ml sample loop. Column conditions were column temperature 70°C, nitrogen gas flow rate 32 ml/min, hydrogen gas flow rate 125ml/min, air flow rate 43ml/min, according to Yaegaki and Sanada[2].

Salivary putrefaction experiments

Eight of the volunteers participating in the clinical experiments also provided saliva samples (2 males and 6 females, age range 30 to 46 years). Between 9.00 and 10.00am, 1-2h following normal daily dietary intake and oral hygiene routines, each subject chewed a paraffin wax tablet for 1 min while swallowing normally, before collection of 10ml whole saliva. Saliva samples were shaken thoroughly following collection prior to 1ml aliquots being pipetted into separate test tubes with screw lids. 10µl aliquots of the test solutions were added to the 1ml saliva aliquots. The samples were incubated overnight at 37°C. Two untreated saliva samples were included for each test subject.

After 24–30 h incubation the tubes were shaken for 15s according to Kleinberg and Codipilly[18], a sample of the saliva headspace was withdrawn from the test tube using a 10ml syringe, and the sample was analysed for VSC directly in the gas chromatograph.

Calculation of synergy

Data from the mouth rinsing experiments were calculated as percentage of the original concentration of H_2S (control), at each of the measurement times for each test subject. Data from the salivary putrefaction experiment was also calculated as percent of the control VSC. The mean values for the results for all test subjects were used to evaluate possible synergistic effects according to the fractional inhibitory index (FIC index) as described by Berenbaum[19] and used in similar studies[20,21]:

$$\text{FIC index} = \frac{A+B}{A} + \frac{A+B}{B}$$

A = effect of antibacterial agent, B = effect of zinc, A + B = effect of the combination of an antibacterial agent and zinc. A FIC index of <1 indicates a synergistic (or complimentary) effect, FIC =1, an additive effect, and FIC >1 an antagonistic effect.

Results

Figure 1 shows the results of mouth rinsing with the different test solutions. 0.3 per cent Zn had a very strong anti-VSC effect after 1h, but this effect diminished relatively fast. 0.025 per cent CHX had only a moderate anti-VSC effect after 1h, but this effect diminished only slightly with time. A similar result was seen for 0.025 per cent CPC after 1h, but the effect of this agent deteriorated more rapidly. The anti-VSC effect of the combination of Zn and CHX was surprisingly marked and long lasting. Scarcely any reduction in anti-VSC effect was observable after 3h, in contrast to the effect of Zn alone. The combination of Zn and CPC had a good anti-VSC effect after 1h, but minimal anti-VSC effect above that of Zn alone at all three measurement times. *Synergy*: The FIC indices for the mouth rinse combination Zn + CHX were 0.56, 0.35 and 0.38 at 1, 2 and 3h respectively, the combination thus having a synergistic effect (see * in *Figure 1*) at all three measuring periods. The Zn + CPC mouth rinse combination had FIC indices of 1.45, 0.88 and 1.13 at 1, 2 and 3h respectively, thus only synergistic at 2h.

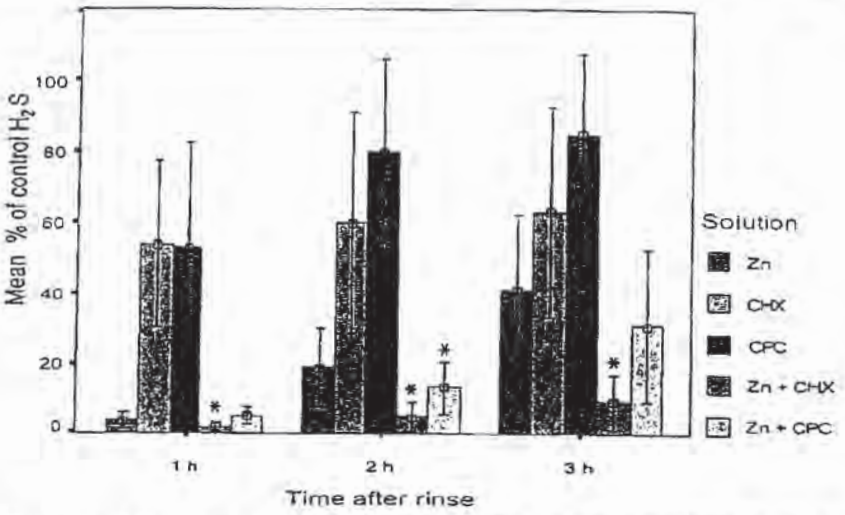

Figure 1. Inhibition of hydrogen sulphide production (mean per cent of control H_2S, ±2 s.d.) at 1, 2 and 3h after mouth rinses with individual test solutions and combinations. Test solutions: 0.3 per cent zinc acetate (Zn), 0.025 per cent chlorhexidine diacetate (CHX), 0.025 per cent cetyl pyridinium chloride (CPC) and combinations of Zn + CHX and Zn + CPC. A lower per cent of control value indicates a more effective anti-VSC agent (control value = 100 per cent). Synergistic anti-VSC effect, * FIC < 1.

The results of the salivary putrefaction experiment for methyl mercaptan are shown in *Figure 2*. A similar pattern of VSC inhibition could be observed as for the mouth rinsing, whereby Zn was the most effective of the individual agents, and both combinations (Zn + CHX and Zn + CPC) were more effective than the individual agents. The results for inhibition of hydrogen sulphide production were in line with the clinical results and are not shown. *Synergy*: Both mouth rinse combinations showed synergistic anti-VSC effects, showing FIC indices of 0.63 and 0.26, for Zn+CHX and Zn+CPC, respectively (see * in *Figure 2*).

Figure 3 shows the results of the extended mouth rinse experiment. The combination of 0.3 per cent zinc acetate and 0.025 per cent chlorhexidine diacetate had a marked anti-VSC effect even after 9h. It should be noted that the mouth rinsing experiments, performed using cysteine rinses according to the cysteine challenge model[18], provided information almost exclusively related to one VSC component, hydrogen sulphide.

Discussion

Previous studies have shown a correlation between oral malodour measured organoleptically and measurements of VSC by gas chromatography[5,22] thereby indicating the relevance of VSC measurements to oral malodour. Similar correlation has been shown using the portable sulphide monitor[23–26].

Cadaverine and putrescine have not been demonstrated as components of oral malodour *per se*[27]. Previously, Tonzetich[28] did not find any non-sulphur compounds involved in oral malodour. More recently Tonzetich[29] suggested that

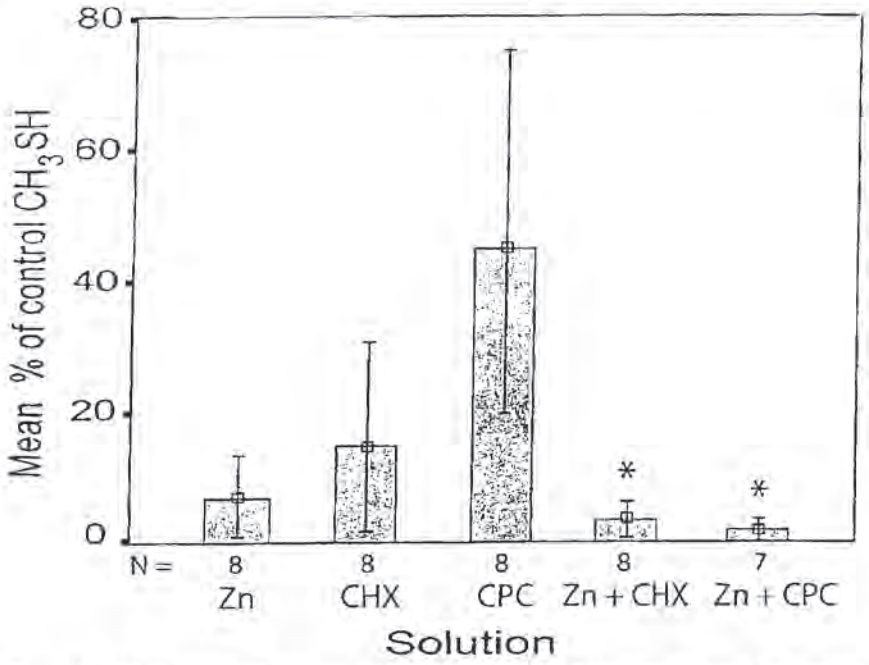

Figure 2. Inhibition of methyl mercaptan production in incubated saliva (mean per cent of control CH_3SH, ±2 s.d.) by test solutions and combinations. Test solutions: 0.3 per cent zinc acetate (Zn), 0.025 per cent chlorhexidine diacetate (CHX), 0.025 per cent cetyl pyridinium chloride (CPC) and combinations of Zn + CHX and Zn + CPC. A lower per cent of control value indicates a more effective anti-VSC agent (control value = 100 per cent). Synergistic anti-VSC effect, * FIC < 1.

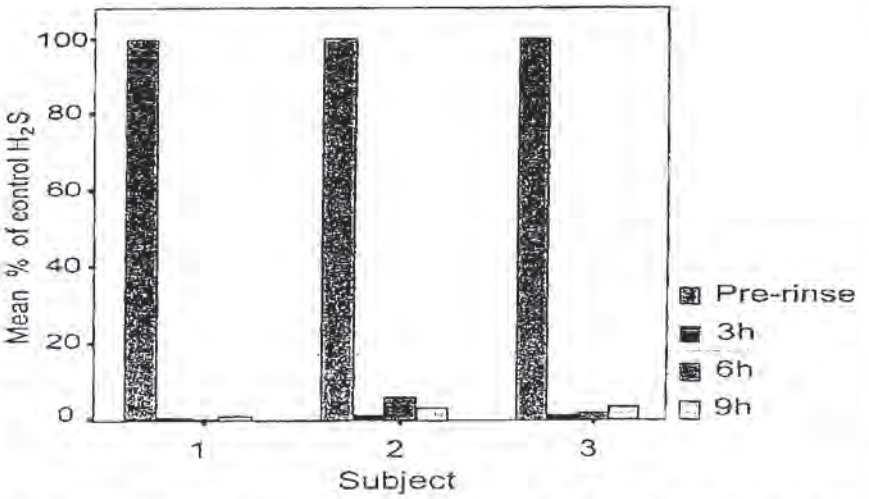

Figure 3. Inhibition of hydrogen sulphide production in three test subjects (mean per cent of control H_2S) over 9 h, by the combination of 0.3 per cent zinc acetate and 0.025 per cent chlorhexidine diacetate. Control =100 per cent.

minor components that are unable to produce oral malodour themselves, occurring in amounts below the threshold of organoleptic perception can still alter the intensity and quality of oral malodour in combination with VSC. The ratio of H_2S and CH_3SH is known to contribute to the quality of oral malodour, at least in periodontal patients[2]. It is also known that zinc ions that inhibit oral malodour also inhibit VSC, as mentioned above. The experimental data cited above indicates that the method involving the use of oral VSC measurements by gas chromatography can be considered as valid and directly related to oral malodour.

Mouth rinses with cysteine to enhance formation of oral VSC in the test panel were used in the present study[18]. This method lends itself to clinical testing of inhibitors of oral VSC production and oral malodour, as discussed by these authors. The reduction in VSC formation subsequent to a single rinse with an inhibiting agent is compared with the original VSC value observed, and any reduction taken as caused by the inhibitor. Additional cysteine rinses at an hourly interval, challenge the effect of the inhibitor and provide data concerning the duration of the effect. A limitation of this method is that only hydrogen sulphide is formed in the oral cavity immediately following cysteine rinses, as mentioned previously[4,13,17]. However, supplementing the clinical experiments with salivary putrefaction experiments can compensate for this limitation. This involves measurement of the effect of an oral malodour inhibitor on the production of both hydrogen sulphide and methyl mercaptan in incubated human saliva. The results for methyl mercaptan can be seen in *Figure 2*.

A comparison of the anti-VSC results for the individual agents and those for the combinations, showed that the combination of zinc ions and chlorhexidine had a better anti-VSC effect than that of zinc ions or chlorhexidine alone (*Figure 1*). The hypothesis to be tested in the present study was thus supported for this combination. This was confirmed by the calculated FIC index, demonstrating a synergistic effect at 1, 2 and 3h after rinsing. That result was further supported by the findings from the salivary putrefaction experiment (*Figure 2*) demonstrating that the combination of zinc ions with both chlorhexidine diacetate and cetylpyridinium chloride showed synergistic anti-VSC effects. In a recent study on effect of antibacterial agents on cariogenic organisms, a different interpretation of drug interactions was used[20]. According

to Isenberg[31], if the FIC index = $0.5 > x < 1.0$, this is described as partial synergy. Using this interpretation in the present study, the mouth rinse experiments showed that the combinations Zn + CHX at 1h, and Zn + CPC at 2h were partially synergistic, while the *in vitro* experiments demonstrated partial synergy for the Zn + CHX combination.

In the long-term experiment the combination of zinc and chlorhexidine provided a reduction of more than 95 per cent of the baseline VSC level even 9h after rinsing (*Figure 3*). This result should be considered very satisfactory especially taking into account the low concentration of the ingredients. Each mouth rinse with cysteine appears likely to consume any zinc retained in the mouth. Under normal conditions without cysteine challenges it may be safe to conclude that the mouth rinse as tested in the long-term experiment could be effective for 12 hours or more.

The moderate clinical anti-VSC effect of the cationic antibacterial agents alone was most likely due to the low concentrations used in order to avoid untoward side effects. Zinc has a metallic taste. The 0.1 per cent and 0.3 per cent zinc acetate solutions used in this experiment are dilute compared with concentrations used in some earlier clinical experiments (4 per cent or more). Pilot experiments by the current authors have shown that even a combination of as low as 0.1 per cent zinc and 0.01 per cent chlorhexidine had an anti-VSC effect, though this was not as long lasting as the presently used concentrations.

