

Effect of Eucalyptus-Extract Chewing Gum on Oral Malodor: A Double-Masked, Randomized Trial

Muneo Tanaka,* Masahiro Toe,* Hideki Nagata,* Miki Ojima,* Masae Kuboniwa,* Katsumasa Shimizu,† Kenji Osawa,† and Satoshi Shizukuishi*

Background: Eucalyptus extracts were found to possess an antibacterial activity against some oral pathogens that produce oral malodor compounds in vitro; however, the clinical effects with respect to oral malodor in humans remain unproven. In the present investigation, a randomized clinical study was designed to test the hypothesis that eucalyptus-extract chewing gum can reduce oral malodor in the general adult population.

Methods: Subjects were randomly assigned to the following three groups: a high-concentration (0.6% eucalyptus extract) group (n = 32), a low-concentration (0.4% eucalyptus extract) group (n = 32), and a placebo group (n = 33). The intake period was 12 weeks. The organoleptic score, level of volatile sulfur compounds (VSCs), and tongue-coating score were recorded at baseline and 4, 8, 12, and 14 weeks. Treatment-to-time interactions among groups were evaluated by repeated-measures analysis of variance (ANOVA) followed by the Games-Howell pairwise comparison test.

Results: Relative to baseline readings, significant reductions in clinical parameters, including organoleptic and tongue-coating scores in the high- and/or low-concentration groups, occurred at 4, 8, 12, and 14 weeks ($P < 0.05$). In addition, group-time interactions revealed significant reductions in the organoleptic score, VSCs, and tongue-coating score in both concentration groups compared to the placebo group ($P < 0.05$).

Conclusions: Eucalyptus-extract chewing gum had long-term effects on the organoleptic score, levels of VSCs, and tongue-coating score. These findings suggest that eucalyptus-extract chewing gum may reduce oral malodor by decreasing the accumulation of tongue coating. *J Periodontol* 2010; 81:1564-1571.

KEY WORDS

Chewing gum; eucalyptus; halitosis; randomized controlled trial; sulfur compounds.

Halitosis is a condition commonly experienced by the general population. In $\approx 80\%$ of all cases, halitosis is caused by oral conditions. In particular, volatile sulfur compounds (VSCs) formed from coatings on the dorso-posterior tongue region are major causes of oral malodor.¹ VSCs are mainly produced through the putrefactive activities of Gram-negative strict anaerobes.² In addition, some products that do not contain sulfur compounds (e.g., volatile fatty acids, putrescine, cadaverine, and skatole) are also derived from the metabolism of peptides and amino acids by bacteria.^{2,3}

Any effective strategy with respect to the control of oral malodor is thought to include the reduction of tongue-coating accumulation and dental-plaque formation via antimicrobial treatment. Numerous studies⁴⁻⁹ appeared in the literature regarding the clinical effect of antimicrobial mouthrinse and toothpaste in the treatment of oral malodor. However, most of these studies^{4,6-8} did not involve randomized controlled trials and were conducted over short evaluation periods, often limited to a few hours. Additionally, the long-term usage of some antiseptic agents such as chlorhexidine may be complicated by the staining of teeth and the development of microbial resistance.⁹

Eucalyptus extracts have been used as a food source for many centuries. Recently, the antibacterial activity of

* Department of Preventive Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan.

† Lotte Central Laboratory, Saitama, Japan.

eucalyptus extract against several periodontopathic bacteria including *Porphyromonas gingivalis* and *Prevotella intermedia* has been documented.¹⁰ These periodontopathic bacteria were found to contribute greatly to VSC production.¹¹ In a double-masked randomized trial, Nagata et al.¹² reported that eucalyptus-extract chewing gum reduces plaque accumulation on tooth surfaces and decreases gingivitis. Thus, it was hypothesized that eucalyptus extract can decrease oral malodor in humans. Oral malodor has been the subject of considerable public interest in industrialized countries.¹³⁻¹⁵ However, subjects used in many other past investigations¹⁶⁻¹⁸ were merely patients with oral malodor or periodontal problems. Recently, it was shown that chewing gum which contains antibacterial agents may be effective in reducing oral malodor.^{19,20} However, these results were shown in the experiment in vitro or in the clinical effect in a short period. The aim of the current investigation was to evaluate the long-term effect of eucalyptus-extract chewing gum on oral malodor using volunteers from the general population in a double-masked, randomized controlled trial.

