The effect of different mouth rinse products on intra-oral halitosis

Abstract: Aim: To evaluate the effect of different mouth rinses 12 h after rinsing on genuine intra-oral halitosis. Materials and Methods: Twenty-four adults with halitosis were included in a double-blind, crossover, randomized clinical trial. Halitosis was evaluated 12 h after rinsing with placebo and five mouth rinse products containing zinc acetate and chlorhexidine diacetate; zinc lactate, chlorhexidine and cetylpyridinium chloride; zinc acetate and chlorhexidine diacetate with reduced amounts of mint and menthol; zinc acetate and chlorhexidine diacetate with reduced amounts of mint and menthol; and chlorine dioxide using the organoleptic method and a gas chromatograph. Test periods were separated by 1 week. Results: Hydrogen sulphide (H$_2$S), methyl mercaptan (MM) and the organoleptic scores (OLS) were significantly reduced 12 h following rinsing with all substances compared to placebo ($P < 0.05$). H$_2$S was more effectively reduced after rinsing with zinc acetate and chlorhexidine diacetate and zinc acetate and chlorhexidine diacetate with reduced amounts of mint and menthol compared to rinsing with zinc chloride and essential oil ($P < 0.05$), and significantly lower values of MM were obtained after rinsing with zinc acetate and chlorhexidine diacetate compared to zinc lactate, chlorhexidine and cetylpyridinium chloride ($P < 0.05$). The percentage effectively treated individuals (H$_2$S ($<$112 ppb), MM ($<$26 ppb) and OLS score $<$2) varied from 58% percentage (zinc acetate and chlorhexidine diacetate) to 26% (zinc chloride and essential oil). Conclusion: All treatments resulted in reduction in halitosis 12 h after rinsing compared to placebo. H$_2$S and MM were most effectively reduced by zinc acetate and chlorhexidine diacetate.

Key words: bad breath; halitosis; hydrogen sulphide; mouth rinses; volatile sulphur compounds

Introduction

Genuine halitosis is subdivided into extra-oral and intra-oral halitosis (1). Available data indicate that 10–30% of the population may have a significant problem with intra-oral halitosis (2, 3). Individuals with intra-oral halitosis present higher concentrations of volatile sulphur compounds (VSCs), such as hydrogen sulphide (H$_2$S), methyl mercaptan (MM) and dimethyl sulphide (DMS) in air from the oral cavity (4). The oral cavity is considered as the major source for intra-oral halitosis (5, 6). Data have indicated that H$_2$S and MM are the main VSCs in participants with intra-oral halitosis, whereas DMS is the main VSC in participants with extra-oral halitosis (5, 7).

Different methods have been used to diagnose intra-oral halitosis. The organoleptic scoring system (OLS) is a subjective method evaluating the strength of halitosis in exhaled air using a scale between 0 and 5 (8).
Recently, more objective methods have been introduced to assess the presence of volatile sulphur compounds in exhaled air. A combined total sum of the volatile sulphur compounds (T-VSCs) in exhaled air can be measured using a sulphide monitor (Halimeter® Interscan Corporation Chatsworth, CA, USA). The T-VSC is measured in parts per billion (ppb) (8). Using a simplified gas chromatograph (OralChroma™, Abilit Corporation, Osaka, Japan), three different gases related to intra-oral halitosis (H₂S, MM and DMS) can be measured separately (9–11).

Different substances have been used in the treatment of intra-oral halitosis (12–14). Mouth rinses containing metal salts, essential oils, chlorhexidine, chlorine dioxide and cetlypyridinium chloride have been shown to reduce VSCs (14–16). Some of these agents are also known to have an antibacterial effect (16–20). Mouth rinses with a combination of different agents, claiming to reduce intra-oral halitosis, are presently available on the market. There are, however, few randomized controlled trials comparing the effectiveness of different mouth rinses on intra-oral halitosis (13).

The aim of the present randomized double-blind clinical trial was to compare the outcome after rinsing with different commercially available mouth rinse products and with a placebo in patients with genuine oral halitosis.

Materials and methods

Advertisement was made at message boards and at the web page of the University of Kristianstad, Sweden, to recruit participants for the study. The individuals were screened, and to be included, they had to present with true intra-oral halitosis as defined below. These screening appointments were performed at different time points of the day between eight and two o’clock.

The study was approved by the Ethical Committee at the University of Lund (Etik 2009/411). The study was performed between August and November 2009 at the Department of Oral Health Sciences, Kristianstad University.

The following inclusion and exclusion criteria were used:

**Inclusion criteria:**
- (i) halitosis of intra-oral origin,
- (ii) an organoanleptic score ≥2 (8),
- (iii) a level of T-VSC >160 ppb determined with a portable sulphur compound detector (Halimeter®) and
- (iv) values exceeding the cut-off levels as recommended by the manufacturer for at least two of the three examined gases by the OralChroma™ (H₂S 112 ppb, MM 26 ppb and DMS 8 ppb) (11).

