Efficacy of a 2-Phase Oil:Water Mouthrinse in Controlling Oral Malodor, Gingivitis, and Plaque


The purpose of the study was to examine the anti-malodor, anti-gingivitis, and plaque reducing properties of a 2 phase oil:water mouthrinse compared with a control mouthrinse. Fifty subjects rinsed with one of the two rinses for 30 seconds twice a day over 6 weeks, while continuing their normal oral hygiene habits. Measurements were made at time zero (prior to beginning the rinsing regimen), and ≥ 9 hours following rinsing, at intervals of 1, 3, and 6 weeks. Malodor of whole mouth, as well as tongue dorsum anterior and posterior, was assessed on a 0 to 5 semi-integer scale by two odor judges. Volatile sulphide compounds (VSC) were determined using a sulphide monitor. Gingival, plaque, and bleeding indices were recorded for Ramfjord teeth. Oral microbial levels were assessed using the oratost. Salivary levels of dianines (putrescine and cadaverine) were analyzed by HPLC. Results were analyzed by 2-tailed covariant ANOVA, with the time zero value as covariant. Dramatic improvements were observed in parameters associated with malodor, periodontal health, plaque accumulation, and microbial levels in both groups. As compared to time zero scores, whole mouth odor, tongue dorsum anterior and posterior odors decreased continuously over time, attaining 80%, 79% and 70%, reductions, respectively following 6 weeks, in the 2-phase mouthrinse group, versus 70%, 77% and 59% for the control group. For whole mouth and tongue dorsum posterior, the reductions observed in the 2-phase mouthrinse group were significantly greater than those obtained with the control mouthrinse (P = 0.026 and P = 0.025, respectively), suggesting that the 2-phase mouthrinse is superior to the control mouthrinse in long-term reduction of oral malodor. For bleeding index, gingival index, oral microbial levels, and VSC, differences between the groups were not significant. Diamine levels were not significantly reduced in either group. The control mouthrinse reduced plaque index more significantly than the 2-phase mouthrinse (P < 0.005). The results of this randomized clinical trial suggest that the 2-phase oil:water mouthrinse formulation is superior to the control mouthrinse in long-term reduction of oral malodor. J Periodontol 1996;67:577–582.

Key Words: Halitosis/prevention and control; gingivitis/prevention and control; dental plaque/prevention and control; mouthrinses/therapeutic use; comparative study.

Mouthrinsing is a common oral hygiene practice dating back to ancient times. The major concern which leads to frequent use of mouthrinses is halitosis (bad breath). Bad breath usually originates within the oral cavity itself, due to production of gases (primarily volatile sulphide compounds, VSC) by sequestered deposits of microorganisms, generally under anaerobic conditions. Periodontal diseases appear, in most studies, to be related to bad breath. Other loci of oral microbial putrefaction include the tongue dorsum, areas of food impaction, and improper dental restorations. Secondary reasons for use of a mouthrinse include control of plaque and gingivitis when used as an adjunct to mechanical means.

Much of the evidence of the efficacy of mouthrinses in reducing bad breath is anecdotal, and there are very few publications in the scientific literature on this subject. Long-term malodor reduction has rarely, if ever, been addressed. For example, in investigations carried out by
Pitts et al., Schmidt and Tarbet, and Tonzetsich data on malodor reduction were collected only up to 3 hours following use.

Research has shown that 2-phase, oil/water formulations containing cationic surface-active agents such as cetlypyridinium chloride (CPC), efficiently bind and desorb oral microorganisms. Two studies have been published on the anti-malodor effects of a prototype 2-phase mouthwash formulation of this type. In the first study, Rosenberg et al. compared the 2-phase prototype rinse with a placebo rinse and an antiseptic mouthrinse containing 0.2% chlorhexidine gluconate. Subjects performed oral rinsing prior to bedtime and in the morning of the following day. Measurements were taken in the late afternoon (e.g., at least 8 hours following rinsing) of the second day and were compared to those taken the previous day afternoon prior to rinsing. The data demonstrated that mouthwashing with the 2-phase mouthwash resulted in a daylong reduction in bad breath-associated parameters. In another study, Yaegaki and Sanada showed dramatic reductions in volatile sulphide levels 3.5 hours after a single use of the same prototype mouthrinse, as compared to time zero measurements, as well as in comparison to a commercial mouthwash.

Since these experiments were performed, a reformulated 2-phase oil/water mouthrinse containing CPC was introduced in the Israeli market. The purpose of the present study was to test the efficacy of this mouthrinse in controlling oral malodor, plaque, and gingivitis over a 6-week period, as compared with a mouthrinse which has been previously shown to be effective in reducing levels of odor-related microorganisms, as well as plaque and gingivitis.