However, despite the relatively low concentrations involved, it was surprising to observe how much the combination of the antibacterial agents improved the effect of zinc ions, both in the mouth rinsing and salivary putrefaction experiments. It appears likely that the specific effect of zinc against sulphur and the unspecific antibacterial effect against the bacterial membranes, in particular related to chlorhexidine, are the mechanisms behind the synergism of the combination. However, previous experiments have indicated that antibacterial agents, and in particular chlorhexidine, can split disulphide bonds[32]. Chlorhexidine is known to be a strong denaturing agent. A splitting of disulphide bonds would be beneficial, as oral bacteria mainly contain desulphydrases, as demonstrated by the results of the present experiment. The splitting of disulphide bonds could provide an explanation for the observed marked and long lasting effect of the mouth rinse containing an aqueous combination of chlorhexidine and zinc ions.

It may be speculated that a further beneficial effect of the antibacterial agents could be to inhibit any additional foul, volatile non-sulphur bacterial products in the oral cavity. Zinc ions alone would have negligible effect on the formation of such products.

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A New Mouthrinse Combining Zinc and Chlorhexidine in Low Concentrations Provides Superior Efficacy Against Halitosis Compared to Existing Formulations: A Double-Blind Clinical Study

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A New Mouthrinse Combining Zinc and Chlorhexidine in Low Concentrations Provides Superior Efficacy Against Halitosis Compared to Existing Formulations: A Double-Blind Clinical Study

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Abstract

- **Objective:** Volatile sulfur compounds (VSC), mainly derived from bacteria located in deep crypts at the back of the tongue and from periodontal pockets, are responsible for approximately 90% of halitosis (bad breath, malodor). The objective of this double blind clinical study was to assess the clinical efficacy of a new formulation for halitosis containing a combination of zinc (0.3% Zn) and chlorhexidine (0.025% CHX) in low concentrations. The new formulation was compared to some widely used and commercially available formulations containing various enzymes and antibacterial agents in a clinical setting under controlled conditions.
- **Methodology:** Ten healthy volunteers participated in this study (5 female, 5 male, mean age: 46.6, range: 26–79). Each participant served as their own control, and neither the investigator nor the ten test subjects knew which formulation they were testing at any given time (double-blind design). Baseline H_2S data were obtained by cysteine rinsing for 30 seconds, 90 seconds mouth closure, and gas chromatographic (GC) analysis of mouth air. On separate days, each participant then rinsed for 60 seconds with 10 ml of each of the eight various formulations. Cysteine rinses were repeated at 1 hour, 2 hours, and 3 hours, and GC measurements of oral H_2S levels were again recorded.
- **Results:** The test rinse (0.3% Zn + 0.025% CHX) reduced the intraoral H_2S levels to 0.16% of control (range: 0.01–0.54%) after 1 hour, 0.4% after 2 hours, and 0.75% after 3 hours, providing superior efficacy in reducing H_2S compared to the other formulations tested ($p < 0.05$).
- **Conclusion:** A combination of Zn and CHX in low concentrations seems to be the most efficient way to remove the VSC that causes bad breath at present. Studies are underway to further explore the extraordinary efficacy of this combination (close to 100%), suggesting a specific mode of action and a synergistic effect of these two components.

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Introduction

Offensive odor emanating from the oral cavity, often termed halitosis, is responsible for approximately 90% of bad breath cases.[1-3] Halitosis is mainly caused by volatile sulfur compounds (VSC) derived from Gram negative anaerobic bacteria, mostly found in periodontal pockets and in the crypts at the back of the tongue.[4,5] Hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and, to a lesser extent, dimethyl sulfide (CH_3SCH_3) are the major components of the VSC that originate from the degradation of the sulfur-containing amino acids, cysteine, and methionine.[6] They have an unpleasant odor, even in extremely low concentrations.[7] In addition to causing halitosis, VSC may play an important role in the etiology of periodontal disease.[8,9] In particular, methyl mercaptan has been shown to penetrate the various tissues in periodontal pockets,[10] and increase the degradation of collagen, as well as inhibiting the protein synthesis of gingival fibroblasts,[11] thus adversely affecting critical events in the development of periodontitis.[8,9]

The authors of this paper, and other researchers, have shown that certain metal ions, zinc (Zn) in particular, can be used to inhibit the formation of VSC[12-14] and subsequently reduce or eradicate halitosis. Moreover, it has been shown that certain antibacterial agents such as chlorhexidine (CHX) or cetylpyridinium chloride (CPC) may also inhibit VSC formation and thus reduce halitosis.[15-17] If zinc ions and antibacterial agents operate by different mechanisms with regard to oral VSC inhibition, it is conceivable that combinations of two or more of these agents may provide an enhanced or synergistic anti-VSC effect.[18] However, the opposite might also be the case; one or two components might reduce or block the effect of the other. In order to examine this further it was decided to: a) evaluate the clinical effectiveness of a new anti-halitosis formulation (SB12®, Antula AB, Stockholm, Sweden) combining low concentrations of Zn (0.3%) and CHX (0.025%); b) use a double-blind clinical protocol to allow an unbiased comparison with other anti-halitosis formulations containing various enzymes and antibacterial agents, as shown in Table I; c) use a specially modified gas chromatograph particularly suited for measurements of low concentrations of VSC and considered the "gold standard" of halitosis measurements;[6,19] and d) use cysteine rinsing according to Kleinberg and Codipilli[20] to introduce bad breath in healthy volunteers in order to avoid some of the problems with including "patients," as well as enabling each participant to serve as his or her own control.

The aim of the present study was to examine the effectiveness of a new anti-halitosis formulation combining low levels of Zn and CHX, and to compare it with other widely used formulations

Table I
A Summary of the Active Ingredients
Listed on the Bottles of the Rinses Used in the Experiment

Kode	Mouthrinse	Active Ingredients
A	Zendium®	Zinc gluconate and various enzymes: amyloglycosidase, glycoxidase, and lactoperoxidase
B C	Listerine® Citrus and Cool Mint	Antibacterial agents: eucalyptol 0.092%, menthol 0.042%, menthyl salicylate 0.060%, and thymol 0.064%
D	Halita®	Chlorhexidine digluconate 0.05%, cetylpyridinium chloride (CPC) 0.05% and zinc lactate 0.14%
E	retarDEX®	Antibacterial agent (cloSYSII®)
F G	Dentyl® Refreshing Clove and Smooth Mint	Antibacterial agents: cetylpyridinium chloride, triclosan
H	SB12® test rinse	Zn acetate 0.3% and chlorhexidine diacetate 0.05%

against halitosis in a double-blind clinical design. The hypothesis to be tested was that Zn combined with CHX in low concentrations effectively inhibits H_2S production induced in healthy individuals, and moreover, is comparatively more effective than other currently used antibacterial agents and/or enzymes.

Materials and Methods

Oral Rinses

Eight different oral rinses were included in the study. All the oral rinses were commercially available at the time of the study except SB12® which was provided free-of-charge by the manufacturer (Antula AB, Stockholm Sweden). This study was performed at the Clinical Research Laboratory, Dental Faculty, University of Oslo, Norway. The following oral rinses were included in the experiment:

A. Zendium® Munnskölj med Zink (Opus Healthcare, Malmö, Sweden)
B. Listerine® Natural Citrus (Pfizer Consumer Healthcare, Morris Plains, NJ, USA)
C. Listerine® Cool Mint (Pfizer Consumer Healthcare, Morris Plains, NJ, USA)
D. Halita® Dentaid (S.L. Parc Tecnològic del Vallès, Cerdanyola, Spain)
E. retarDEX® (Periproducts Ltd, Middlesex, UK)
F. Dentyl® Refreshing Clove (Fresh Breath Ltd, London, UK)
G. Dentyl® Smooth Mint (Fresh Breath Ltd, London, UK)
H. SB12® (Antula Healthcare AB, Stockholm, Sweden)

A summary of the active ingredients of the various rinses, as listed on the bottles, is shown in Table I.

Test Subjects and Protocol

Ten healthy volunteers participated in this study. They were recruited from the research staff at the Dental Faculty, comprising five females and five males, mean age: 46.6, range: 26–79. All test subjects took part in the experiment with informed consent, after having received an explanation of the protocol. They did not have any medical history that in any way could relate to halitosis. The trial followed a crossover, double-blind design.

On test days, the subjects refrained from their normal oral hygiene and presented at the laboratory at 9:00 a.m. The participants rinsed for 30 seconds with 5 ml of a 6 mM solution of L-cysteine (Sigma Chemical Co., St Louis, MO, USA) at pH 7.2. Subsequently, they kept their mouths closed for 90 seconds, after which mouth air samples were aspirated into a 3 ml sample loop connected to the auto injector of a gas chromatograph (Shimadzu, Kyoto, Japan), modified for this purpose as previously described.[19] The obtained mouth air samples were thereafter analyzed directly by separation in the gas chromatograph using a Teflon column (3.66-mx 0.32 cm, temperature 70°C, nitrogen gas flow 32 ml min^{-1}, hydrogen gas flow rate 125 ml min^{-1} and airflow rate 43 ml min^{-1}) packed with polyphenol ether (5%)—phosphoric acid (0.05%) on 40/60 mesh Chromosorb T and a flame photometric detector.[6] The standardized H_2S formation in the mouth that was obtained after the cysteine rinsing constituted the baseline as a control for each tested subject. Immediately following, each subject rinsed for 30 seconds with one of the eight test solutions (A-H). Thereafter, cysteine rinses followed by mouth air analyses were repeated at 1, 2, and 3 hours. The H_2S levels were subsequently compared with the baseline levels for each subject. At least one non-test day between uses of the different test solutions was introduced to avoid a putative cross-over effect between the different test solutions.

Statistical Analyses

Concentration of H_2S in breath samples from the control measurement, and from measurements taken 1, 2, and 3 hours after treatment were obtained from gas chromatograph software (EZStrat 7.2) as AUC (area under the curve) for the chromatogram peak. Those raw data were furthermore calculated as a % of control for each of the test subjects.

Differences between the examined mouthrinses were statistically tested by one-way ANOVA and LSD multiple comparisons. These tests were performed on both AUC (presented in Table II) and % of control (Figures 1, 2, and 3). The outcomes of the statistical analyses were similar in both cases. It was further investigated whether different active ingredients have or do not have an inhibitory effect on oral H_2S formation; results greater than 100% were considered as "not having" inhibitory effect. The reason for those results greater than 100% needs closer investigation.

Results

A significant inhibition of H_2S production was observed in mouth air samples taken 1, 2, and 3 hours after the rinse with a combination of Zn and CHX in low concentration (H) compared to the H_2S baseline in all the 10 subjects tested. A great inter-individual variation in H_2S levels was observed between the different test subjects. The results are summarized in Table II.

A great variation in effectiveness among the various formulations was observed, ranging from virtually no observed effect (A, F) to almost 0% of control (H) over the whole testing period (3 hours). The results of the rinsing experiment (AUC) comparing the eight different anti-halitosis formulations are summarized in Table II and illustrated as % of control in Figures 1–3.

Table II
Comparison of Oral H_2S Formation Before and After Treatment with the Different Mouthrinses

	H_2S Formation in AUC (Untreated Control and 1, 2, 3 Hours after Treatment)							
Mouthrinse	Control	± Std. Error	1 h	± Std. Error	2 h	± Std. Error	3 h	± Std. Error
A	10526730	1996725	*10399169	1642165	*11016940	1893715	*11062133	1777625
B	8034153	2261068	*6952575	1469653	*6806082	2025882	*7630772	2105447
C	9393820	2207629	*4727536	2138393	*5526099	1870641	*8212024	2650699
D	8659070	1343685	1130869	878992	1477441	1135042	146372	597131
E	6915213	165857	2985235	673863	4028744	1090056	3211370	9375354
F	8303359	2418222	*9731853	1689299	*9476981	1916136	*8223760	1427925
G	7758629	2341766	*6585337	2333692	*8376508	2750260	*7402215	1680240
H	13677005	5266525	12213	5013	48234	23353	87059	41391
One-way ANOVA	$p > 0.05$		$p < 0.05$		$p < 0.05$		$p < 0.05$	
LSD	$p > 0.05$		$p < 0.05$		$p < 0.05$		$p < 0.05$	

* Significantly different from test rinse, H—$p < 0.05$

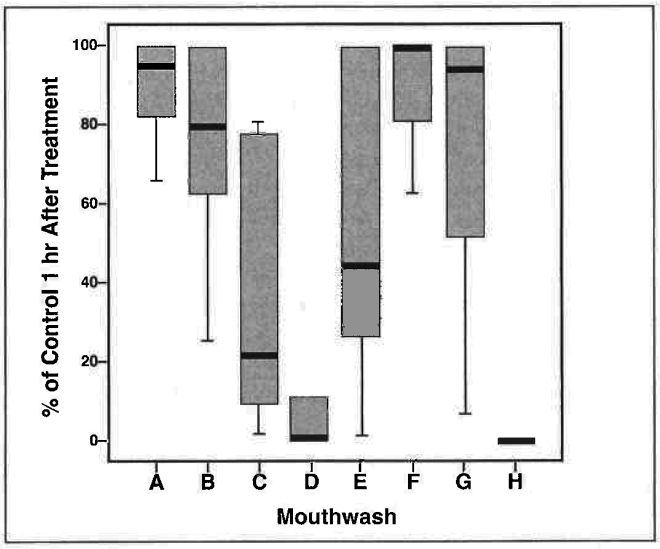

Figure 1. *Box plot of the results showing the inhibition of oral H_2S formation (percentage of control baseline H_2S) obtained 1 hour after mouth rinse. The lines within the boxes indicate the medians. Top and bottom boundaries of each box show 75th and 25th percentiles, respectively. Whiskers indicate the maximum/minimum points.*