**Exclusion criteria:**
- (i) untreated periodontitis, defined as the presence of more than one periodontal pocket with a probing pocket depth ≥ 6 mm,
- (ii) open carious lesions,
- (iii) pregnancy,
- (iv) systemic medication related to oral dryness,
- (v) systemic antibiotic therapy within the preceding 3 months of the study,
- (vi) a current smoking habit and
- (vii) extra-oral halitosis.

A total of 32 individuals underwent a separate screening visit to verify that they fulfilled the inclusion criteria. This screening visit was performed during daytime. Twenty-four healthy adults (17 females) fulfilling the inclusion and exclusion criteria were selected in the study. The treatment procedures were fully explained to the participants who met the inclusion criteria. Before entering the study, the participants signed an informed consent. A detailed medical history was obtained. The participants were given a detailed written instruction on how to behave before rinsing with the solution, that is (i) not consuming any food containing onions, garlic or hot spices during the prior 48 h, (ii) not drinking alcoholic beverages in the preceding 12 h, (iii) not using a tongue scraper during the study, (iv) not using any other mouth rinse during the study, (v) not using scented cosmetics or aftershave lotions in the morning of the examination and (vi) not to eat or drink after rinsing with the solution until after the examination 12 h later the following day.

All participants participated in six test periods, each with duration of 12 h. The test periods were separated by a washout period of 1 week. The participants received the rinse solutions in coded bottles according to the randomization in Table 1. A computer-based randomization program IBM SPSS 18.0 (IBM, corporations Somers, NY, USA) was used to randomize the participants in a different order for the six study protocols. The solutions were delivered in small non-transparent coded bottles and packed in envelopes with instructions on how long and how much of the solution the participants should use for rinsing. These instructions were in accordance with the manufacturers’ recommendations for the different test solutions.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Water)</td>
<td>B (SB12®), C (Halita®), D (SB12® Mild), E (Listerine® Total Care) and F (RetarDEX®),</td>
</tr>
</tbody>
</table>

Table 1. Presentation of Latin square design, the procedure of products used
rinsing time and the amount of solution used for each product is presented in Table 2.

The study participants were instructed to rinse with the solutions in the evening after brushing their teeth 12 h before their appointment the following day. They received a text message by phone to remind them of when to rinse in the evening. They were not allowed to eat or drink anything after rinsing with the solution until after the examination 12 h later the following day. The participants returned the bottle with the remaining rinse solution at the examination appointment 12 h after rinsing.

Registrations

The following registrations were made at the clinic 12 h after rinsing: (i) evaluation of organoleptic scores using an arbitrary 0–5 scale (0 = no halitosis to 5 = offensive halitosis) (8), (ii) measurements of H2S, MM and DMS levels in air from the oral cavity using a portable gas chromatograph (OralChroma™) and (ii) the participants made a self-evaluation of the treatment effect using a VAS scale from 0 to 100. One and the same investigator (SEA) performed all the registrations except the self-evaluation which was performed by de
cient subjects.

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 participants should provide statistical power (85%). The statistical package SPSS 18.0 for Windows was used for all analyses. The Kolmogorov–Smirnov test was used to test for normal distribution of the data. A Kruskal–Wallis ANOVA was employed to determine differences by treatment and this was followed using Wilcoxon signed-rank test to determine whether significant differences existed among the five active treatments (B–F) and for each active treatment (B–F) compared to the placebo (A). The same test was used for self-evaluation of the treatment effect. The statistics was performed by a statistician who was blinded to group assignment. Significance was declared at \( P < 0.05 \).

Results

Seventeen female and seven male participants with a mean age of 48.6 years (SD: ± 11.0, range 31–68) participated in the study. All 24 participants completed the study.

The mean ± SD and median values of the organoleptic scores are presented in Table 3. The statistical analyses demonstrated a significant difference 12 h after rinsing with solutions B, C, F \((P < 0.001)\), D \((P < 0.01)\) and E \((P < 0.05)\), respectively, compared to the non-active treatment A. No statistical difference was found between the different active treatments when evaluated by organoleptic scoring.

Measurements of H2S, MM and DMS using the OralChroma™

The mean ± SD and median values for H2S, MM and DMS are presented in Table 3. The H2S values were significantly reduced for all the treatments compared to the non-active control treatment. Comparing treatment effects, the active treatments B and D reduced H2S more than treatment E \((P < 0.05)\). Methyl mercaptan was also significantly reduced following rinsing with the active solutions in comparison with the non-active treatment. A significant difference in reduction in MM was found between the active treatments B and C \((P < 0.05)\). The DMS was significantly reduced by all active treatments except E compared to the non-active treatment. Product F was more effective in reducing DMS compared to treatments, B, C, D \((P < 0.01)\) and treatment E \((P < 0.05)\).