**MATERIALS AND METHODS**

The experiment consisted of a 6-week, randomized clinical trial of 50 volunteers (mean age 24 years; 37 females). Volunteers were recruited by advertisements placed on the campus of Tel Aviv University and were remunerated. Smokers and partial denture wearers were excluded. Following randomized distribution into one of the two mouthrinse groups, subjects received either the 2-phase mouthwash (N = 26) or the control mouthrinse (N = 24). Volunteers rinsed morning (following breakfast and toothbrushing) and evening (directly prior to bedtime, following toothbrushing). Each rinse consisted of 30 seconds vigorous mixing in the mouth and gargling. For the 2-phase mouthwash, volunteers were instructed to shake the bottle before use. Volunteers were asked to refrain from rinsing with water, eating, or drinking, for at least 30 minutes following rinsing. During the course of the 6-week trial, the participants continued their usual oral hygiene and dietary habits but were instructed to refrain from using other commercial mouthrinses. Measurement days were day zero (baseline prior to rinsing), and 1, 3, and 6 weeks. Volunteers were not subjected to any prophylactic treatment. On measurement days, volunteers were instructed not to apply scented products; not use the mouthrinse before arrival; and to refrain from eating, drinking, or chewing gum for at least 2 hours prior to examination.

**Measurement Parameters**

*Soft tissue examination.* A complete intraoral soft tissue examination was carried out at each appointment to evaluate the condition of the oral mucosa. The buccal, labial, and sublingual mucosa; the tongue; the hard and soft palate; the uvula; and the oropharynx were examined for inflammation, ulcerations, or other lesions. Aberrations were recorded, their severity assessed, and a judgement made as to whether they were attributable to the mouthrinse preparations and regimens utilized.

**Gingival index.** Modified gingival index was determined according to Lobene et al., employing Ramfjord teeth. Results were scored as mean of all surfaces examined.

**Plaque index.** Plaque area was scored by the Turesky modification of the Quigley-Hein index, employing Ramfjord teeth. Results were scored as mean of all surfaces examined.

**Bleeding index.** Papillary bleeding index was determined according to Muhlemann, employing Ramfjord teeth. Results were scored as mean of all surfaces examined.

**Volatile sulfur compounds (VSC).** Quantitative measurement of intraoral volatile sulphones (VSC) was carried out using a sulphide monitor 1 ppm full-scale, connected to a pen recorder. Measurement was essentially as previously reported, except that disposable straws, rather than teflon tubing, were employed. Volunteers were asked to refrain from talking for several minutes prior to measurement. The monitor was zeroed on ambient air, and measurement performed by inserting a disposable ¼” plastic straw approximately 4 cm into the partially opened oral cavity. The volunteer was instructed to breathe through his/her nose during measurement. Results were recorded as peak ppb sulphide equivalents.

**Oral microbial levels.** Oral microbial levels were determined using the orat test, a technique which measures the rate of oxygen depletion in expectorated milk samples. The technique has been previously described and shown to correlate with microbial counts and clinical parameters. Briefly, volunteers rinsed for 30 seconds with 10 mL of sterile milk. Following expectoration, 3 mL of the samples were added to test tubes containing.

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1 Assuta, Shemen Stool Industries, Ltd., Haifa, Israel.
2 Listerine, Warner-Lambert, Morris Plains, NJ.
3 Model 1170, Interscan Corp., Chatsworth, CA.
Table 1. Reductions in Malodor Related Parameters over Time*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole mouth (scale of 0-5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>2.14 ± 0.88</td>
<td>0.85 ± 0.83</td>
<td>0.69 ± 0.69</td>
<td>0.42 ± 0.55</td>
</tr>
<tr>
<td>Control</td>
<td>2.40 ± 1.00</td>
<td>1.38 ± 0.84</td>
<td>1.29 ± 0.78</td>
<td>0.71 ± 0.64</td>
</tr>
<tr>
<td>Tongue dorsum anterior (scale of 0-5)</td>
<td>1.45 ± 0.85</td>
<td>not determined</td>
<td>0.59 ± 0.39</td>
<td>0.30 ± 0.26</td>
</tr>
<tr>
<td>Control</td>
<td>1.91 ± 0.98</td>
<td>not determined</td>
<td>0.95 ± 0.66</td>
<td>0.43 ± 0.45</td>
</tr>
<tr>
<td>Tongue dorsum posterior (scale of 0-5)</td>
<td>2.50 ± 0.79</td>
<td>not determined</td>
<td>1.34 ± 0.86</td>
<td>0.75 ± 0.60</td>
</tr>
<tr>
<td>Control</td>
<td>2.87 ± 1.04</td>
<td>not determined</td>
<td>1.95 ± 0.76</td>
<td>1.18 ± 0.82</td>
</tr>
<tr>
<td>Peak volatile sulphide (ppb hydrogen sulphide equivalents)</td>
<td>94 ± 36</td>
<td>58 ± 14</td>
<td>52 ± 11</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>Control</td>
<td>79 ± 40</td>
<td>69 ± 33</td>
<td>58 ± 16</td>
<td>56 ± 16</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± standard deviation.
*Significantly lower over time as compared with control (P = 0.026).
**Significantly lower over time as compared with control (P = 0.025).