MATERIALS AND METHODS

Subjects

Between February 2006 and June 2006, participants residing in the suburbs of Osaka, Japan, were recruited through advertisements in newspapers for this study. Subject ages ranged from 20 to 50 years. These subjects were previously introduced.¹² Individuals were examined in terms of the gingival index (GI)²¹ and periodontal probing depth (PD) at Osaka University Dental Hospital, Osaka, Japan. Additionally, blood tests and urinalysis were conducted. Exclusion criteria were: 1) antibiotic treatment or periodontal treatment within the previous 3 months, and/or 2) a history of systemic disease, and/or 3) abnormal findings on blood tests and/or urinalysis (HbA1c >5.8% and/or glucoseuria positive and/or aspartate aminotransferase >40 IU/L and/or alanine aminotransferase >49 IU/L and/or γ -glutamyl transpeptidase >80 IU/L and/or urobilinogenuria positive), and/or 4) decreased number of teeth (<24 teeth), and/or 5) absence of gingivitis (GI = 0), and/or 6) existence of deep periodontal probing depth (>6 mm) at \geq one site.

All subjects provided written informed consent. The study protocol was approved by the Ethical Committee for Clinical Research of Osaka University Graduate School of Dentistry.

Chewing Gum

Chewing gum,[†] with and without eucalyptus extract, was used in this study. The components in sugarless chewing-gum tablets, other than eucalyptus extract, were identical to those found in sugarless chewing-

gum tablets currently on the market. The weight of each tablet was 1.5 g. The proportions of compounds contained in the test and placebo gums were previously described.¹²

Study Design

Eligible subjects were randomly assigned to one of the following groups by the study coordinator:[§] a high-concentration group (0.6% eucalyptus extract chewing gum [90 mg/day], a low-concentration group (0.4% eucalyptus extract chewing gum [60 mg/day], and a placebo group (chewing gum without eucalyptus extract). Randomization was performed according to the method of minimization.²² To keep the balance of the distribution of the confounder, the weight factor was set differently for the stratification factors (GI = 10, age = 8, and gender = 7). All investigators and study personnel were masked to the treatment assignment for the duration of the study.

Two weeks prior to the use of chewing gum, all participants received full-mouth supragingival scaling. During the intake period (12 weeks), subjects chewed two chewing-gum tablets for 5 minutes, five times per day. Subjects were instructed to chew the gum after three main meals and between meals (two periods); thus, a designated time to chew the gum was not indicated. Malodor assessment and an oral examination were conducted at baseline and 4, 8, 12, and 14 weeks (Fig. 1).

Flow of Subjects Through the Study

Figure 2 shows the flow of subjects through the study. One hundred subjects were randomly assigned to one of three groups: the high-concentration group (n = 33), the low-concentration group (n = 33), and the placebo group (n = 34). Three participants were excluded before the baseline examination. One individual (in the high-concentration group) was lost after the baseline examination; however, the data at baseline were included in the intention-to-treat analysis. All other subjects were followed to their final examination. As a result, 97 subjects were analyzed (high-concentration group = 50.0% male, mean age: 33.7 years, n = 32; low-concentration group = 40.6% male, mean age 33.4 years, n = 32; and placebo group = 57.6% male, mean age: 34.7 years, n = 33). No statistical differences were detected in the mean age and gender among the three groups.

Oral Examinations

A tongue-coating score was calculated by multiplying the thickness score by the area score.²³ The area was reported as a score of 0 to 3 (0 = no tongue coating, 1 = tongue coating covering less than one-third of tongue dorsum, 2 = tongue coating covering one-third to two-thirds of the tongue dorsum, and 3 = tongue coating

[†] Supplied by Lotte Central Laboratory.