Self-assessment of intra-oral halitosis

The mean ± SD and median values for the self-evaluation are presented in Table 3. A significant difference in self-assessment scores was reported following active treatments B and E \((P < 0.01)\) as well as C and D \((P < 0.05)\) compared to the non-active treatment A. The participants considered treatment B \((P < 0.05)\) and E \((P < 0.01)\) to be significantly better then treatment F.

Table 2. Presentation of products used, their active ingredients, volume and time used when rinsing

<table>
<thead>
<tr>
<th>Product</th>
<th>Active ingredients</th>
<th>Volume used</th>
<th>Rinsing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Water</td>
<td>10 ml</td>
<td>1 min</td>
</tr>
<tr>
<td>B</td>
<td>Zinc acetate (0.3%) and chlorhexidine diacetate (0.025%)</td>
<td>10 ml</td>
<td>1 min</td>
</tr>
<tr>
<td>C</td>
<td>Zinc lactate (0.14%), chlorhexidine (0.05%) and cetylpyridinium chloride (0.05%)</td>
<td>15 ml</td>
<td>1 min</td>
</tr>
<tr>
<td>D</td>
<td>Zinc acetate (0.3%) chlorhexidine diacetate (0.025%) (less amount of mint and menthol then SB12®)</td>
<td>10 ml</td>
<td>1 min</td>
</tr>
<tr>
<td>E</td>
<td>Zinc chloride (0.9%) and essential oil (thymol, eukalypitol, methyl salicylate)</td>
<td>20 ml</td>
<td>30 s</td>
</tr>
<tr>
<td>F</td>
<td>Chlorine dioxide</td>
<td>10 ml</td>
<td>30 s</td>
</tr>
</tbody>
</table>

A (Water), B (SB12®), C (Halita®), D (SB12® Mild), E (Listerine® Total Care) and F (RetarDEX®).
Effectively treated 12 h after rinsing

To evaluate efficacy of treatment cut-off levels for H₂S (<112 ppb), MM (<26 ppb) and OLS, score <2 was used to define a successful treatment 12 h after rinsing. The percentage effectively treated individuals 12 h after rinsing varied from 58% (solution B) to 26% (solution E) (Table 4).

Discussion

The present study included only non-smoking individuals with genuine intra-oral halitosis. The majority of the individuals in this study were female. It is possible that this could have influenced the results as it has been reported that VCS sores may be related to the menstrual cycle. Calil et al. (2008) reported the oral concentration of VSC scores to be higher in women in the premenstrual and menstrual phases in comparison with the follicular phase and in men. However, although the menstrual cycle has been pointed out as a factor that may influence VSC scores, this relationship has not yet been clarified (21).

Table 3. Overview of the median, 25th and 75th percentiles, mean values, standard deviation (SD) and statistically significant differences of the different treatments. Both the results of the statistical comparison between the five active treatments (B-F) and comparison between the placebo (A) and each active treatment are shown

<table>
<thead>
<tr>
<th>Variable</th>
<th>Values</th>
<th>Treatment</th>
<th>Significance between active treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>OLS</td>
<td>Median</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>25th%</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>75th%</td>
<td>3.0</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>H₂S</td>
<td>Median</td>
<td>338.0</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>25th%</td>
<td>177.0</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>75th%</td>
<td>903.8</td>
<td>84.3</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>490.8</td>
<td>67.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>432.5</td>
<td>129.3</td>
</tr>
<tr>
<td>MM</td>
<td>Median</td>
<td>105.0</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>25th%</td>
<td>46.8</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>75th%</td>
<td>230.5</td>
<td>62.3</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>184.3</td>
<td>46.0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>247.7</td>
<td>63.9</td>
</tr>
<tr>
<td>DMS</td>
<td>Median</td>
<td>26.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>25th%</td>
<td>14.0</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>75th%</td>
<td>60.0</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>37.4</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>33.5</td>
<td>27.8</td>
</tr>
<tr>
<td>Self-assessment</td>
<td>Median</td>
<td>7.1</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>25th%</td>
<td>5.0</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>75th%</td>
<td>8.6</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>6.8</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.3</td>
<td>2.4</td>
</tr>
</tbody>
</table>
The intention of this study was to include individuals with a genuine halitosis. To find such participants, several individuals had to be screened. To categorize individuals into the halitosis group eligible to be included in the study, cut-off levels that previously had been found reliable were used. (8, 11). The crossover study design using a one-week washout between test periods has been demonstrated to be useful. Using this model, design statistical analyses failed to identify differences in baseline values of H2S, MM and DMS by four treatment periods separated by a one-week washout (23).