0.12 mL of methylene blue solution (0.1%), and the time required for a blue-to-white color change over a 6 mm diameter at the bottom of the test tube recorded. The test was performed directly following the VSC measurements.

Odor judge measurements. Organoleptic measurements were made by two odor judges, based on the whole mouth expiration, as well as odor assessment from the anterior and posterior of the tongue dorsum.27,28 For whole mouth malodor, following a 2-hour fast, subjects were instructed to exhale briefly through the mouth, at a distance of about 10 cm from the nose of the judge. Odor from the anterior tongue dorsum was determined by having the volunteers lick their wrist with their extended tongue. After 5 seconds, the odor judge smelled the odor at a distance of about 5 cm from the wrist of the volunteer. For assessment of the posterior tongue dorsum, samples were obtained by mild scraping of the area with a plastic spoon. Following 5 seconds, the odor judge scored the odor at a distance of about 5 cm from the spoon. Results of each malodor assessments were rated on a semi-integer scale from 0 to 5 with descriptions as follows: 0: no appreciable odor; 1: barely noticeable odor; 2: slight, but clearly noticeable odor; 3: moderate odor; 4: strong odor; 5: extremely foul odor. The average of the two judges scores was used for each measurement. Both judges were blinded to one another’s scores, as well as to the mouthwash used by each volunteer.

Diamine analysis. Unstimulated saliva samples (200 μL) were passed through 0.45 μm pore filters and were then analyzed essentially as described by Goldberg et al.29 Samples from 8 subjects could not be filtered and were not included in the analysis. For high performance liquid chromatography (HPLC) analysis, samples were first derivitized using O-phthalaldehyde which was prepared by dissolving 50 mg of O-phthalaldehyde in 4.5 mL methanol, together with 0.5 mL borate buffer (0.9 M, pH = 9.5) and 50 μL of β-mercaptoethanol. The solution was diluted 1:10 in methanol and kept at -20°C. Eighty μL borate buffer and 25 μL of the diluted reagent solution were added to saliva samples (20 μL). The HPLC system consisted of pump,** an injection valve equipped with a 20 μL sample loop, and a C18 reverse phase column†† (250 × 4 mm). Samples were injected at a flow rate of 1 mL/min. The mobile phase was composed of a filtered, degassed 30:70 mixture of 12.5 mM sodium phosphate buffer (pH = 7.2):acetonitrile. Detection was carried out using a UV detector‡‡ at a wavelength of 231 nm.

Statistical analysis. To approximate normal distributions, bleeding index levels, oral microbial level scores and salivary concentrations of putrescine and cadaverine were ln transformed. Statistical comparisons of these parameters were thus performed on ln transformed values.14,24-26 Since, in several instances, significant differences were found between the two groups at baseline when compared using the unpaired T test, the data were subsequently analyzed by 2-tailed ANCOVA, with repeated measures, using the time zero value as covariant. Pearson correlation coefficients were employed to test for correlations among the results of the different measurement techniques on all 50 participants at baseline and following 3 weeks.

RESULTS

Results of the oral malodor-related scores (average of judge scores of whole mouth, tongue dorsum anterior, and tongue dorsum posterior, as well as VSC), are summarized in Table 1. Results of the oral indices (gingival index, bleeding index, plaque index) and oral microbial lev-

**Eldez 9600, Eldex Laboratories, Inc. Napa, CA.
†Lichrosphere 100 PR-18, Merck, Darmstadt, Germany.
‡‡Spectro Monitor 3.200 LDC Analytical, Orlando, FL.
gingival index scores were similarly reduced in the 2-phase mouthrinse and control groups by 52% and 49%, respectively. Comparable reductions in mean bleeding index scores were also observed following 2 weeks (33% and 37% for the 2-phase mouthrinse and control groups, respectively). In the case of plaque index, highly significant reductions over time were observed in both groups, as compared to baseline ($P < 0.0001$). Whereas mean reduction in both bleeding and gingival indices were comparable in both experimental and control groups, the mean reduction in plaque index in the control group (62%) was significantly lower than the reduction observed in the 2-phase group (49%; $P = 0.005$). Highly significant increases ($P < 0.0001$) were observed in oratest level scores in both groups, indicative of reductions in microbial levels. Differences in experimental versus control scores were not significant over time.