[§] Japan Medical Laboratory, Osaka, Japan.

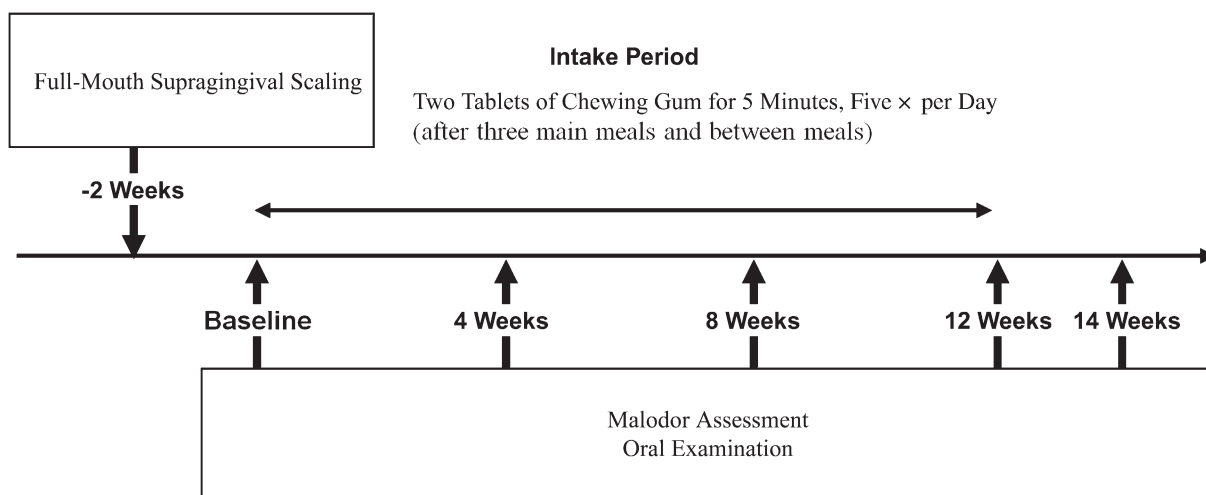


Figure 1.
Outline of the clinical study.

covering greater than two-thirds of the tongue dorsum). Thickness was reported as a score of 0 to 2 (0 = no tongue coating, 1 = thin tongue papillae visible, and 2 = thick tongue papillae invisible). A significantly high κ value ($\kappa = 0.82$; 95% confidence interval [CI]: 0.64 to 1.00; $P < 0.001$) for the tongue-coating score was obtained.

Malodor Assessment

Bad breath was assessed via the measurement of VSCs with a gas chromatograph and an organoleptic score at 4:30 pm to 6:30 pm at all patient appointments. Prior to their appointments for odor assessment, subjects were asked to refrain from oral activities including eating, drinking, and chewing for 4 hours and brushing and mouth rinsing for one half day.

For obtaining an organoleptic score, subjects remained quiet and maintained a closed mouth for a period of 30 seconds. Subjects were then requested to exhale through the mouth with moderate force into a sampling bag^{||} for 2 to 3 seconds to prevent the dilution of odor with lung and room air. This procedure was repeated three or four times at the same visit. Three evaluators (MT, MT, and HN), who were trained to perform an examination standardized by the Japan Bureau of Environmental Health, Tokyo, Japan, estimated the odor at a distance of ~10 cm from the sampling bag.²⁴ The organoleptic score was estimated based on a scale of 0 to 5; subsequently, the mean values of the three judges were used. The percentage of agreement in organoleptic scores among the three examiners was always >71% ($\kappa = 0.77$).