In the present study, three different methods were used to register intra-oral halitosis. OLS have been considered to be the golden standard (24). The method is relatively easy to perform but requires training of the examiner. It should, however, be noticed that it is a subjective method and it may therefore be valuable to combine different methods when assessing intra-oral halitosis. A high correlation between the three methods to evaluate oral halitosis used in this study was reported by Saad et al. (25). The authors concluded that any of these methods on its own could be sufficient in evaluating oral halitosis. The sulphide monitor has a high sensitivity for H2S, but a low sensitivity for MM. The use of a gas chromatograph has the advantage to distinguish between different gases related to oral halitosis. The use of OLS scores, often considered as the gold standard, may be affected by menthol and other flavours used in the mouth rinses. To increase the reliability of the results in this study, both the OLS scoring system and the more objective gas chromatograph were used.

To reduce the possible effects of bias, the clinical examiner in this randomized clinical trial was at all times blinded to the sequence of group assignment. The rinse products were bottled in the same type of non-transparent bottles, and the rinse instructions were delivered together with the rinse solution in a coded envelope to keep the examiner and participant unaware of the product used. Additionally, the organoleptic scores were always obtained before using the gas chromatograph.

The results from the present study demonstrated that the tested mouth rinses were all effective in reducing VSCs.
(38) reported up to 12 h reduction in VSCs. Our study accordingly confirms the results previously reported on the effectiveness of rinse solution C.

Treatment F in the present study significantly reduced H₂S, MM, DMS and OLS compared to water and was significantly better in reducing DMS compared to all other active treatments tested. This may, however, be of limited value for the individuals suffering from intra-oral halitosis as Tangerman & Winkel (5) reported that DMS is the main contributor to intra-oral halitosis. Several studies have demonstrated that chlorine dioxide reduces oral halitosis (37, 39–43). Rinsing with a solution containing chlorine dioxide reduced H₂S, MM, and DMS and OLS up to 4 h (39, 44) and 6–11 h after rinsing (37, 40). These and our results differ from the ones reported by Silwood et al. (41) who noted a relapse to baseline values 5 h after rinsing with a solution containing chlorine dioxide. This could not be confirmed in the present study. On the contrary solutions, B and F were the ones resulting in the best sustained reduction in intra-oral halitosis 12 h after rinsing.

In this study, an attempt to evaluate efficacy of treatment was performed. Individuals presenting with scores below the cut-off scores used to define oral halitosis in conjunction with an OLS score below 2 was considered effectively treated. The composite outcome variable may be debated, but it still gives an indication on the overall treatment outcome.

Self-evaluation of oral halitosis is difficult and self-perceived results should be interpreted with caution (45, 46). Nevertheless, a self-perceived effect may be of importance when the individual is about to choose between different treatment options. In this study, treatments B and E were considered by the participants to be more effective than treatment F. One reason for this may be that treatment F has no added flavour and/or fragrance to give the user a sensation of freshness like other mouth rinses may do (39).

Conclusion

Compared to placebo 12 h after rinsing, the tested active treatments were effective in reducing intra-oral halitosis. H₂S and MM were most effectively reduced by treatment B.

Clinical relevance

Scientific rationale for study

Intra-oral halitosis is a significant problem for many individuals. Mouth rinses containing zinc and antimicrobial agents have been used to reduce volatile sulphur compounds in the exhaled air. This study aimed at evaluating the effect of different mouth rinses with varying ingredients 12 h after rinsing in patients with diagnosed intra-oral halitosis.

Principal findings

All active treatments resulted in a reduction in intra-oral halitosis compared to placebo 12 h after use. Higher reductions in hydrogen sulphide and methyl mercaptan (MM) were obtained after rinsing with a zinc acetate- (0.3%) and chlorhexidine diacetate (0.025%)-containing mouth rinse. A chlorine dioxide-containing rinse most effectively reduced dimethyl sulphide.

Practical implications

In participants with intra-oral halitosis, a mouth rinse containing zinc acetate/lactate, chlorhexidine, cetylpyridinium chloride or chlorine dioxide could be recommended for daily use.

Acknowledgement

The authors would like to acknowledge the financial support from Antula Health Care AB, Stockholm, Sweden, and The Research foundation at Kristianstad University, Kristianstad, Sweden.

Conflict of interest

Antula Health Care AB, Stockholm, Sweden, partially supported this trial. Antula Health Care AB was, after the study was completed, acquired by Meda OTC AB. Professor Renvert is presently consulting for Meda OTC AB. The other authors declare no conflict of interest.

Source of funding

The study was supported by the Research Foundation at Kristianstad University, Sweden. The study was also partly supported by a research grant from Antula Health Care AB, Stockholm, Sweden.

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