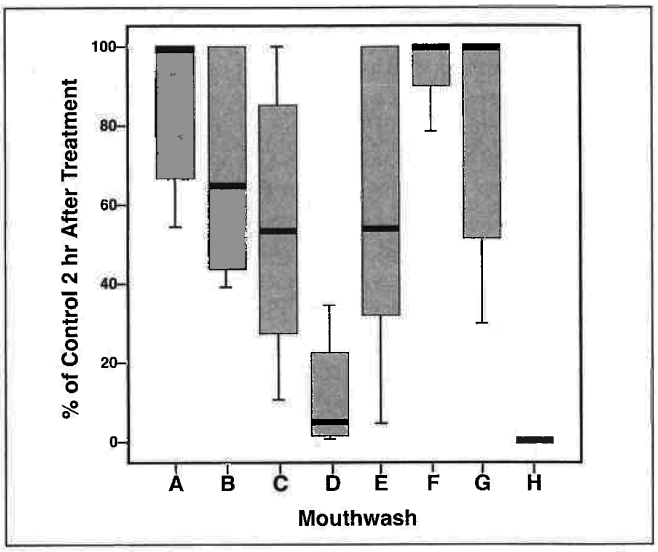

Figure 2. *Box plot of the results showing the inhibition of oral H_2S formation (percentage of control baseline H_2S) obtained two hours after mouth rinse.*

Discussion

Given the design of this study with each test subject serving as their own control, the great inter-individual variation in H_2S levels that was observed did not adversely influence the overall quality of the results. Moreover, by inducing halitosis in healthy volunteers, the difficulty with putative interference with various diseases and medication (drugs) that might influence H_2S production was avoided. Halitosis is a symptom and not a disease, that often occurs in otherwise healthy individuals mainly due to local conditions in the mouth; *i.e.*, putrefaction of anaerobic bacteria in crypts at the back of the tongue and in periodontal pockets.[1,4-6,8] The choice of test subjects thus seemed appropriate. The subjective nature of bad breath *per se*, as well as rather subjective (organoleptic, nasopalatinal index)[23] and less sensitive and specific measurement methods (*i.e.*, portable sulfide monitor, *e.g.*, Halimeter®),[24] further complicate this picture making it more difficult to perform reliable comparative studies, as well as

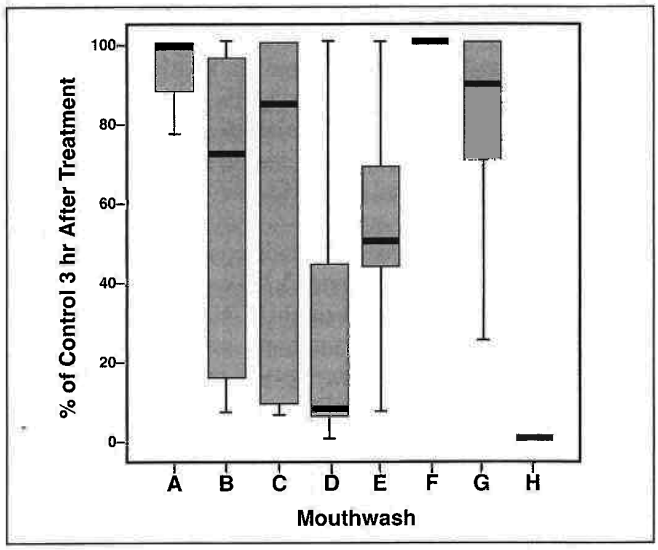

Figure 3. *Box plot of the results showing the inhibition of oral H_2S formation (percentage of control baseline H_2S) obtained three hours after mouth rinse.*

assessing the relative amounts (and contribution) of H_2S and CH_3SH to halitosis. The introduction of the gas chromatograph, with further modifications of this equipment to suit this purpose (separate and measure VSC obtained directly from the mouth *in vivo*),[6,19] has greatly improved the quality of data, and allows direct comparison of various mouth rinses and combinations of active ingredients used to inhibit bad breath. These modifications include changing the sample injection system to allow application of air samples directly from the mouth to the GC, and a specially made Teflon column to allow better separation of large samples and higher sensitivity readings for low concentration of sulfur gases which smell badly at extremely low concentrations, particularly CH_3SH.

Although the results supported our original hypothesis that a combination of Zn and CHX in low concentrations was the most efficient way to inhibit H_2S formation and thus halitosis, the degree of effectiveness was surprising (almost 0% of control in H_2S even after 3 hours). Additional studies are underway to further explore this effect, as well as its apparent long-lasting effectiveness. It moreover supports a previous pilot study indicating some H_2S inhibitory effect even after 12 hours, and given the low concentration of the active components (Zn and CHX), suggests a synergistic effect of the two.[18] It further indicates that Zn and CHX in low concentrations have specific mechanisms of action, separate binding sites, and might even work in a different way than when applied in concentrations most widely used (and significantly higher; Zn 0.3 % vs. 2-5 % and CHX 0.025 % vs. 0.2 %). No side effects have moreover been observed when they are used in such low concentrations[16-18] compared to some reported side effects (such as discoloration, metal taste, mucosal desquamation, and possible disturbance of the normal micro flora of the mouth) of current formulations.[25-27]

We speculate that the mechanism of action is mostly a direct inhibition of the gas *per se* (H_2S) and, to a lesser extent, the antibacterial effect that is well known for both CHX[28,29] and Zn[30] in higher concentrations. We suggest there is a two-step mechanism where CHX initially splits SH bindings, rendering S^- available for positive Zn^{2+} ions to bind, resulting in the formation of insoluble non-odorous Zn-sulfides that are passed through the GI tract and eventually excreted. Further studies of this hypothetical mechanism of action are clearly needed, and the potent inhibitory effect of this new formulation may also include other mechanisms working in parallel. Clearly, more information is needed to better understand how CHX and Zn work in such low concentrations.

The results from comparing various commercially available and widely used oral rinses against halitosis were rather surprising. Our working hypothesis that CHX and Zn, taken in combination and in low concentrations, was the most efficient way to inhibit halitosis, was substantiated by the finding that the two most efficient oral rinses (D and H) contained such a formulation. The combination of CHX, Zn, and cetylpyridinium chloride (CPC; D) seemed to be less effective that CHX and Zn alone (F). This might be due to some unwanted inhibitory effect, the most likely being Cl^- in CPC binding to the positively charged CHX as we have previously shown.[15,18] The origin of the active ingredient (kind of salts added) differs and might also account for some of these differences. The active ingredients of H are chlorhexidine diacetate and Zn acetate, compared with D, chlorhexidine digluconate and Zn lactate, with slightly different concentrations involved.[19] No side effects have been reported with either formulations (D and H), except for a slight discoloration of the tongue in some individuals after using D, and the effect of both on halitosis, as well as other relevant parameters, seems well documented. [16,17] The clinical effectiveness of B and C, particularly as antibacterial agents, is also well documented.[26,28] This effect was supported by our comparative study; B and C had a H_2S inhibitory effect ranging from 20–0% of control, depending on the exact formulation (taste and color) and time (1–3 hours). However, B and C are mainly prescribed as plaque and gingivitis inhibitory agents and are significantly less effective against bad breath than D and H. The halitosis-inhibitory effect is probably secondary to an inhibition of the oral microflora, including some anaerobic sulfur-producing species. Some H_2S-inhibitory effect was also observed after rinsing with E (50% of control after 3 hours) as well as G (90–100% of control), whereas A and F did not show any effect after 3 hours. Formulation A contains Zn in addition to enzymes, and although Zn has been shown to have an effect against VSC,[12-14] it does not work against H_2S in this formulation. F and G both show very little effect against H_2S, although one of its active ingredients (CPC) has been shown to be active against VSC.[15-17] Moreover, they contain triclosan which is known as a potent plaque inhibitor.[26,28] The conclusion to be drawn from these observations is that even if a rinse contains ingredients previously shown to be active against VSC, this does not necessarily mean that they work against VSC in the present formulation. Most of the active ingredients are charged molecules, easily neutralized by other components of the rinse. It furthermore suggests that all new formulations or changes in old ones should be thoroughly tested for anti-halitosis effect, preferably applying the more sensitive and reliable GC method before introduction to the market. B, C, E, F, and G contain antibacterial agents as their active ingredients (CloSYS II® and CPC + triclosan in combination) and probably work mainly through inhibiting the oral micro flora,[29] the anti-halitosis effect being secondary to an inhibition of sulfur-producing bacteria. D and H seem to be more specifically addressing the responsible gases (VSC) given the low concentrations of both Zn, CPC, and CHX used, suggesting that the antibacterial component of these formulations seems to be less dominant.

In conclusion, given the important role of the oral microflora in preserving oral health and protecting against foreign intruders, including infectious micro-organisms, food proteins, and other potentially immune-activating substances, it seem logical to recommend cautious use of local antibacterial agents in general. When the indication is clear, the most efficient and specific formulations (*i.e.*, a patented combination of CHX and Zn in low concentrations [31]) targeting the VSC that are major components in bad breath should be preferred. This formulation is also less likely to cause unwanted side effects.[16,27]

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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.

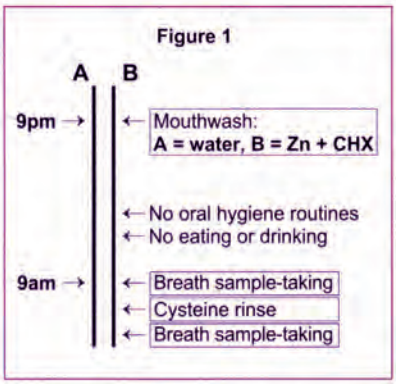

Figure 1: Experimental design. Timelines (A and B) showing the order in which the sequence of mouthwash, cysteine challenge rinsing and taking of breath samples occurred.

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.

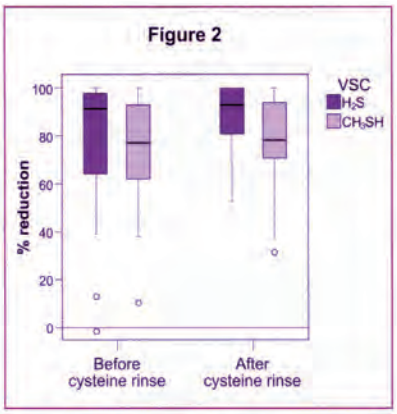

Figure 2: Boxplot-and-whisker plot of the results showing the mean percent reduction of VSC formation in mouth gas samples obtained 12 hours after the mouthwash with Zn + CHX. The lines within the boxes indicate the medians. Top and bottom boundaries of each box show 75th and 25th percentiles, respectively. 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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Comparative effects of various commercially available mouth-rinse formulations on halitosis

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Abstract

This double-blind clinical study aimed to compare a new mouthwash for halitosis – SB12® (0.3% Zinc plus 0.025% Chlorhexidine) – with seven commercially available formulations. Hydrogen sulphide (H_2S) was used as a representative measure of halitosis. Baseline H_2S data were obtained by cysteine rinsing and gas chromatographic (GC) analysis. Each of the eight formulations was assessed by this methodology after 1, 2 and 3 hours, with one day between tests to avoid any cross-over effect.

The non-parametric Kruskal-Wallis one way analysis of variance on ranks was used to compare groups and the Tukey test for all pair-wise multiple comparisons.

H_2S production was significantly inhibited after rinsing with the new formulation, mean reduction 99.27% after 1 hour, 98.51% after 2 hours and 91.48% after 3 hours. The new formulation was significantly more effective ($p>0.05$) in reducing H_2S than all the other formulations tested, which varied widely in their effectiveness.

In conclusion, Zn and CHX at low concentrations show a remarkable efficacy in removing H_2S, a significant cause of bad breath. This is likely due to a synergistic effect of these two agents. SB12® is therefore recommended as the most specific and effective formulation for the treatment and prevention of bad breath.

Key words: halitosis, oral malodour, mouth rinse, volatile sulphur compounds, hydrogen sulphide, zinc, chlorhexidine, gas chromatography, anaerobic bacteria, cysteine challenge.

Introduction

There are many mouthwashes available in Europe which may be used as part of a daily oral hygiene routine. Many users expect these mouthwashes to help them combat bad breath, even though this may not be the main claim for the product. In this study, a cross section of mouthwashes with different formulations was tested to investigate *in-vivo* to what extent they are effective against bad breath, or halitosis.

The origin of approximately 80% of cases of halitosis is the activity of mainly Gram negative, anaerobic bacteria in the oral cavity.[1] The bacteria are commonly located in deep crypts at the back of the tongue and, to a lesser extent, in periodontal pockets.[1,2] These bacteria produce volatile sulphur compounds (VSC) by catabolisation of organic substrates, particularly cysteine.[3,4] The main VSC in halitosis is hydrogen sulphide (H_2S), although methyl mercaptan (CH_3SH), and dimethyl sulphide $(CH_3)_2S$ are also involved.[5,6] These VSC all have an unpleasant odour even at extremely low concentrations.[7] In addition to producing bad breath, VSC have been implicated in the aetiology of periodontal disease resulting in tooth loss.[8] It is well documented that solutions of certain metal ions, in particular zinc (Zn), can be used to reduce or inhibit the formation of VSC.[9,10] Moreover, certain

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National Committee for Research Ethics in Norway. (REK-Sør: S-02260).

Gas chromatography-VSC analysis

The VSC analysis system included a GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame photometric detector, a 12ft x 1/8 inch Teflon column packed with 5% polyphenyl ether-0.05% phosphoric acid on 40/60 mesh Chromosorb T, and an auto-injection system with a 3-ml sample loop. Column conditions were column temperature 70oC, nitrogen gas flow rate 32 ml/min, hydrogen gas flow rate 125 ml/min, air flow rate 43 ml/min, according to a previously described methodology.[17] Mouth air samples were aspirated using a 10-ml syringe connected to the outlet of the auto-injector, and analysed for VSC directly in the gas chromatograph. Immediately after this procedure the subjects rinsed for 1 min with 10-ml of one of the test solutions, and the solutions were expectorated. Cysteine rinsing and mouth air analyses were repeated at 1 h, 2 h and 3 h after rinsing with the respective solutions.

Statistical analysis

Concentration of H_2S in breath samples from the control (baseline) measurements and from measurements taken 1, 2 and 3 hours after treatment with one of the eight mouthrinses were obtained from GC software (EZStrat 7.2) as area under the curve (AUC) for the chromatogram peak. The raw data were, in addition, calculated as % of control for each test subject. Differences between the mouthrinses were statistically tested by Kruskal-Wallis one way analysis of variance and all pair-wise multiple comparisons by using the Tukey test. These tests were performed on both AUC and % of control.