Levels of VSCs were analyzed with a gas chromatograph[¶] equipped with a flame photometric detector system.²³ Mouth air (10 ml) was aspirated with a gas-tight syringe. Subsequently, samples were

injected onto the gas chromatograph column at 70°C. The glass column was packed with 25% $\beta\beta$ -oxydipropionitrile on a 60- to 80-mesh support system,[#] and the glass was treated with phosphoric acid. The concentration of each sulfur compound was determined with a standard sample of hydrogen sulfide, methylmercaptan, or dimethyl sulfide prepared with a permeator.^{**} The level of VSC was defined as parts per million (ppm) of the total concentrations of hydrogen sulfide, methylmercaptan, and dimethyl sulfide.

Statistical Analyses

The sample size for this study was ≥ 30 individuals per group. In general, a smaller sample size, in the range of 10 to 20 subjects per group, is sufficient to implement the method as determined in a previously described pilot study.²⁵ The sample size was based on repeated-measures analysis of variance (ANOVA) with a significance level of 0.05, a power level of 0.8, and with an obtained effect size f (organoleptic score: 0.14; VSCs: 0.16; tongue-coating score: 0.22). The required sample size for the organoleptic score, VSCs, and tongue-coating score were 15, 12 and seven individuals per group, respectively. The sample size used in this investigation was sufficient.

Differences in variables among groups at baseline were tested with χ^2 exact tests for proportions and ANOVA for means. Dunnett tests were used to compare means at designated times with those at baseline within each group. Repeated-measures ANOVA, both unadjusted and adjusted for baseline values of the

^{||} GL Science, Tokyo, Japan.

[¶] Shimadzu GC-14B, Shimadzu, Kyoto, Japan.

[#] Chromosorb W AW-DMCS-ST, Shimadzu.

^{**} PD-1B, Gastec, Kanagawa, Japan.

