Comparative effects of various commercially available mouth-rinse formulations on halitosis

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

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

About the authors:
The first author Per Stanley Thrane is a doctor (MD) and a dentist (DDS) and specialist in Periodontics. He currently holds a position as Professor of Periodontics, Institute for Clinical Odontology, University of Oslo. This work has been done at the University Clinic and the Clinical Research Laboratory, Dental Faculty, University of Oslo where Thane is Deputy Leader. Thrane and co-workers have published extensively in this field of research with some 200 articles in international reviewed journals over the years.

Address for correspondence:
Per S. Thrane, Department of Periodontology, Faculty of Dentistry, University of Oslo, Oslo, Norway. Telefax: +47 22852390 E-mail: thrane@odont.uio.no

1. Department of Periodontology, Faculty of Dentistry, University of Oslo, Oslo, Norway

2. Oral Research Laboratory, Faculty of Dentistry, University of Oslo, Oslo, Norway

3. Department of Cariology and Gerodontology, Faculty of Dentistry, University of Oslo, Oslo, Norway

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

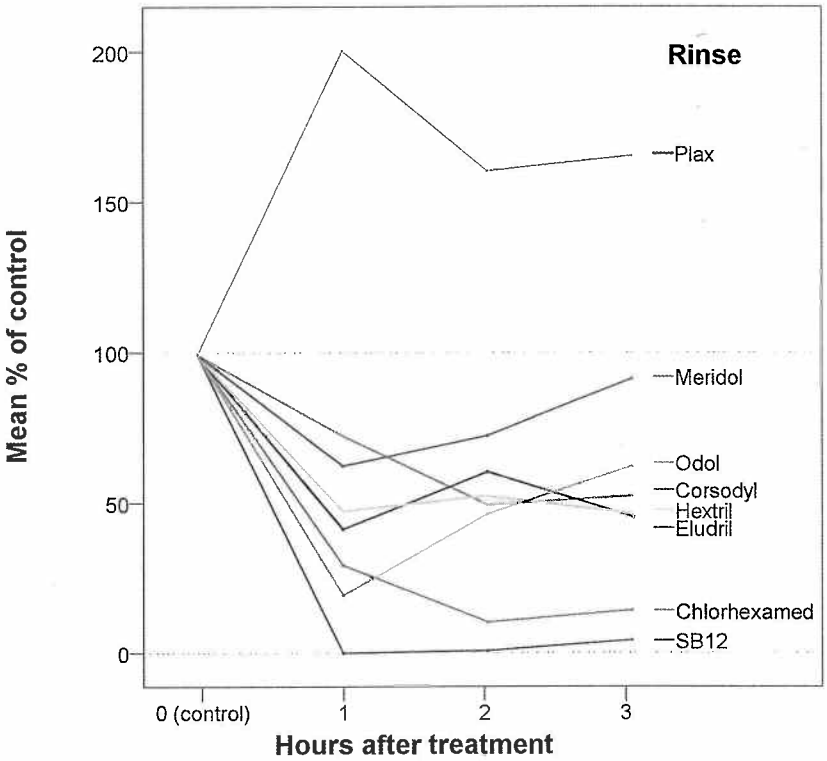

Figure 1. Graphical representation of concentration of H_2S in breath samples taken 1, 2 and 3 hours after use of various mouthrinses as a mean % of control

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.

References

1. van den Broek AMWT, Feenstra L, de Baat C. A review of the current literature on management of halitosis. *Oral Diseases* 2008; **14**: 30-39.

2. Jonski G, Young A, Wåler SM, Rölla G. Insoluble zinc, cupric and tin pyrophosphates inhibit the formation of volatile sulphur compounds. *Eur J Oral Sci* 2004; **112**: 429-32.

3. Kleinberg I, Westbay G. Oral malodour. *Crit Rev Oral Biol Med* 1990; **1**: 247-259.

4. Scully C, El-Maaytah M, Porter SR, Greenman J. Breath odor: aetiopathogenesis, assessment and management. *Eur J Oral Sci* 1997; **105**: 287-293.

5. Quirynen M. Management of oral malodour. *J Clin Periodontol* 2003; **30**(Suppl 5): 17-18.

6. Suarez FL, Furne JK, Springfield J, Levitt MD. Morning breath odor: influence of treatments on sulfur gases. *J Dent Res* 2000; **79**(10): 1773-77.