Results

The results of the rinsing experiment comparing the eight different formulations are summarised in Table 2 and illustrated in Figure 1. The results are also illustrated as percentage of control values in Figure 2. Of the eight mouthrinses tested in this double-blind clinical study, SB12® was clearly superior ($p<0.05$) to all of the others at all time points, providing a 99.27% reduction in H_2S levels after 1 hour, 98.51 % reduction after 2 hours and 91.48 % reduction after 3 hours. Of the other mouthrinses tested, the effectiveness of Odol® came closest to SB12®, with an 80% reduction after 1 hour, followed by Chlorhexamed®, which provided a 70% reduction after 1 hour. Interestingly, after 2 and 3 hours Chlorhexamed® proved to be more effective than Odol®, Corsodyl®, Eludril® and Hextril®, all providing a VSC-reducing effect of approximately 50%. Meridol® proved much less effective with a reduction in H_2S levels of only 8% after 3 hours. Plax® appeared to have little or no effect in preventing the formation of H_2S elicited by cysteine challenge in this study.

Mouth air samples taken 1, 2 and 3 hours after the rinse with the combination of Zn and CHX in low concentrations (SB12®) showed significant ($p<0.05$) inhibition of H_2S production compared to the H_2S baseline in all of the 10 subjects tested. This result occurred despite the inter-individual variation in H_2S levels

Table 2. Relative concentrations of H_2S in breath samples taken 1, 2 and 3 hours after use of various mouthrinses as percentage of control (baseline) values

		1h after treatment	2h after treatment	3h after treatment
		Mean	*Mean*	*Mean*
Rinse	SB12®	0.73	1.49	8.52
	Chlorhexamed®	30.36	11.23	15.02
	Corsodyl®	73.82	50.27	53.25
	Eludril®	42.46	61.92	46.07
	Hextril®	48.63	53.34	47.07
	Meridol®	63.42	73.56	92.86
	Odol®	20.53	47.40	63.05
	Plax®	201.24	161.63	166.57

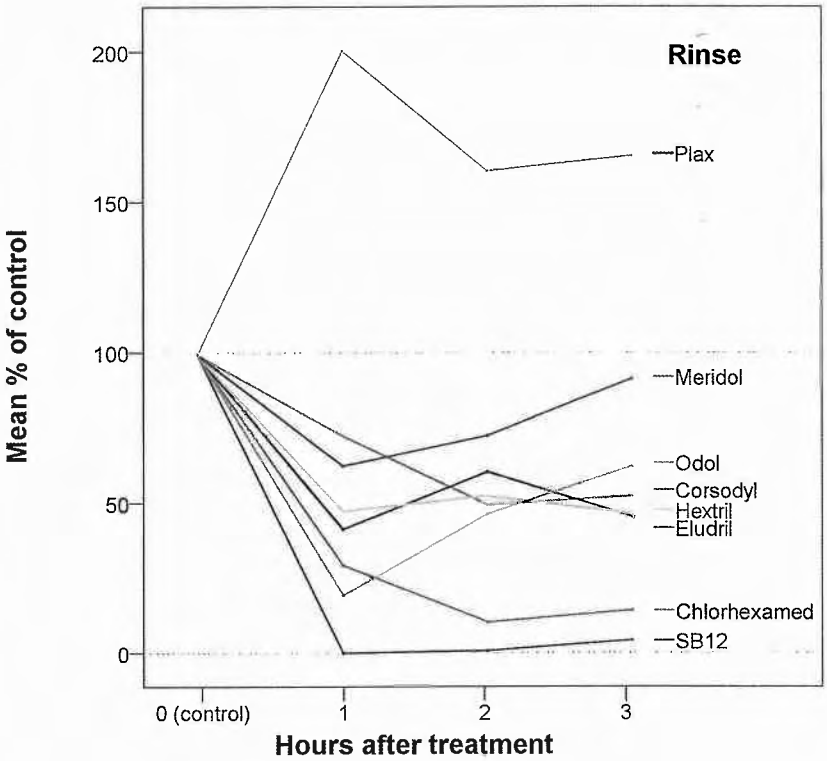

Figure 1. Graphical representation of concentration of H_2S in breath samples taken 1, 2 and 3 hours after use of various mouthrinses as a mean % of control

observed between the different test subjects. These results are summarised in Table 2 and Figure 1.

Marked variation in effectiveness was observed among the other mouthrinse formulations, ranging from no observed effect (Plax®), little effect (Corsodyl® , Meridol®) to moderate effect (Chlorhexamed®, Eludril®, Hextril® and Odol®). These latter four mouthrinses were all significantly less effective than SB12® ($p<0.05$).

The statistical analyses for AUC and % of control gave similar results, both in favour of SB12.

Discussion

A close correlation between halitosis measured organoleptically, with a portable sulphide monitor and measurement of VSC by GC support the relevance of VSC measurements to oral malodour.[7, 18] The experimental data cited above indicate that the method involving the use of oral VSC measurements by GC can be considered as valid and directly related to oral malodour. Mouthrinses with cysteine were used in this study in a standardised way to enhance the formation of oral VSC in the test panel.[15] This method is well documented and highly suited to clinical testing of inhibitors of oral VSC production and halitosis.[1, 19, 20] This is important as it enabled the use of well-motivated, healthy volunteers with an interest in dental health and hygiene to take part in the study. However, the study results should translate well to the real-world situation of halitosis, as cysteine is one of the key substrates for the anaerobic bacteria responsible for these conditions.[13, 15]

The reduction in VSC formation subsequent to a single rinse with an inhibiting agent was compared with the original VSC value observed and any reduction was then considered to be caused by the inhibitor formulation. Additional cysteine rinses at hourly intervals further challenged the effect of the inhibitor and provided data concerning the duration of its effect over a 3 hour period. Previous studies that included salivary putrefaction experiments or GC studies of non-cysteine-stimulated VSC support the relevance of H_2S studies as a measure of bad breath, as

H_2S is the most abundant component of VSC.[19, 21]

The authors, and others, have previously shown that a combination of low concentrations of Zn and CHX is a highly effective inhibitor of VSC and thus bad breath.[5, 11, 12, 22] A recent study comparing eight commercially available anti-halitosis formulations also concluded that this combination was superior to the other seven formulations and that this superiority was most likely due to a synergistic effect of Zn and CHX.[16]

Odol® contains a combination of zinc and CPC, which has also previously been shown as effective against VSC.[11,12,16,22] Chlorhexamed® contains relatively high concentrations of CHX with a known antibacterial and anti-halitosis effect.[9,10] CHX, because of its structure and positive charge, is also known for its retentive properties and thus long-lasting effect in the mouth.[23] These properties were further supported by the findings in this study. Unfortunately, the use of CHX is complicated by side-effects such as tooth discolouration, particularly when used in high concentrations for long periods and this limits its clinical use.[24] Of the anti-halitosis rinses tested in this study, Corsodyl®, Eludril® and Chlorhexamed® all contain CHX in relatively high concentrations, thus increasing the risk of tooth discolouration when used over the long-term. Hextril® contains a CHX analog, hexitidine, with similar properties. However, from the results of this study, these formulations appear to be much less effective against bad breath than the combination of low concentrations of CHX and Zn in SB12®. CHX is still the most efficient plaque-inhibiting agent commercially available, and is particularly useful when mechanical plaque control is disrupted, for example, immediately after dental surgery.[23] However, given the important role of the normal oral microflora in preserving oral health and protecting against foreign intruders, including infectious microorganisms, food proteins and other potentially immune-activating substances, it seems logical to recommend caution about the use of local antibacterial agents in general. This is the first study to demonstrate *in-vivo* that a combination of Zn and CHX is more effective at reducing H_2S than CHX alone. When there is a clear indication, the most efficient and specific formulation that targets VSCs including H_2S, the major components of bad breath, should be preferred. The present study indicates that the patented combination of CHX and Zn in low concentrations is the most efficient in this regard.[25] This formulation is also the least likely to cause unwanted side effects, based on the low concentrations of CHX and Zn relative to the other CHX formulations.[22,23,24,26]

Meridol®, containing a combination of aminofluoride and stannous fluoride did not prove to be very effective against bad breath. We have previously shown that stannous fluoride has some VSC inhibiting effect, as have aminofluorides, but apparently the combination of the two partly blocks the overall VSC inhibiting effect.[20] Plax® is most widely used as an oral antibacterial as an alternative to CHX. It contains a combination of sodium fluoride and CPC, the latter with demonstrated anti-VSC effect when used alone.[11] However, it appears that adding sodium fluoride may block this effect, because the combination exhibited no H_2S inhibiting effect in our study. A meta-analysis of data on the effectiveness of Plax® on oral health in 2003 also concluded that there was no conclusive evidence of its effectiveness in reducing plaque levels or gingivitis.[27]

The superior efficacy of SB12®

compared to the other mouthrinses and the low concentrations of the active substances (Zn and CHX) suggest a specific mechanism of action. A hypothesis has been proposed that the synergistic effect observed is caused by a coordinated attack on the soluble VSC. This involves CHX splitting the disulphide bonds, thus allowing Zn to bind to the sulphur ions more efficiently. [16] This results in the formation of insoluble zinc sulphide that is subsequently swallowed or expectorated.

In conclusion, a combination of CHX and Zn in low concentration, such as SB12®, is very effective against H_2S a VSC which plays a significant role in bad breath and should be the preferred treatment for this particular problem.

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ORAL DISEASES

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ORIGINAL ARTICLE

Comparative effects of various commercially available mouthrinse formulations on oral malodour

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OBJECTIVES: The primary aim of this study was to compare a new mouthwash (SB12®) containing 0.025% chlorhexidine and 0.3% zinc for oral malodour reduction against four commercially available mouthwashes and negative control. A secondary aim was to compare the two methods for measuring volatile sulphur compounds (VSC) by halimetry and OralChroma.

METHODS: Organoleptic scale, halimeter and the OralChroma were used to assess oral malodour and VSC. The effects of five test formulations and water (negative control) were assessed after 30, 60, 90 and 180 min, with 1 week between the treatments to avoid any cross-over effect.

RESULTS: Reduction in H_2S by halimetry and malodour levels by organoleptic assessment ranged from, slight (LacerFresh®) ($P > 0.05$), moderate (BreathRx®, SmartMouth®) ($P < 0.01$) to marked effects (SB12®, Listerine®) ($P < 0.001$) at all time points compared with water. The largest differences were observed at 30 min and decreased with time. SB12® showed separation from Listerine® at 180 min, using ANOVA plus Bonferroni's Multiple Comparison post-test ($P < 0.05$). Relationships between organoleptic, halimeter and OralChroma were between $R^2 = 0.795$ and 0.926.

CONCLUSION: SB12 shows a consistent and reproducible inhibitory effect on oral malodour parameters, which in turn correlate well with each other.

Oral Diseases (2011) **17**, 180–186

Keywords: oral malodour; mouth rinse; volatile sulphur compounds; hydrogen sulphide; zinc; chlorhexidine; organoleptic; Halimeter; OralChroma; anaerobic bacteria

Introduction

A significant source of oral malodour is from organisms on the surface of the tongue with microbes inhabiting the tongue biofilm being responsible for approximately 80% of cases of oral malodour (Yaegaki and Sanada, 1992; Rosenberg and Leib, 1995; Van den Broek *et al*, 2008). The particular papillary surface of the tongue with its large number of crypts and fissures allows it to harbour a high number of bacteria in a relatively anaerobic environment (Tonzetich, 1977). The extremely diverse microflora particularly Gram-negative anaerobes possess enzymes that allow biotransformations of sulphur substrates (cysteine, methionine and glutathione) into volatile sulphur compounds (VSC) (Kleinberg and Westbay, 1990; Scully *et al*, 1997). The main VSC in oral malodour is believed to be hydrogen sulphide (H_2S), although methyl mercaptan (CH_3SH) (Tonzetich, 1971; Yaegaki and Sanada, 1992) and dimethyl sulphide $(CH_3)_2S$ may also play a role (Suarez *et al*, 2000; Quirynen, 2003). In addition to producing bad breath, VSC produced by periodontopathogens in the gingival crevice have been implicated in the aetiology of periodontal disease resulting in tooth loss if left untreated (Shapiro and Dworkin, 1997; Radcliff and Johnson, 1999). Other volatile organic compounds (VOC) contribute to an unknown extent to oral malodour and they are thought to include indole, amines and acids (Kostlec *et al*, 1980; Goldberg *et al*, 1994; Radcliff and Johnson, 1999).

Numerous mouthwashes are available for use as part of a daily oral hygiene routine. The formulations contain actives that may inhibit microbial growth, enzymatic reactions or may react directly with VSC to reduce their levels in the breath. In addition, these formulations may include flavour compounds, which can mask the effects of odiferous compounds.

Certain metal ions, in particular zinc (Zn), are well known to reduce or inhibit the formation of VSC (Tonzetich, 1971; Yaegaki and Suetaka, 1989; Young *et al*, 2002) as do certain antibacterial agents such as chlorhexidine (CHX) and cetylpyridinium chloride (CPC) with a subsequent reduction in oral malodour (Loe and Schiott, 1970; Lang *et al*, 1973; Denton, 1991; Grossman *et al*, 1996; Young *et al*, 2002; Roldan *et al*, 2003; Winkel *et al*, 2003). The combination of low concentrations of Zn and CHX seems to be particularly

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effective (Young *et al*, 2002; Winkel *et al*, 2003). More evidence is emerging for the efficacy of this combination including double-blind comparisons with several widely used formulations against halitosis. The studies have mainly used gas chromatography (GC) to measure VSC (Tonzetich *et al*, 1991; Yaegaki and Sanada, 1992; Rosenberg, 1996).