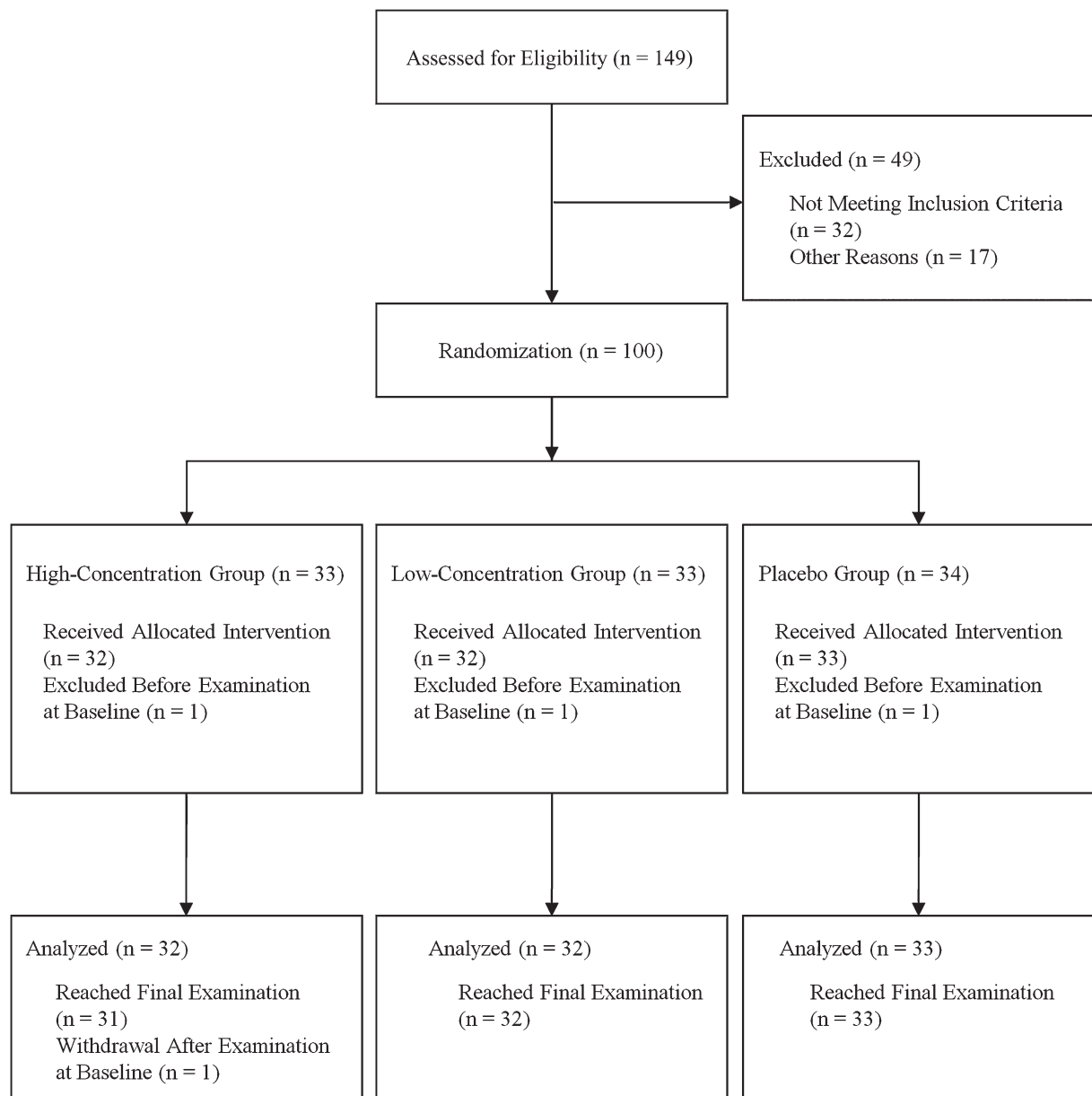


Figure 2.
Flow of patients through the study.

organoleptic score, VSCs, and tongue-coating score, was used to compare the time patterns of outcomes among the three treatment groups (i.e., the interaction of time and treatment). All statistical tests were two-sided. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline demographics are shown in Table 1. The three groups were balanced in terms of baseline characteristics. No statistical differences with respect to malodor assessments and clinical parameters at baseline were observed among the three groups. The mean values for oral malodor and clinical parameters

during the examination period are summarized in Table 2. Relative to that at baseline, the organoleptic score decreased significantly at 4, 8, 12, and 14 weeks in the low- and high-concentration groups ($P < 0.05$) but not in the placebo group. In addition, VSC levels in the high-concentration group also decreased markedly at 8 and 12 weeks ($P < 0.05$). The tongue-coating score in the high-concentration group was significantly decreased at 8, 12, and 14 weeks ($P < 0.05$) compared to baseline values. Repeated-measures ANOVA that were adjusted for baseline values of the organoleptic score, VSCs, and tongue-coating score revealed significant interactions

Table 1.
Baseline Demographics (mean \pm SD)

| Demographic | High-Concentration Group (n = 32) | Low-Concentration Group (n = 32) | Placebo Group (n = 33) | P* |
|----------------------|-----------------------------------|----------------------------------|------------------------|----|
| Males/females (n) | 16/16 | 13/19 | 19/14 | NS |
| Age (years) | 33.7 \pm 8.6 | 33.4 \pm 8.7 | 34.7 \pm 8.8 | NS |
| GI | 0.83 \pm 0.31 | 0.85 \pm 0.36 | 0.80 \pm 0.34 | NS |
| Organoleptic score | 1.85 \pm 0.55 | 1.98 \pm 0.52 | 1.72 \pm 0.63 | NS |
| Total VSCs (ppm) | 0.25 \pm 0.38 | 0.25 \pm 0.40 | 0.15 \pm 0.26 | NS |
| Tongue-coating score | 1.53 \pm 1.32 | 1.53 \pm 1.72 | 1.03 \pm 1.22 | NS |

NS = not significant.

* Chi-square exact test was used for proportions, and ANOVA was used for continuous variables.

between treatment and time in the organoleptic score, VSCs, and tongue-coating score (Table 2).