The salivary levels of cadaverine and putrescine at baseline and following 3 weeks are compared in Table 3. In contrast to all other parameters, significant reductions were not observed in either group.

Pearson correlation coefficients were computed to compare associations among the various parameters. At baseline, significant correlations were observed between oral microbial level scores and both whole mouth odor ($P = 0.021$), as well as tongue posterior odor ($P = 0.010$); a marginal association was observed between oral microbial level results and bleeding index scores ($P = 0.062$). Putrescine levels were inversely related to tongue anterior odor scores ($P = 0.024$). As expected, diamine scores were significantly associated with one another ($P = 0.005$). Similarly, whole mouth, and tongue anterior and tongue posterior scores were all closely related to one another ($P < 0.01$). Gingival index was closely related to bleeding index ($P < 0.001$), and was associated with plaque index ($P = 0.010$) and tongue anterior odor ($P = 0.038$). Correlations were also sought in comparing the differences between baseline and 3 weeks scores for all subjects. Changes in oratest level scores were associated with changes in both tongue posterior odor ($P = 0.012$) and cadaverine ($P = 0.020$). Changes in putrescine were also associated with changes in posterior tongue odor ($P = 0.27$), as well as with changes in bleeding index scores ($P = 0.001$). The changes in levels of the two diamines following 3 weeks were highly correlated with each other ($P < 0.001$).

**DISCUSSION**

The main purpose of the present study was to test the efficacy of a novel 2-phase mouthrinse on oral malodor and dental-associated parameters. A mouthrinse with previously demonstrated effectiveness in reducing malodor, gingivitis, bleeding, and plaque and oral microbial levels was employed as a control. Subjects were measured at baseline, and were then instructed to use one of
the rinses twice a day over a 6-week period, as a supplement to their usual oral hygiene habits. Measurements were conducted at 1, 3, and 6 weeks.

The data show that the 2-phase mouthwash is highly effective in reducing bad breath parameters. Significantly greater reductions in whole mouth and tongue posterior malodor were observed in the 2-phase mouthwash group, as compared with the control mouthrinse. Considering the fact that measurements were performed 9 to 18 hours following the previous rinsing, the mean reductions observed (e.g., 80% in the 2-phase mouthwash group, following 6 weeks) are particularly noteworthy.

Highly significant and comparable reductions in gingivitis levels, as measured by gingival index, were observed in both mouthrinse groups. The mean reductions observed in the experiment and control groups (52% and 49% respectively) are comparable to the results found in a 6-week study conducted by Axelsson and Lindhe. Furthermore, reduction in bleeding and microbial load were highly significant and similar in both groups.

Although both mouthrinses significantly reduced plaque levels in comparison to baseline, the control significantly outperformed the two-phase mouthrinse, with a 63% mean reduction following 6 weeks, as compared to 49%. In the Axelsson and Lindhe study, mean plaque reduction by the control was 51%, and was found to be comparable to that achieved by 0.1% chlorhexidine (54%). The role of supragingival plaque in malodor is at present unclear. In several investigations, plaque index was significantly associated with oral malodor. However, in a more recent study, a significant correlation was not observed. Similarly, in the present study, baseline plaque levels were not associated with any of the odor-related parameters measured (P > 0.05). Since the 2-phase mouthrinse appeared more effective than the control in reducing malodor (especially with respect to the tongue dorsum posterior), but was relatively less effective in supragingival plaque reduction, we may postulate that the superior malodor-reducing effect of the 2-phase mouthrinse is related primarily to its efficacy in reducing the load of odorogenic microorganisms on the tongue dorsum posterior.

Previous investigations have suggested that the potent anti-malodor and antibacterial properties of 2-phase oil-water formulations stem mainly from the desorption of oral bacteria and debris by oil droplets. Investigations are currently underway to determine whether additional mechanisms, such as slow release of CPC, potentiated by the oil droplets, may also play a role in the efficacy of such compositions.

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