Two common approaches for assessing oral malodour include halimetry and organoleptic measurements by a trained odour judge (Rosenberg *et al*, 1991; De Boever *et al*, 1994). More recently, another instrument has been commonly employed – a portable GC system (OralChroma®, ABIMEDICAL Corporation, Japan). Organoleptic assessments by a trained judge have been shown to correlate with halimetry (Rosenberg *et al*, 1991; De Boever *et al*, 1994) but the relationship between these measurements and Oral Chroma has not been widely studied.

The aim of this study was to compare a combination of low concentrations of Zn and CHX (SB12®) with several commercially available mouthwash preparations and a negative control using both organoleptic measures and a halimeter. A secondary aim was to compare these results with those obtained using an OralChroma®.

The hypothesis to be tested in this study was that the combination of Zn and CHX in low concentrations is at least as effective as a selection of other currently used antibacterial agents/mouth rinses against malodour and VSC concentrations. By testing the active formulations against a negative control (water), information could be gained as to the efficacy of test compounds in terms of their immediate (within 30 min) and intermediate (3 h) effects.

Materials and methods

Human subjects

Fourteen volunteers from the University of the West of England were selected from a database of volunteers previously screened for inclusion in malodour trials. The panel included eight women and six men with a mean age at onset of 39 years (range 23–64). They were all healthy adults with no sign of oral disease.

Study design

The study was double-blind and neither judge, technician nor panellists knew which product was administered for all test days. Test days were 1 week apart. Each subject was randomly assigned a label 1–14. The mouthwashes were assigned letters A to F. All products were dispensed into 15 ml volumes by an independent technical member of staff. The volunteers rinsed for 2 min for each mouthwash. Each subject received all test products in random order thereby acting as their own control.

Eligibility criteria included informed consent and availability at the specified study intervals and sampling times plus a baseline organoleptic malodour score of >2 on each study morning. Exclusion criteria included: medical history of infectious diseases (e.g. hepatitis, HIV, tuberculosis); obvious gingival inflammation, active or severe caries, gingivitis or advanced periodontitis and oral thrush; antibiotic medication within 1 month prior to the start of the trial or during the trial period; consumption of medicated sweets containing antimicrobial agents; changes in oral hygiene practices during the trial; consumption of foods associated with oral malodour (such as garlic, spices or alcohol) on the day prior to, and on the sampling day; using strongly perfumed cosmetics on the sampling day; and substantial false dentition. On the evening prior to the test day, volunteers were instructed to continue their normal oral hygiene habit but on the morning of their assessments, they were asked to avoid oral hygiene (brushing their teeth) and food intake.

All participants were provided with their individual protocol, a diary and appointment dates/times for attending the laboratory. An adverse reaction form was available on request from the principal investigator. With the exception of the treatment mornings, subjects were not asked to alter their normal oral hygiene regime throughout the 6-week study.

Oral test rinses

Five oral rinses, all of which are commercially available, were compared along with water as the negative control: SB12®, Listerine®, BreathRX®, Smarth Mouth® and Lacer Fresh®. Table 1 lists the mouthrinses, the manufacturers and a summary of their ingredients (and amounts) as far as this information is available.

Ethics and study conduct

The protocol and informed consent form were approved by the local Ethics Committee. The study was conducted in a manner consistent with the ethics encompassed within the 'Declaration of Helsinki'.

Organoleptic assessment

One trained odour judge scored breath odour levels using the 0–5 organoleptic scale as outlined by Rosenberg *et al* (1991) and modified in term of odour descriptives by Greenman *et al* (2004), 0 = no odour, 1 = barely noticeable, 2 = slight odour, 3 = moderate odour, 4 = strong odour, 5 = very strong odour (saturation).

Instrumental analysis

Measurements using Halimeter and OralChroma were taken according to the manufacturer's instructions. Two halimeter readings were taken and the calculated average was recorded as ppb. OralChroma readings were taken using a 1 ml gas sample from a '2-min' closed mouth via plastic syringe. H_2S was obtained by measurements of area-under-curve (AUC) of the separated chromatographic peaks from the output trace. However, because of the 10-min time period required for running samples, only one sample per person per time point was taken.

Trial procedures

On the test day, volunteers reported to the breath odour judge who carried out a baseline breath assessment. Two assessments were taken within 5 min and an average

Table 1 Mouthrinse, ingredients and code

Code	*Mouthrinse*	*Ingredients*
A	Listerine antibacterial Mouthwash-Total Care® **Pfizer Consumer Healthcare Walton-on-the-Hill, Surrey, UK**	Aqua, alcohol, sorbitol, aroma, poloxamer 407, benzoic acid, eucalyptol, methyl salicylate, thymol, menthol, sodium fluoride, zinc chloride, sucralose, sodium saccharin, sodium benzoate, benzyl alcohol, CI 16035, CI 42090. Contains sodium fluoride (0.022% w/v 100 ppm F).
B	BreathRX® **Discus Dental, Europe, Rotterdam, The Netherlands**	Aqua, sorbitol, propylene glycol, PEG-40, hydrogenated castor oil, poloxamer 407, xylitol, aroma (mint, thymol and eucalyptus oil), zinc gluconate, cocamidopropyl betaine, cetylpyridinium chloride, sodium saccharin, citric acid, CI 42090.
C	SmartMouth Wash® **Triumph Pharmaceutical Inc., St. Louis, MO, USA**	Solution 1: purified water, sodium benzoate, benzoic acid, and sodium chlorite. Solution 2: purified water, glycerine, poloxamer 407, propylene glycol, benzoic acid, flavour, poloxamer 124, sodium benzoate, sodium chloride, sodium saccharin, zinc chloride, D&C Yellow No 10, and FD&C Blue No 1.
D	LacerFresh Mouthwash® **Lacer, S.A Sardenya, Barcelona, Spain**	Triclosan 0.15%, zinc chloride 0.05%, sodium fluoride 0.05% (225 ppm), xylitol 1%
E	SB12® **Antula Healthcare, Stockholm, Sweden**	Zinc acetate (0.3%), chlorhexidine diacetate (0.025%), sodium fluoride (0.05%), mint/menthol flavour (in alcohol)
F	Control	Water

value calculated for each time point. Following organoleptic assessment, the laboratory technician undertook baseline halimeter and OralChroma readings. The volunteers were then given 15 ml of one of six test mouthrinses, in a randomized and double-blind manner and instructed to rinse the mouth for 2 min. The breath assessments and instrumental readings were repeated at 30, 60, 90 and 180 min after test or control 'treatment'. The volunteers were not allowed to eat or drink between sampling.

Statistical analysis

Organoleptic scores, VSC concentrations (by halimetry) and H_2S (by OralChroma) were taken at baseline, 30, 60, 90 and 3 h per person, per treatment. GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to log transform, plot (as change in readings from time zero) and statistically analyse the data using ANOVA plus Bonferroni's multiple comparison post-test. Correlation tests were performed using Excel Microsoft and goodness of fit expressed as the coefficient of determination (r^2).

Results

Table 2 shows the range and overall average readings for the pretreatment (baseline) conditions for the three measured parameters. As this involved readings from 14 individual trialists on six different occasions (five treatments and control), the total data points are $n = 84$. The mean and range are suitable for a designed study to show reductions in malodour parameters. Efficacy in terms of reduction in VSC compared with control substance F (water) as measured by the Halimeter (Figure 1 and Table 3) varied among the mouthrinse formulations, ranging from no significant effect with product D (LacerFresh®), moderate effect with products B (BreathRX®) and C (SmartMouth®) $P \leq 0.01$, to good marked effect with products E (SB12®) and A (Listerine®) $P \leq 0.001$.

Table 2 Average, minimum and maximum values of baseline readings (pretreatment measurements) for organoleptic scores, VSC and H_2S (from OralChroma) recorded for 14 trialists

Measurements	*Average (±s.d.; n = 84)*	*Minimum (±s.d.)*	*Maximum (±s.d.)*
Organoleptic score	3.55 (0.23)	2.50 (0.00)	4.33 (0.25)
Halimeter readings	156 (49.21)	36.16 (3.65)	379.50 (86.00)
H_2S OralChroma	416.40 (213)	50.75 (27.80)	544 (61.09)

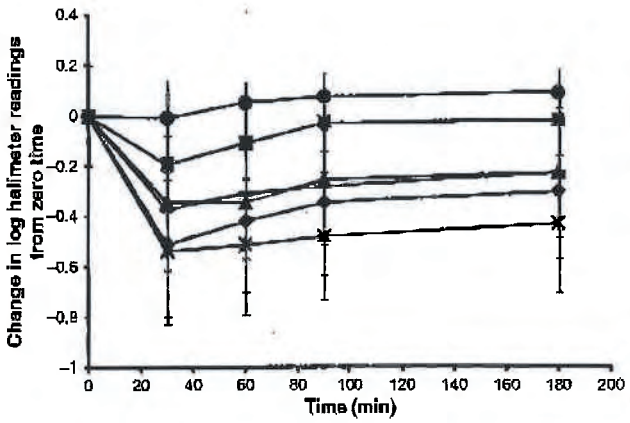

Figure 1 Log_{10} halimeter changes for five products plus control (*SB12, ◆Listerine, BreathRX, ▲SmartMouth, ■LacerFresh, ●Control)

Comparing the Halimeter™ (Interscan Corporation, Chatsworth, CA, USA) readings between products, SB12® ($P \leq 0.01$) and Listerine® ($P \leq 0.05$) both showed statistical separation from LacerFresh at all time points using ANOVA plus Bonferroni's Multiple Comparison post-test. The separation for SB12 was larger and was maintained throughout the 3-h observation period.

Efficacy as measured by reduction in breath odour using the organoleptic scale showed water to have a very

Table 3 Summary of ANOVA plus Bonferroni statistical data

Products	*Time*	P-*values* *Halimeter*	P-*values* *Organoleptic*
A and B	All time points	-	-
A and C	All time points	-	-
A and D	30	$P < 0.05$	$P < 0.05$
	60	$P < 0.01$	$P < 0.001$
	90	$P < 0.01$	$P < 0.001$
	180	$P < 0.05$	$P < 0.001$
A and E	30	-	-
	60	-	-
	90	-	-
	180	-	$P < 0.01$
A and F	30	$P < 0.001$	$P < 0.001$
	60	$P < 0.001$	$P < 0.001$
	90	$P < 0.001$	$P < 0.001$
	180	$P < 0.001$	$P < 0.001$
B and C	All time points	-	-
B and D	30	-	-
	60	-	$P < 0.001$
	90	-	$P < 0.001$
	180	-	$P < 0.001$
B and E	30	-	-
	60	-	-
	90	-	-
	180	-	$P < 0.01$
B and F	30	$P < 0.01$	$P < 0.001$
	60	$P < 0.01$	$P < 0.001$
	90	$P < 0.01$	$P < 0.001$
	180	$P < 0.01$	$P < 0.001$
C and D	30	-	-
	60	-	$P < 0.001$
	90	-	$P < 0.001$
	180	-	$P < 0.001$
C and E	30	-	-
	60	-	-
	90	-	$P < 0.05$
	180	-	$P < 0.01$
C and F	30	$P < 0.01$	$P < 0.001$
	60	$P < 0.01$	$P < 0.001$
	90	$P < 0.01$	$P < 0.001$
	180	$P < 0.01$	$P < 0.001$
D and E	30	$P < 0.01$	$P < 0.001$
	60	$P < 0.001$	$P < 0.001$
	90	$P < 0.001$	$P < 0.001$
	180	$P < 0.001$	$P < 0.001$
D and F	30	-	$P < 0.001$
	60	-	$P < 0.001$
	90	-	$P < 0.05$
	180	-	-
E and F	30	$P < 0.001$	$P < 0.001$
	60	$P < 0.001$	$P < 0.001$
	90	$P < 0.001$	$P < 0.001$
	180	$P < 0.001$	$P < 0.001$

(-) Not Significant
A = Listerine; B = BreathRX; C = SmartMouth; D = Lacerfresh; E = SB12; F = Water

slight breath reduction at 30 min, but then odour levels increase to above the initial, time zero, pretreatment level. All products separated statistically from water at all time points (range $P \leq 0.05$–0.001). As can be seen in Figure 2, product D (LacerFresh®) had the least benefit, products A (Listerine®), B (BreathRX®) and C (SmartMouth®) show more marked reductions, while product E (SB12®) reduced breath odour levels to a measurably greater extent than all other products, and maintained the reduction up to 180 min.

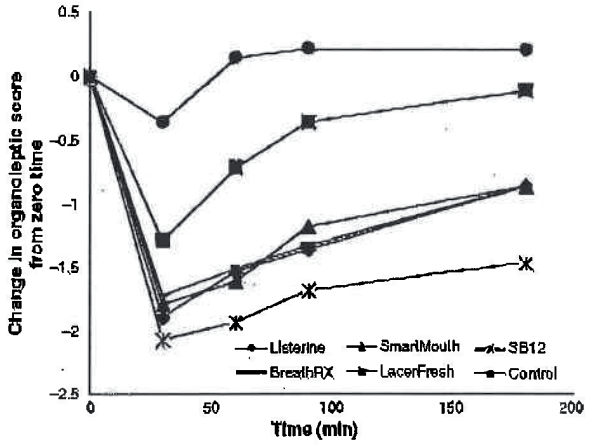

Figure 2 Organoleptic changes for five products plus control (*SB12, ♦Listerine, BreathRX, ▲SmartMouth, ■LacerFresh, ●Control)

SB12®, (Figure 2, Table 3), showed statistical separation from LacerFresh® at all time points ($P < 0.001$), from SmartMouth® at 90 min ($P \leq 0.05$) and at 180 min ($P < 0.001$), and from Listerine® and BreathRX® at 180 min ($P < 0.01$). Listerine® showed statistical separation from LacerFresh® at all time points ($P \leq 0.05$). Smart Mouth® and BreathRX® separated from LacerFresh® at 60 min ($P < 0.001$).

The organoleptic data support the Halimeter™ results with all products maintaining their positions of efficacy F < D < C < B < A < E. Figure 3 shows the results obtained for H_2S using the OralChroma™. These data followed a similar profile of reduction and recovery over time as halimetry or organoleptic scores. Relationships between organoleptic scores, Halimeter™ and OralChroma were between $R^2 = 0.795$ and 0.926 as seen in Figures 4–6.