As illustrated in Figure 3, in the experimental period, high- and low-concentration groups showed significant decreases from placebo groups in the organoleptic score (high-concentration group = mean difference: -0.66 [95% CI: -1.01 to -0.30], $P = 0.0004$; low-concentration group = mean difference: -0.63 [95% CI: -0.98 to -0.27], $P = 0.0007$), VSCs (high-concentration group = mean difference: -0.23 ppm [95% CI: -0.44 to -0.03], $P = 0.0252$; low-concentration group = mean difference: -0.23 ppm [95% CI: -0.43 to -0.02 ppm], $P = 0.0292$), and tongue-coating score (high-concentration group = mean difference: -1.08 [95% CI: -1.82 to -0.34], $P = 0.0047$; low-concentration group = mean difference: -0.93 [95% CI: -1.66 to -0.19], $P = 0.0137$). Repeated-measures ANOVA revealed a significant interaction of treatment and time for the organoleptic score, VSCs, and tongue-coating score ($P < 0.001$, $P = 0.0342$, and $P = 0.0029$, respectively). We used contrast analysis for post hoc test (Games-Howell pairwise comparison test) following ANOVA to compare groups which confirmed a significant reduction in values for the high- and low-concentration groups relative to the placebo ($P < 0.05$).

DISCUSSION

The most important finding of this study is that long-term usage of eucalyptus-extract chewing gum compared to the use of a placebo reduced tongue coating and decreased oral malodor. Group-time interactions revealed significant reductions in the means of the organoleptic score, VSCs, and tongue-coating score in the high- and low-concentration groups relative to the placebo group. These results were analyzed by repeated-measures ANOVA followed by the Games-Howell pairwise comparison test. The effectiveness of eucalyptus-extract chewing gum on oral malodor

was also confirmed per the following results: statistically significant reductions in organoleptic and tongue-coating scores at 4, 8, 12, and 14 weeks and in levels of VSCs at 8 and 12 weeks relative to baseline in the high-concentration group. Other studies²⁶⁻²⁹ indicated that a mouthrinse or toothpaste that contains antiseptic ingredients produces significant reductions in VSC levels and in the organoleptic score. However, these reductions were found to be transient. In addition, the use of active antiseptic ingredients, including chlorhexidine, cetylpyridinium chloride, and triclosan, is often accompanied by side effects. In the present investigation, abnormal effects on teeth oral mucosa and tongue were not detected on visual examination or with detailed questioning of subjects regarding their oral condition. To the best of our knowledge, in the present study, we report the first long-term efficacy analysis of chewing gum that contained antimicrobial agents on oral malodor.

The control of oral malodor by reducing the responsible microorganisms and preventing the overgrowth of opportunistic pathogens was shown to be effective.⁹ Toothpastes and mouthrinses with antimicrobial properties can chemically reduce plaque accumulation by reducing the number of causative microorganisms.³⁰ In the present investigation, eucalyptus extracts functioned as an antiseptic agent. Eucalyptus extracts inhibit the growth of periodontopathic bacteria¹⁰ and affect virulence factors of *P. gingivalis*.³¹ Therefore, the reduction in the tongue-coating score in the test groups may be associated with an effect of eucalyptus extracts on oral microorganisms. Periodontal pathogens on the tongue dorsa may contribute greatly to VSC production;¹¹ furthermore, the origin of oral malodor appears to be the coating on the dorso-posterior region of the tongue.¹ Thus, a reduction in VSC and organoleptic scores may be due to the reduction of the tongue-coating score. However, Shinada et al.³² recently reported that the experimental and placebo