7. Niles HP, Gaffar A. Relationship between sensory and instrumental evaluation of mouth odor. *J Soc Cosmet Chem* 1999; **44**: 101-107.

8. Radcliff PA, Johnson PW. The relationship between oral malodour, gingivitis and periodontitis. A review. *J Periodontol* 1999; **70**: 485-489.

9. Young A, Jonski G, Rölla G. The oral anti-volatile sulphur compound effects of zinc salts and their stability constants. *Eur J Oral Sci* 2002; **119**: 31-34.

10. Yaegaki K, Suetaka T. The effect of zinc chloride mouthwash on the production of oral malodour, the degradations of salivary cellular elements, and proteins. *J Dent Health* 1989; **39**: 377-386.

11. Young A, Jonski G, Rölla G. Inhibition of orally produced volatile sulfur compounds by zinc, chlorhexidine or cetylpyridinium chloride – effect of concentration. *Eur J Oral Sci* 2003; **111**(5): 400-404.

12. Winkel EG, Roldan S, van Winkelhoff AJ, Herrera D, Sanz M. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc-lactate on oral halitosis. A dual-center, double blind placebo-controlled study. *J Clin Periodontol* 2003; **30**: 300-306.

13. Rosenberg M. Clinical assessment of bad breath: Current concepts. *J Am Dent Assoc* 1996; **127**: 475-482.

14. Acceptance Program Guidelines Products Used In the Management of Oral Malodor. American Dental Association. Council on Scientific Affairs 2003. Available at: http://www.ada.org/ada/seal/standards/index.asp

15. Kleinberg I, Codipilly DM. Cysteine challenge testing: a powerful tool for examining oral malodour processes and treatments *in vivo*. *Int Dent J* 2002; **52**: 221–228.

16. Thrane PS, Young A, Jonski G, Rölla G. A new mouthrinse combining zinc and chlorhexidine in low concentrations provides superior efficacy against halitosis compared to existing formulations: A double-blind clinical study. *J Clin Dent* 2007; **18**: 82-86.

17. Yaégaki K, Sanada K. Volatile sulphur compounds in mouth air from clinically healthy subjects and patients with periodontal disease. *J Periodont Res* 1992; **27**: 233-238.

18. Rosenberg M, Kulkarni GV, Bosy A et al. Reproducibility and sensitivity of oral malodour measurements with a portable sulphide monitor. *J Dent Res* 1991; **70**: 1436-1440.

19. Young A, Jonski G, Rölla G. Combined effect of zinc ions and cationic antibacterial agents on intraoral volatile sulphur compounds (VSC). *Int Dent J* 2003; **53** (4): 237-242.

20. Young A, Jonski G, Rölla G, Waaler SM. Effects of metal salts on the oral production of volatile sulfur-containing compounds (VSC). *J Clin Periodontol* 2001; **28**: 776-781

21. Thrane PS, Jonski G, Young A, Rölla G. Twelve-hour duration of effect on morning breath odour confirmed for a mouthwash combining low concentrations of zinc and chlorhexidine. *Dental Health* 2008; **3**: 8-12.

22. Roldan S ,Winkel EG, Herrera D, Sanz M, van Winkelhoff AJ. The effect of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc lactate on the microflora of oral halitosis patients: a dual-centre, double-blind placebo-controlled study. *J Clin Periodontol* 2003; **30**: 427-434.

23. Addy M. Chlorhexidine compared with other locally delivered antimicrobials. A short review. *J Clin Periodontol* 1986; **13**: 957–964.

24. Ellingsen JE, Rölla G Eriksen HM. Extrinsic stain caused by chlorhexidine and other denaturing agents. *J Clin Periodontol* 1982; **9**: 317-322.

25. Rölla G. Oral composition for inhibiting oral malodor. *United States Patent*, Patent No: US 6,344,184, B1, Feb.5, 2002, Assignee: In2Group SVAB.

26. Winkel EG, Roldan S, van Winkelhoff AJ, Herrera D, Sanz M. Clinical effects of a new mouthrinse containing chlorhexidine, cetylpyridinium chloride and zinc lactate on oral halitosis. A dual-center, double blind placebo-controlled study. *J Clin Periodontol* 2003; **30**: 300-306.

27. Angelillo I, Nobile G, Pavia M. Evidence of clinical effect of Plax prebrushing rinse on oral health is inconclusive. *J Evid Base Dent Prac* 2003, **3**: 8-9.