Discussion

Five oral rinses, SB12® (containing a low concentration of Zn and CHX), Listerine®, BreathRX®, SmartMouth® and Lacer Fresh®, all of which are commercially available, were compared along with water as the

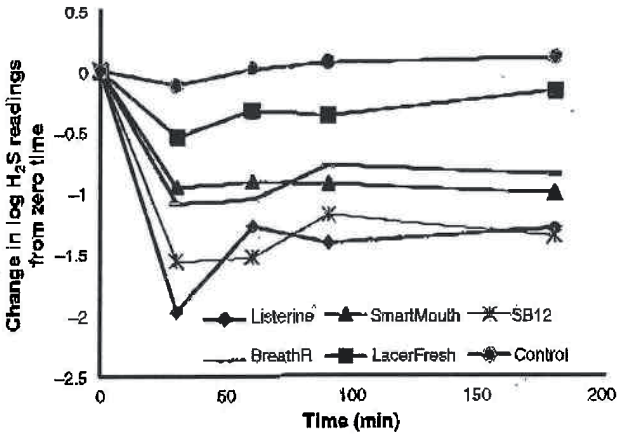

Figure 3 Log_{10} Hydrogen sulphide changes for five products plus control (*SB12, ♦Listerine, BreathRX, ▲SmartMouth, ■LacerFresh, ●Control)

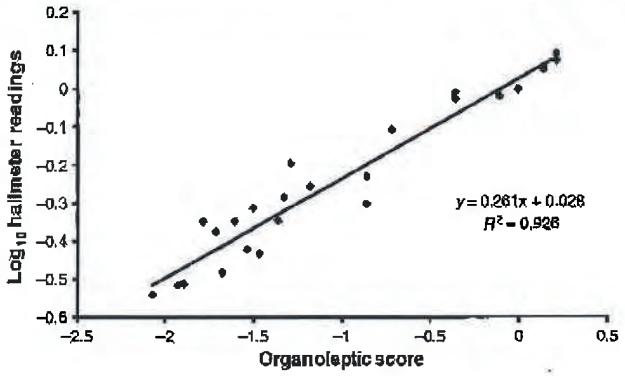

Figure 4 Correlation between organoleptic score and Log_{10} H_2S readings from Halimeter

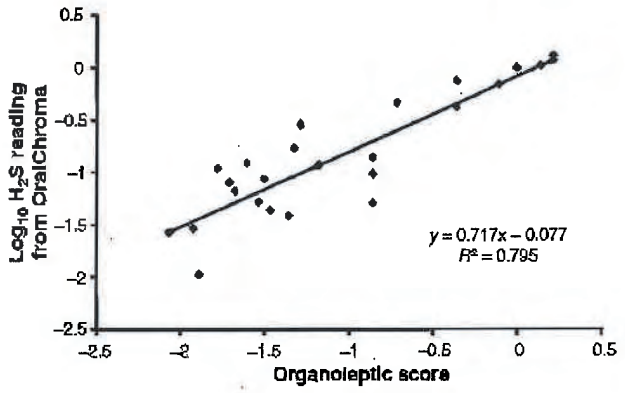

Figure 5 Correlation between organoleptic score and Log_{10} H_2S readings from Oral Chroma™

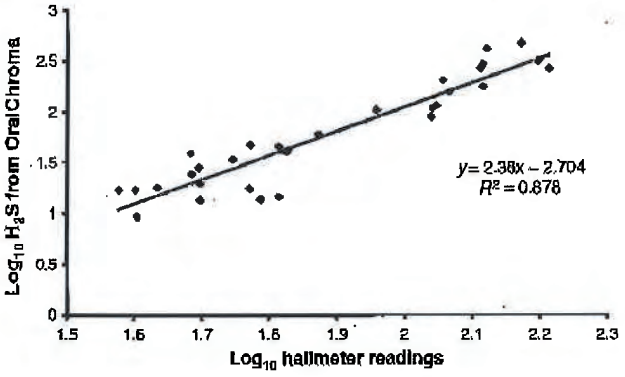

Figure 6 Correlation between Log_{10}VSC readings from Halimeter™ and Log Oral Chroma™

negative control. The odour-inhibiting capacity of the mouthwash formulations was determined using the organoleptic scale, the Halimeter and the OralChroma. Malodour levels of 14 orally healthy volunteers were assessed at baseline and at the same time periods during the day. The organoleptic assessment of individuals prior to and after treatment was performed by one trained odour judge in a completely double-blind manner. It is well accepted that humans have the capacity to determine the strength (i.e. concentration) of odour molecules. Models relating the organoleptic score to the occupancy of odour binding sites (degree of receptor saturation) have been proposed (Greenman *et al*, 2004, 2005). Judges can be trained to score the strength of odour (0–5) and it is clear that to have a useful meaning, a zero score must relate to no detectable odour and a five must be as strong as it gets. When subjected to pure odour compounds of known concentrations, judges are able to discriminate and respond in a dose-dependent manner even when the order of concentrations is randomized. Moreover, the judges can repeat their measurements at a later date and be shown to give similar (reproducible) responses. Another useful method to validate the organoleptic judge is to see how their scores compare with other objective measurements of the same or similar samples, using an instrumental gas sensor (e.g. Halimeter) or GC.

In this study, it was important to see whether any correlations between sensory and instrumental measurements existed so that one type of measure could be used to validate the other. Although some reports have shown a relationship between organoleptic score and either halimeter or GC (Rosenberg *et al*, 1991; Winkel and Tangerman, 2005; Doran *et al*, 2007; Van den Velde *et al*, 2009), no relationships between all three methods have been reported. In the present study, it was noticed that whether an inhibitory effect from an active mouthwash was calculated as a change in malodour value from time zero or as an absolute measurement at each time point, the correlations between the three methods of breath measurement were high. This finding implies that all methods are equally capable of assessing oral malodour and that any method on its own might also be sufficient.

The inhibitory effects on H_2S and oral malodour can be described as follows: slight effect (Lacer Fresh®), a moderate effect (BreathRX®, SmartMouth®) and a marked effect (Listerine®, SB12®). However, in comparison with a clinically proven mouthwash such as Listerine (Pitts *et al*, 1983), SB12 was shown to be numerically and at some time points, statistically, superior.

Chlorhexidine, a cationic bis-biguanide with low mammalian toxicity and broad spectrum activity against Gram-negative and Gram-positive bacteria (Denton, 1991), has been used for *in vitro* and *in vivo* studies (Kimminent *et al*, 1996; Jones, 1997). The cationic properties of CHX explain how its electrostatic attraction by the anionic bacterial surfaces may lead to membrane disruption, increased permeability and cell death and as a result, to a reduction in bacterial load (Jones, 1997; Kuyyakanond and Quesnel, 1992; Quirynen *et al*, 2002) and malodour. Chlorhexidine is also known for its high substantivity to buccal surfaces and has been shown to reduce gingival inflammation and dental plaque (Cummins and Creeth, 1992; Andy and Moran, 1997; Bollen and Quirynen, 1996). The strong antimicrobial action and increased substantivity in the mouth of CHX justify its use for malodour reduction (Bosy *et al*, 1994; De Boever, 1996). More recently, CHX has been used in association with other anti-malodour agents such as CPC and Zn and the efficacy of this combination was shown to be more effective than

CHX alone (Roldan *et al*, 2003; Quirynen *et al*, 2002) suggesting a more synergistic effect by CHX when present with other active compounds.

The efficacy of CHX against microbes has been shown to be both dose- and time-dependent (Quirynen *et al*, 2002) and different product formulations may use CHX at different concentrations, which might explain the variability of side effects such as discolouration of the oral mucosa and teeth as well as an alteration of taste (Flötra *et al*, 1971; Bosy *et al*, 1994; Quirynen *et al*, 2002).

From the 1970s onwards, zinc has been extensively studied either on its own or in association with other compounds used to control oral malodour, (Tonzetich, 1977; Schmidt and Tarbet, 1978; Wåler, 1978; Young *et al*, 2001). In addition to its antimicrobial properties, zinc is relatively non-toxic, non-cumulative and gives no visible colouration (Quirynen *et al*, 2002). It is believed that zinc binds to the membrane of microorganisms, interfering with, and reducing cell growth rate (Sugarman, 1983; Radke *et al*, 1994). It has also been suggested that zinc reacts with VSC by forming an insoluble complex (ZnS), which is non-volatile and thus non-odiferous (Boulware *et al*, 1985).

In previous clinical trials using mouthwashes containing zinc, volunteers have reported an unpleasant metallic taste (Young *et al*, 2003). It has also been shown that low concentrations of zinc alone do not produce an unpleasant taste but are not very effective against oral malodour. Likewise, CHX at high concentrations produces taste effects as well as staining. Young *et al* showed that low concentrations of CHX still maintained an effect over time. It could be that a low concentration of CHX may reduce the staining of the teeth without loosing all of its anti-malodour properties. A synergistic effect between low zinc and low CHX, previously observed by others (Young *et al*, 2003; Thrane *et al*, 2007), may reduce oral malodour and decrease the above-mentioned side-effects. It is likely that zinc and CHX have different high-affinity binding sites within the cell, and that occupation of one type of site makes the cell more sensitive to the inhibitory or cidal effects of the other type of ligand.

In conclusion, a combination of CHX and Zn in low concentration, such as SB12, was shown to reduce significantly (for up to 3 h) H_2S in the oral cavity, which is considered to be the main contributor to oral malodour.

Acknowledgements

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ORIGINAL ARTICLE

Comparison of different treatment modalities for oral halitosis

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Abstract

Objectives. To assess the effects on intra-oral halitosis by a mouth rinse containing zinc acetate (0.3%) and chlorhexidine diacetate (0.025%) with and without adjunct tongue scraping. ***Materials and methods.*** Twenty-one subjects without a diagnosis of periodontitis were randomized in a cross-over clinical trial. Organoleptic scores (OLS) were assessed to define intra-oral halitosis by total volatile sulfur compound (T-VSC) measurements and by gas chromatography. ***Results.*** Twenty-one subjects with a mean age of 45.7 years (SD: ±13.3, range: 21–66). The OLS were significantly lower following active rinse combined with tongue scraping ($p < 0.001$) at all time points. Immediately after, at 30 min, and at day 14, the T-VSC values were lower in the active rinse sequence than in the negative rinse sequence ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively). At 30 min and at day 14, the hydrogen sulfide (H_2S) and methyl mercaptan (MM) values were lower in the active rinse sequence compared to the inactive rinse sequence ($p < 0.001$). The inactive rinse sequence with tongue scraping reduced T-VSC at 30 min ($p < 0.001$) but not at 14 days. Similar reductions in T-VSC, H_2S and MM were found in the active rinse sequence with or without tongue scraping. ***Conclusion.*** The use of a tongue scraper did not provide additional benefits to the active mouth rinse, but reduced OLS and tongue coating index.

Key Words: *halitosis, mouth rinse, tongue scraper*

Introduction

Halitosis is considered as a social and a psychological problem. Available data suggest that the prevalence of halitosis with an oral etiology (intra-oral halitosis) is high [1–3]. Oral halitosis can be caused by several intra-oral factors such as tongue coating, periodontal diseases, tooth decay, unclean dentures, mucosal ulcerations and diseases, mouth breathing and poor oral hygiene [3]. Approximately 40% of individuals affected by halitosis have no underlying organic disease [4]. Extra-oral halitosis may be caused by respiratory tract conditions such as sinusitis, tonsillitis, bronchiectasis, lung or liver disease [5].

Intra-oral halitosis has been associated with bacterial production of hydrogen sulfide (H_2S), methyl mercaptan (MM) and dimethyl sulfide (DMS) [6,7]. Anaerobic bacteria in periodontal pockets and on the dorsum of the tongue can degrade sulfur-containing amino acids, resulting in the formation of volatile sulfur compounds (VSC) [8–12]. Recent data also suggest that β-galactosidase activity in saliva is an important factor in intra-oral halitosis [13]. It is of interest that the activity of this enzyme was not related to the presence of bacteria associated with periodontitis, suggesting that intra-oral halitosis may in certain cases be present independent of such bacteria [13].

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Intra-oral halitosis has been studied with different methods including a subjective organoleptic scoring system (OLS) with a scale between 0–5 [14]. OLS is considered as the 'gold standard' to diagnose intra-oral halitosis. Objective assessments of intra-oral levels of VSCs can be performed with a device assessing total volatile sulfur compounds (T-VSC) or by gas chromatography [15,16]. Currently, gas chromatography is considered as the most accurate device to detect VSC in breath air [17].

Different treatment strategies including mechanical debridement of teeth, rinsing with antimicrobial agents and/or the use of metal salts have been proposed for the management of intra-oral halitosis [18]. The treatment of intra-oral halitosis in patients with periodontitis has focused on periodontal therapy, improvement of oral hygiene and the use of a tongue scraper by the patient [19,20]. Thus, data suggest that the use of a tongue scraper may reduce the level of intra-oral halitosis also in subjects who do not have periodontitis [21,22]. The data are, however, contradictory. Although a significant reduction in VSC may occur over 3 months the mean VSC scores at 3 months remained at much higher levels than suggested as the cut-off level of VSC by gas chromatography to define absence of VSC causing intra-oral halitosis [23].

In a recent systematic review, the authors found no evidence that diet modification, the use of a sugar-free chewing gum, tongue cleaning by brushing, scraping the tongue or the use of zinc containing toothpaste resulted in clinically important results in regards to the control for intra-oral halitosis [4].

The aim of the present randomized single blinded cross-over clinical trial was to compare the efficacy of four intervention modalities to control for intra-oral halitosis in subjects with a diagnosis of intra-oral halitosis but without a diagnosis of periodontitis.

Materials and methods

The Ethics Committee at the University of Lund, Sweden, approved the study. All subjects signed an informed consent. Advertisements in the local newspaper, on message boards and on the web page at the University of Kristianstad, Sweden, were used to recruit subjects. The study was conducted between 2008 and 2009 and was performed at the dental clinic of the University of Kristianstad, Sweden.