Table 2.
Malodor and Tongue-Coating Scores During the Follow-Up Period

| Parameter | Baseline* | 4 Weeks | 8 Weeks | 12 Weeks | 14 Weeks | <i>p</i> † |
|--------------------------|------------------|------------------|------------------|------------------|------------------|------------|
| Organoleptic score | | | | | | 0.032 |
| High-concentration group | | | | | | |
| Mean (95% CI) | 1.9 (1.7 to 2.1) | 1.5 (1.3 to 1.7) | 1.5 (1.2 to 1.7) | 1.4 (1.2 to 1.6) | 1.5 (1.3 to 1.7) | |
| <i>p</i> ‡ | | 0.029 | 0.019 | 0.010 | 0.050 | |
| Low-concentration group | | | | | | |
| Mean (95% CI) | 2.0 (1.8 to 2.2) | 1.6 (1.4 to 1.7) | 1.6 (1.5 to 1.8) | 1.7 (1.5 to 1.9) | 1.7 (1.5 to 1.8) | |
| <i>P</i> | | 0.004 | 0.018 | 0.047 | 0.030 | |
| Placebo group | | | | | | |
| Mean (95% CI) | 1.7 (1.5 to 1.9) | 1.6 (1.5 to 1.8) | 1.7 (1.5 to 2.0) | 1.9 (1.7 to 2.1) | 2.0 (1.8 to 2.2) | |
| <i>P</i> | | 0.950 | 1.000 | 0.453 | 0.130 | |
| Total VSCs (ppm) | | | | | | 0.023 |
| High-concentration group | | | | | | |
| Mean (95% CI) | 0.3 (0.1 to 0.4) | 0.2 (0.0 to 0.3) | 0.1 (0.1 to 0.1) | 0.1 (0.1 to 0.1) | 0.2 (0.1 to 0.2) | |
| <i>P</i> | | 0.594 | 0.042 | 0.047 | 0.315 | |
| Low-concentration group | | | | | | |
| Mean (95% CI) | 0.3 (0.1 to 0.4) | 0.2 (0.1 to 0.3) | 0.2 (0.1 to 0.2) | 0.2 (0.1 to 0.2) | 0.2 (0.1 to 0.2) | |
| <i>P</i> | | 0.877 | 0.190 | 0.307 | 0.314 | |
| Placebo group | | | | | | |
| Mean (95% CI) | 0.1 (0.1 to 0.2) | 0.2 (0.1 to 0.3) | 0.2 (0.0 to 0.3) | 0.2 (0.1 to 0.4) | 0.3 (0.1 to 0.4) | |
| <i>P</i> | | 0.964 | 0.946 | 0.714 | 0.564 | |
| Tongue-coating score | | | | | | <0.001 |
| High-concentration group | | | | | | |
| Mean (95% CI) | 1.5 (1.1 to 2.0) | 1.4 (1.0 to 1.7) | 0.9 (0.6 to 1.2) | 0.7 (0.5 to 1.0) | 0.7 (0.5 to 1.0) | |
| <i>P</i> | | 0.908 | 0.030 | 0.003 | 0.004 | |
| Low-concentration group | | | | | | |
| Mean (95% CI) | 1.5 (0.9 to 2.1) | 1.4 (0.9 to 1.9) | 1.1 (0.7 to 1.4) | 0.8 (0.5 to 1.1) | 0.9 (0.6 to 1.2) | |
| <i>P</i> | | 0.964 | 0.367 | 0.053 | 0.116 | |
| Placebo group | | | | | | |
| Mean (95% CI) | 1.0 (0.6 to 1.5) | 1.0 (0.7 to 1.3) | 1.1 (0.7 to 1.5) | 1.1 (0.8 to 1.4) | 1.3 (0.9 to 1.7) | |
| <i>P</i> | | 1.000 | 0.998 | 0.998 | 0.713 | |

* No statistical differences were observed among the three groups at baseline.

† Repeated-measures ANOVA adjusted for baseline values of the organoleptic score, VSCs, and tongue-coating score.

‡ Statistical significance between baseline and the observed time point with the Dunnett test.

mouthwash reduced tongue coating because of the mechanical function. The use of chewing gum may affect an accumulation of tongue coating with a mechanical function. In the present investigation, we did not find any reduction of tongue coating with the use of the placebo gum. Additional investigation may be necessary to clarify the effect of eucalyptus extract on bacterial flora on tongue coating in vivo. On the other hand, in a previous study, Nagata et al.¹² indicated that eucalyptus extract exerted a significant effect on plaque accumulation, GI, bleeding on probing, and PDs. Significant dose-response effects were only observed for plaque accumulation. Thus, a decrease in oral malodor may be partly related to the effectiveness of eucalyptus extracts.