- Inclusion criteria: (1) halitosis of intra-oral origin, (2) OLS ≥2 and (3) T-VSC ≥160 ppb, as determined with a Halimeter®.
- Exclusion criteria: (1) untreated periodontitis defined as the presence of more than one periodontal pocket with a probing pocket depth ≥6 mm, (2) open caries lesions, (3) pregnancy, (4) systemic medications known to cause hypo-salivation, (5) systemic antibiotic therapy within the preceding 3 months prior to the study, (6) current smoker or (7) a medical history with a disease known to be associated with extra-oral halitosis.

The subjects were given detailed verbal and written instructions regarding food intake to exclude a diet that may have an impact on oral malodor. They were given routine oral hygiene measures including the sequence assigned rinsing and as defined tongue scraping and what to do before each visit at the clinic. They were specifically asked; (I) not to consume food containing onions, garlic or hot spices within 48 h before assessments, (II) not to drink alcoholic beverages within 12 h before assessments, (III) not to eat or drink within 5 h before assessments (subjects were allowed to drink water until 3 h before assessments), (IV) not to perform oral hygiene measures, tongue cleaning or use any mouth-rinse in the morning of the examination day and (V) not to use scented cosmetics, perfume or after-shave lotions in the same morning as the study assessments were performed. Subjects were instructed not to change their oral hygiene habits during the study period. During each of the four study sequences, the subjects came to the clinic at the same time during the morning hours at baseline, day 1 and at day 14.

At study end-point, all subjects had participated in all four study intervention protocol sequences using: (I) the active test mouth rinse alone, (II) the active test mouth rinse with the use of a tongue scraper, (III) the inactive mouth-rinse alone and (IV) the inactive mouth-rinse with the use of a tongue scraper. The different test sequences were separated by a washout period of 1 week. Subjects were randomly assigned to protocol sequence order (Latin square) (Table I) using a computer-based randomization software program IBM®/SPSS® 18.0 (IBM®, Corporation Somers, NY). The two rinse solutions (active and inactive rinse) were distributed in coded bottles. The study subjects and the examiner (SEA) were unaware of sequence assignment. The subjects were instructed to rinse with 10 ml of the provided solution during 1 min twice daily and then to spit out the rinse solution. The subjects were instructed to rinse after breakfast and before bed-time.

The active mouth-rinse included water, glycerin, sorbitol, alcohol (1.8%), zinc acetate (0.3%), chlorhexidine diacetate (0.025%), sodium fluoride (0.05%),

Table I. Sequencing of cases to protocol order using the design of a Latin square.

Procedure	Sequence			
Active rinse alone	I	II	III	IV
Active rinse + tongue scraping	II	III	IV	I
Negative control rinse alone	III	IV	I	II
Negative control rinse + tongue scraping	IV	I	II	III

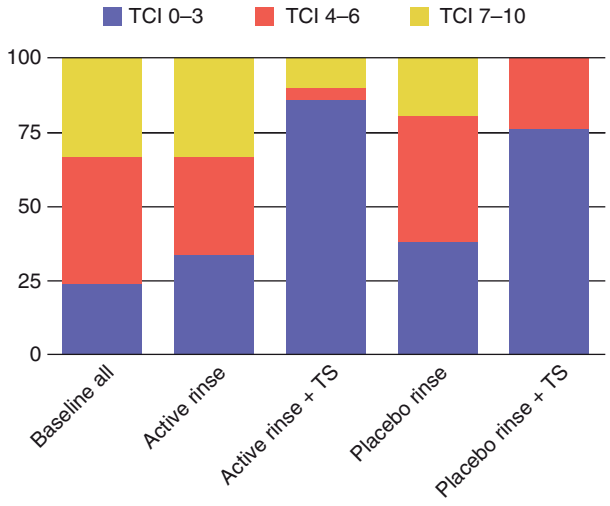

Figure 1. Frequency distribution of tongue coating index (baseline overall frequency distribution) and TCI scores for the four study groups, active rinse, active rinse plus tongue scraping (TS), control rinse and control rinse with tongue scraping (TS), at baseline and at day 14.

hydrogenated Castro oil, citric acid, acesulfame potassium, menthol and *Mentha piperita* (SB12®, Antula Healthcare AB, Stockholm, Sweden). The composition of the inactive mouth-rinse contained the same ingredients except that the inactive mouth-rinse did not include zinc acetate, chlorhexidine diacetate or sodium fluoride.

According to the study protocol, a tongue scraper (Halita®, DentAid, Barcelona, Spain) was used in two of the study sequences. For the adjunct use of the tongue scraper, the subjects were instructed and trained in how to use the tongue scraper. Briefly, they were shown to pull out the tongue, apply the tongue scraper to the dorsum of the tongue and perform five strokes. They were instructed to cover the dorsum of the tongue as far posterior as possible. This procedure was to be performed twice daily and before using the rinsing solution. Study subjects, but not the examiner (SEA), knew, of course, if they, during the specific study sequence, had used the tongue scraper or not. After the conclusion of the study all subjects responded to a questionnaire about the use of rinse and tongue scraper to control for compliance.

Study assessments were performed as follows: (I) Day 1: baseline values before intervention, (II) Day 1: immediately after intervention, (III) Day 1: 30 min after intervention and (IV) Day 14: 8–12 h after the last intervention the evening before. At baseline, the participants had not been eating during 5 h preceding the assessments. One and the same investigator (SEA) performed all registrations. The examiner was trained and calibrated in judging intra-oral halitosis at a clinic specialized in the treatment of intra-oral halitosis. Subjective assessments of intra-oral halitosis were performed using an arbitrary 0–5 scale (0 = no halitosis to 5 = offensive halitosis) [19]. The tongue coating index (TCI) was used to assess the extent of tongue coating [24].

The Halimeter® (Interscan Corporation, Chatsworth, CA, USA) was used to assess total VSC in breath air. The OralChroma™ (ABIMEDICAL Corporation, Kawasaki City, Japan) was used to assess H_2S, MM and DMS in breath air from study subjects and consistent with the use of these devices in other studies of intra-oral halitosis [15,16,17].

Statistics

Sample size was estimated based on the assumption that the negative control rinse would provide limited to no effects on VSCs, whereas the active rinse should reduce VSCs by 40%. Thus, a sample size of 20 subjects should provide statistical power (85%). The Kolmogorov-Smirnov test was used to identify that data for all variables failed to demonstrate a normally distribution pattern. The Kruskal-Wallis ANOVA and Univariate ANOVA with the Bonferroni post-hoc test were used to compare baseline sequence conditions. Further data analysis between and within study sequences for the study group sequences were studied by Wilcoxon signed rank test, by Kruskal-Wallis ANOVA and by repeated Mann Whitney U-tests. Data were also assessed by Spearman rank correlation. Significance was declared at $p < 0.05$.

Results

Subject characteristics

A total of 53 subjects were screened for intra-oral halitosis resulting in the inclusion of 21 adults (10 females) with confirmed intra-oral halitosis. All 21 subjects completed the study. The mean age of these subjects was 45.7 years (SD: ±13.3, range: 21–66).

At baseline in each study sequence, all study subjects had an OLS ≥2. Reliability tests performed between the baseline organoleptic scorings of the four treatments sequences demonstrated a high level of reliability (Cronbach's α varying between 0.63–0.87 ($p < 0.01$ and $p < 0.001$, respectively).

Comparisons by Kruskal-Wallis ANOVA failed to identify baseline sequence differences in the distribution of T-VSC scores ($p = 0.83$), the levels of H_2S scores ($p = 0.62$), the levels of MM scores ($p = 0.46$), the levels of DMS scores ($p = 0.90$) and the levels of T-VSC scores ($p = 0.27$). Univariate ANOVA with the Bonferroni post-hoc test confirmed these results (p-values varying between 0.27–1.0).

Baseline bleeding on probing at ≥20% of surfaces (four per tooth) was, on average, found in 23.8% (5/21) of the subjects and with the highest subject BOP score at 35% of surfaces. Statistical analysis failed to

Table II. Median, 25th and 75th percentiles, mean values and standard deviation (SD) for the active rinse alone at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

						p-values		
Active rinse values expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	Time 0–1	Time 0–2	Time 0–3
OLS	Median	2.0	0.0	0.0	2.0	0.001	0.001	0.01
	25th %	2.0	0.0	0.0	1.0			
	75th %	3.0	0.0	1.0	2.0			
	Mean	2.6	0.1	0.3	1.6			
	SD	0.8	0.3	0.6	0.6			
T-VSC	Median	164.0	64.0	64.0	113.0	0.001	0.001	0.01
	25th %	123.5	58.0	58.0	73.5			
	75th %	277.0	75.5	66.0	150.0			
	Mean	262.9	67.8	62.8	122.0			
	SD	264.6	13.9	7.5	55.3			
Hydrogen sulfide	Median	492.0	0.0	0.0	51.0	0.001	0.001	0.01
	25th %	113.0	0.0	0.0	6.5			
	75th %	1005.5	0.0	0.0	244.5			
	Mean	806.4	0.4	1.8	159.4			
	SD	973.6	2.0	5.2	235.4			
Methyl mercaptan	Median	67.0	30.0	11.0	14.0	0.01	0.001	NS
	25th %	12.5	0.0	0.0	0.0			
	75th %	250.5	50.0	39.0	48.0			
	Mean	165.7	55.2	26.5	50.5			
	SD	247.1	102.5	39.5	93.9			
Dimethyl sulfide	Median	28.0	10.0	8.0	12.0	0.01	0.01	NS
	25th %	13.0	0.0	0.0	0.0			
	75th %	68.0	30.5	23.5	35.5			
	Mean	51.4	24.8	13.3	71.9			
	SD	61.5	40.3	18.8	180.3			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

identify significant correlations between the percentage sites with bleeding and T-VSC, H_2S, MM and DMS scores.

Changes in organoleptic scoring (OLS) results

In comparison to pre-treatment scores, significantly lower OLS were identified immediately after intervention, 30 min after intervention ($p < 0.001$) in both the active rinse sequence alone and in the active rinse plus tongue scraping sequence. Significantly lower OLS scores were also obtained at day 14 for the active rinse alone ($p < 0.01$) and for the active rinse plus tongue scraping sequence ($p < 0.001$). In the negative control rinse group with tongue scraping, significantly lower OLS were found immediately after intervention and at 30 min after intervention ($p < 0.001$). Statistical analysis failed to demonstrate differences in OLS between baseline and 14 days in the negative control rinse sequence alone ($p = 0.32$), but demonstrated significantly lower OLS in the negative control rinse with the tongue scraping sequence ($p < 0.01$). Thus, at day 14 in the active rinse sequence 38.1% of the subjects had a negative OLS score while 66.7% of the subjects in the active rinse with tongue scraping sequence had a negative OLS score. Thus, at day 14 in the negative control rinse sequence 23.8% of the subjects had a negative OLS score while 33.3% of the subjects in the negative control rinse sequence with tongue scraping had a negative OLS score.

Differences in tongue coating index (TCI change) at day 14

The distributions of TCI at baseline and at day 14 are presented (Figure 1). Analysis by Mann-Whitney U-test identified that the change in TCI between baseline and day 14 was significantly lower in the active rinse sequence with tongue scraping than in the sequence with active rinsing alone ($p < 0.001$). The

Table III. Median, 25th and 75th percentiles, mean values and standard deviation (SD) for the active rinse in combination with tongue scraping at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Active rinse and tongue scraping expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	*p*-values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	0.0	1.0	0.001	0.001	0.001
	25th %	2.0	0.0	0.0	1.0			
	75th %	3.0	0.0	0.5	2.0			
	Mean	2.5	0.0	0.3	1.3			
	SD	1	0	0.6	0.7			
T-VSC	Median	154.0	64.0	64.0	91.0	0.001	0.001	0.01
	25th %	110.0	61.5	58.0	71.5			
	75th %	275.0	68.0	71.5	118.0			
	Mean	220.9	65.1	64.1	130.9			
	SD	163.1	5.8	7.8	132.1			
Hydrogen sulfide	Median	311.0	0.0	0.0	38.0	0.001	0.001	0.05
	25th %	82.5	0.0	0.0	0.0			
	75th %	772.0	0.0	0.0	205.0			
	Mean	628.0	0.8	3.8	195.3			
	SD	960.5	3.7	10.6	362.6			
Methyl mercaptan	Median	56.0	10.0	8.0	9.0	0.001	0.001	0.05
	25th %	15.5	0.0	0.0	0.0			
	75th %	139.0	35.0	14.0	42.0			
	Mean	136.4	25.0	10.3	44.6			
	SD	236.9	36.0	15.3	96.7			
Dimethyl sulfide	Median	42.0	8.0	0.0	12.0	0.01	0.001	NS
	25th %	17.5	0.0	0.0	0.0			
	75th %	77.0	18.0	19.5	74.5			
	Mean	59.2	15.8	17.1	41.5			
	SD	64.0	27.9	33.2	55.4			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter.

TCI change was also lower in the negative control rinse with tongue scraping sequence than in the negative control rinse sequence alone ($p < 0.001$). Statistical analysis failed to demonstrate a difference in the TCI change between the active and the inactive rinse sequences without tongue scraping ($p = 0.09$) and between the active rinse sequence with tongue scraping vs the negative control rinse sequence with tongue scraping ($p = 0.64$). Statistical analysis failed to demonstrate a correlation (Spearman rank correlation) between changes in TCI values between baseline and day 14 vs changes in T-VSC, H_2S, MM or DMS.

The intervention effects on VSC levels (within-subject analysis)

Median, 25th and 75th percentiles, mean, standard deviation and the *p*-values in the four study sequences various test combinations are presented for T-VSC, H_2S, MM and DMS at the different time points for the active mouth rinse sequence alone (Table II) and in combination with tongue scraping (Table III), for the inactive control rinse sequence (Table IV) and for the inactive rinse with tongue scraping (Table V). The changes in H_2S and MM levels in the four sequences between baseline and day 14 are presented in box-plot diagrams (Figures 2 and 3).

Comparisons between sequences at the different time points for T-VSC, H_2S, methyl mercaptan and dimethyl sulfide values

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the T-VSC values immediately after ($p < 0.001$), at 30 min ($p < 0.001$) and at day 14 ($p < 0.05$). Further analysis by repeat Mann-Whitney U-tests identified that immediately after, at 30 min and at day 14, the T-VSC values were significantly lower in the active rinse group compared to T-VSC values in the placebo

Table IV. Median, 25th and 75th percentiles, mean values and standard deviation (SD) for the negative control placebo rinse alone at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Negative control rinse values expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	*p*-values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	1.0	2.0	0.001	0.001	NS
	25th %	2.0	0.0	1.0	1.5			
	75th %	3.0	1.0	2.0	3.0			
	Mean	2.6	0.3	1.4	2.3			
	SD	1.0	0.5	0.9	1.0			
T-VSC	Median	180.0	111.0	130.0	183.0	NS	NS	NS
	25th %	99.0	86.0	87.0	85.5			
	75th %	287.0	183.0	259.5	297.5			
	Mean	242.1	227.0	225.0	221.7			
	SD	215.5	310.3	261.5	166.6			
Hydrogen sulfide	Median	239.0	187.0	226.0	272.0	NS	NS	NS
	25th %	89.0	52.5	95.5	92.0			
	75th %	912.0	519.5	514.0	816.5			
	Mean	625.9	754.1	491.3	599.2			
	SD	886.3	1742.4	693.1	690.7			
Methyl mercaptan	Median	51.0	35.0	31.0	115.0	0.05	NS	NS
	25th %	27.0	3.5	9.0	31.0			
	75th %	191.5	91.5	102.5	184.5			
	Mean	215.6	125.6	124.9	140.1			
	SD	420.8	314.0	331.3	164.7			
Dimethyl sulfide	Median	40.0	25.0	17.0	35.0	NS	NS	NS
	25th %	10.0	9.0	10.0	16.0			
	75th %	82.0	52.5	69.5	89.0			
	Mean	54.1	43.2	50.1	185.0			
	SD	56.7	53.3	65.6	600.9			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

rinse group ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively). Statistical analysis failed to demonstrate differences for T-VSC values between the placebo rinse alone and the inactive rinse sequence with tongue scraping (p-values varying between 0.19–0.78). The T-VSC values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse sequence and tongue scraping sequence immediately after procedure ($p < 0.001$), at 30 min after procedure ($p < 0.001$) and at day 14 ($p < 0.05$). Statistical analysis failed to demonstrate differences for T-VSC values between the active test rinse sequence and the active rinse sequence with tongue scraping (p-values varying between 0.33–0.98).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedures for the H_2S values at 30 min and at day 14 ($p < 0.001$). Further analysis by repeat Mann-Whitney U-tests identified that, at 30 min, and at day 14, the H_2S values were significantly lower in the active rinse sequence compared to H_2S values in the inactive rinse sequence ($p < 0.001$). Statistical analysis failed to demonstrate differences for H_2S values between the inactive rinse sequence and the combined rinse and tongue scraping sequence (p-values = 0.90 and 0.91, respectively). The H_2S values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse and tongue scraping sequence both at 30 min and at day 14 ($p < 0.001$). Statistical analysis failed to demonstrate differences for H_2S values between the active test rinse sequence and the active test rinse and tongue scraping sequence (p-values = 0.90 and 0.93, respectively).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the MM values immediately after ($p < 0.05$) and at 30 min after procedure ($p < 0.01$). Repeat Mann-Whitney U-tests identified that, at 30 min and at day 14, the MM values were significantly lower in the active rinse sequence compared to MM values in

Table V. Median, 25th and 75th percentiles, mean values and standard deviation (SD) for the negative control placebo rinse in combination with tongue scraping at the different time points (time 0 = baseline, time 1 = immediately after rinse, time 2 = 30 min after rinse and time 3 = after 2 weeks of rinsing).

Negative control rinse and tongue scraping expressed in ppb	Values	Time 0	Time 1	Time 2	Time 3	*p*-values		
						Time 0–1	Time 0–2	Time 0–3
OLS	Median	3.0	0.0	1.0	2.0	0.001	0.001	0.01
	25th %	2.0	0.0	0.5	1.0			
	75th %	3.0	0.5	2.0	2.5			
	Mean	2.5	0.3	1.3	1.8			
	SD	0.9	0.7	0.9	1.0			
T-VSC	Median	186.0	100.0	90.0	142.0	0.01	0.001	NS
	25th %	108.5	84.0	69.5	87.0			
	75th %	286.0	204.0	176.0	293.0			
	Mean	246.1	172.5	172.9	193.5			
	SD	231.6	166.2	203.0	125.5			
Hydrogen sulfide	Median	367.0	380.0	216.0	271.0	NS	0.01	NS
	25th %	138.5	55.3	48.0	111.0			
	75th %	692.5	667.8	503.5	946.0			
	Mean	847.4	561.8	386.3	606.1			
	SD	1396.0	781.4	553.9	706.1			
Methyl mercaptan	Median	93.0	44.5	33.0	61.0	NS	0.01	NS
	25th %	22.5	2.5	8.0	18.0			
	75th %	265.0	102.3	167.5	173.0			
	Mean	180.1	98.3	85.2	123.7			
	SD	231.5	155.1	106.9	168.1			
Dimethyl sulfide	Median	48.0	27.5	26.0	41.0	NS	NS	NS
	25th %	7.0	2.3	0.0	0.0			
	75th %	101.0	58.3	84.0	117.5			
	Mean	64.6	40.8	42.0	99.5			
	SD	64.6	46.5	42.9	141.7			

OLS, Organoleptic scores; T-VSC, Total volatile sulfur compound (T-VSC) by Halimeter®.

the inactive rinse sequence at 30 min ($p < 0.05$) and at day 14 ($p < 0.001$). Statistical analysis failed to demonstrate differences for MM values between the inactive rinse alone and the inactive rinse sequence with tongue scraping (p-values = 0.86, and 0.27, respectively). The MM values were significantly lower in the active rinse and tongue scraping sequence than in the inactive rinse with tongue scraping sequence both at 30 min ($p < 0.001$) and at day 14 ($p < 0.01$). Statistical analysis failed to demonstrate differences for MM values between the active test rinse sequence and the active test rinse with tongue scraping sequence (p-values = 0.32 and 0.71, respectively).

Analysis of the data by Kruskal-Wallis ANOVA identified significant differences by sequence procedure for the DMS values immediately after ($p < 0.05$) and at 30 min after procedure ($p < 0.01$). Repeat Mann-Whitney U-tests identified that, at 30 min after procedure, the DMS values were significantly lower in the active rinse sequence compared to DMS values in the inactive rinse sequence ($p < 0.01$). At the other study time points, statistical analysis failed to demonstrate DMS differences between these two procedures. Statistical analysis failed to demonstrate differences between the inactive rinse sequence and the inactive rinse sequence with tongue scraping, as well as between active rinse and active rinse with tongue scraping.

Discussion

Baseline data assessments consistently confirmed that, at the beginning of each intervention sequence, baseline T-VSC, H_2S, MM and DMS scores were comparable and that the 1 week wash-out period was sufficient to control for any effect that the preceding study sequence might have had on intra-oral halitosis. Thus, for this type of study of intra-oral halitosis in

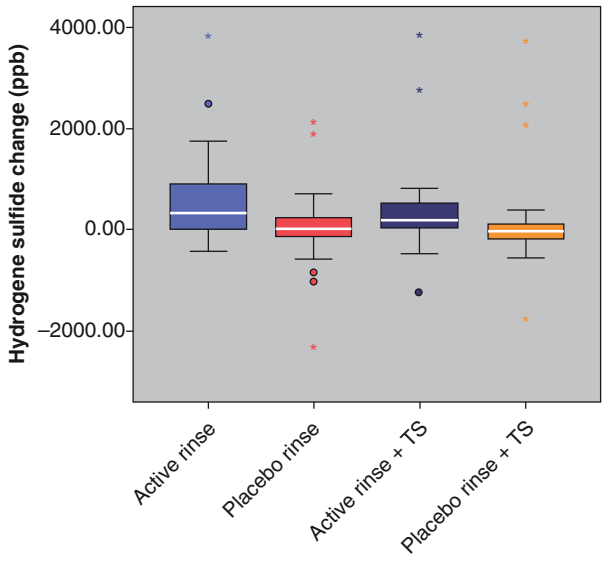

Figure 2. Change for Hydrogen sulfide between baseline and at day 14 for the four interventions. Notice that a positive value suggests a reduction in H_2S at day 14 (λ 0 outlier value, * extreme outlier value) (TS, tongue scraping).

subjects who did not have a diagnosis of periodontitis, the cross-over study design, including a 1-week washout period was appropriate.

The data failed to demonstrate baseline differences at each sequence for the four treatment modalities. Hence, the use of the cross-over design was appropriate. Due to the lack of normal distribution of the T-VSC, H_2S, MM and DMS scores the statistical analysis was performed with non-parametric tests which did not fully allow us to control for study sequence allocation. Nevertheless, the analysis clearly demonstrated statistical differences by sequence

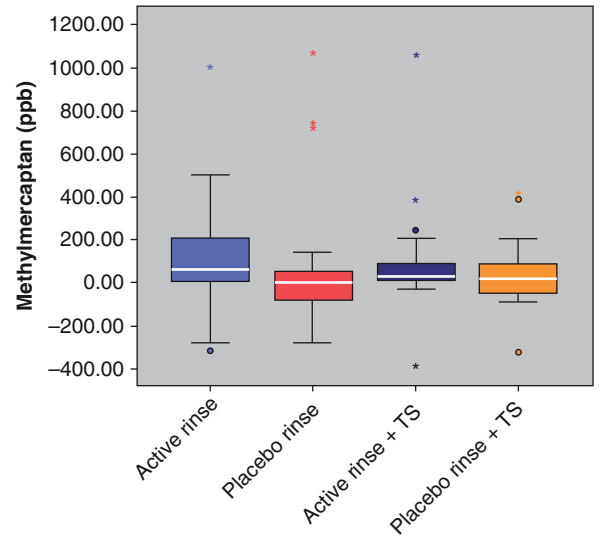

Figure 3. Change for Methyl mercaptan between baseline and at day 14 for the four interventions. Notice that a positive value suggests a reduction in MM at day 14 (λ 0 outlier value, * extreme outlier value) (TS, tongue scraping).

modality and that rinsing alone with the active rinse solution consistently demonstrated the highest ability to reduce the VSCs studied.

The present study design with the inclusion of a tongue scraper did not allow for a fully double-blind design. The rinse products were, however, bottled in the same type of bottles and labeled such that the subjects and the investigator were unaware if the subjects had been using the active or negative control rinse solutions during the dedicated study sequence. The flavoring agent may have affected the professional assessments using the organoleptic scoring system immediately after rinsing and at 30 min following interventions. Therefore, the OLS at these time points may be less accurate than the Halimeter® and OralChroma™ readings.

Other studies assessing the effects of therapy in subjects with intra-oral halitosis and periodontitis have shown that periodontal intervention reduces intra-oral halitosis [20,25]. In the present study, we identified that intra-oral halitosis can occur in subjects who do not have periodontitis. The present study also suggested that the adjunct use of a tongue scraper provided limited impact on intra-oral halitosis.

Compared to 30 min after intervention, less reductions of VSC were observed at day 14. The explanation might be that the participants rinsed with or without tongue scraping the evening before and did not brush their teeth or used the rinsing solution or the tongue scraper 8–12 h before the registrations in the morning of day 14. This 8–12 h time frame may have allowed the accumulation of VSC before the assessments. Bacterial re-growth resulting in elevated production of VSC may have occurred. This could explain the trend of higher values of VSC at day 14. It should, however, be observed that the VSC values at day 14 were lower than at baseline in the active rinse sequences but not in the sequence with the placebo rinse.

Other studies have suggested that mechanical methods including tongue brushing or tongue scraping to clean the dorsum of the tongue reduce the levels of VSC in exhaled air [10,11,26–28]. The present study demonstrated that tongue scraping had limited effects in reducing levels of VSC in comparison to the effects of the active rinse solution. Mechanical cleaning of the tongue may have a short time effect on intra-oral halitosis [21].

The chemicals used in the active mouth rinse had effects on intra-oral halitosis. One possible explanation may be a chemical binding and inactivation of VSC by ingredients of the active mouth-rinse. Other data suggest that the use of chlorite anions and chlorine dioxide in a mouth rinse may have effects on intra-oral halitosis [28,29]. Metal ions, including zinc, have been used for several years in the treatment of intra-oral oral halitosis [30–33]. Dentifrices with either Zn++ or baking soda significantly reduce

VSC levels [6,34]. A reduction of intra-oral halitosis following chlorhexidine rinses has also been reported [12,35]. Furthermore, data suggest a synergistic effect between chlorhexidine and zinc, which may explain the efficacy in binding VSC thereby controlling for intra-oral halitosis [36]. Zinc salts are approved therapeutics by FDA (US Food and Drug Administration) with anti-inflammatory and anti-bacterial effects. Therefore, subjects with intra-oral halitosis could be recommended to use a mouth-rinse with the active ingredients studied (zinc and chlorhexidine) for the control of intra-oral halitosis.

The present study demonstrated that the active rinse alone without the tongue scraping provided the most reliable change (reduction) in intra-oral halitosis as defined by T-VSC, H_2S and MM assessments. The report in a recent systematic review and our findings that the use of a tongue cleaner provides marginal or no effects on intra-oral halitosis are consistent [4].

In conclusion, rinsing with a zinc-acetate and chlorhexidine diacetate containing mouth rinse resulted in a clinically relevant reduction of intra-oral halitosis during a study period of 2 weeks. The use of a tongue scraper did not provide additional benefits to the active rinse. The removal of tongue coating debris with a tongue scraper does not seem to influence VSC levels in breath air in subjects who do not have periodontitis.

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