This study was characterized by several limitations. Patients who displayed oral malodor were deemed suitable for efficacy analysis of topical antibacterial

agents with respect to oral malodor. Participants in the present investigation were not selected on the basis of the olfactory threshold level. In a study by Tanaka et al.,¹¹ subjects with oral malodor were defined on the basis of the olfactory threshold level of total VSCs ≥ 0.25 ppm and/or an organoleptic score ≥ 2 .¹¹ Based on these criteria, participants without oral malodor comprised nearly one-half of each of the three groups in the present study (data not shown). Miyazaki et al.¹³ reported that 6% to 23% of subjects in the general population showed VSC values above the socially acceptable limit at some period during the day. Thus, eucalyptus-extract chewing gum may be applicable for the reduction and prevention of oral malodor in the general population as characterized by very mild to moderate oral malodor in a population approach. Individuals who exhibited gingivitis but not deep periodontal pockets were recruited for

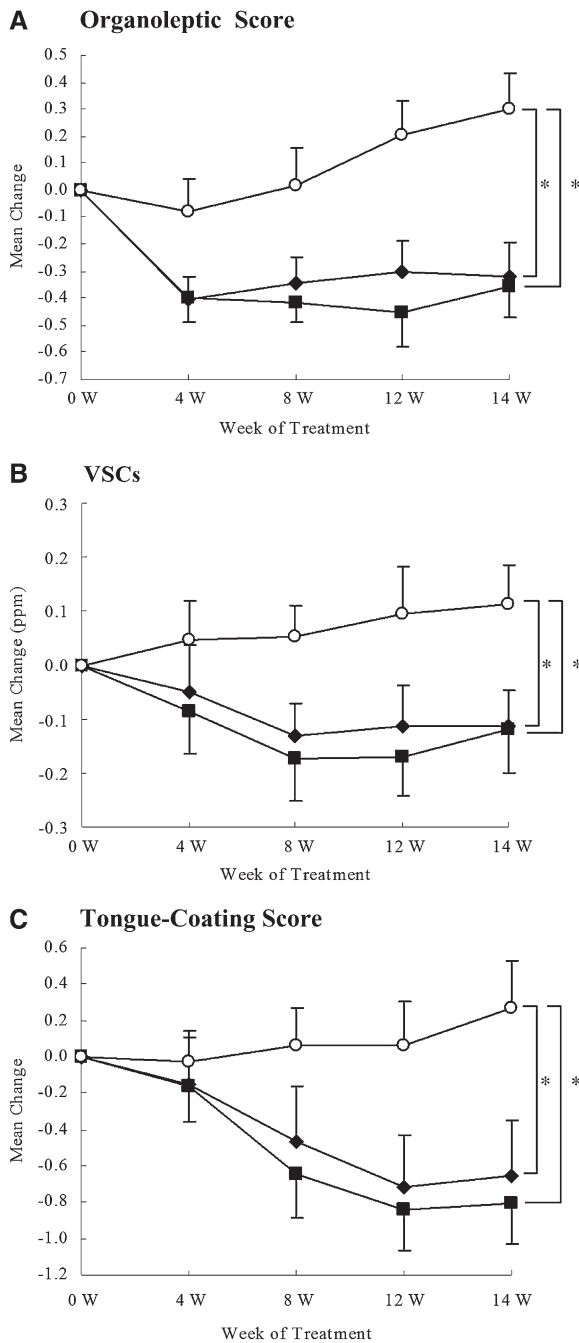


Figure 3.

Effect of eucalyptus-extract chewing gum on oral malodor and tongue coating. Organoleptic score (A), the mean concentration of VSCs (B) determined by gas chromatography, and tongue-coating score (C) were measured at baseline and 4, 8, 12, and 14 weeks (W) after baseline. The data represent the mean change \pm SE from the values at baseline. ■ = high-concentration group ($n = 31$; intake of 0.6% [90 mg/10 tablets] eucalyptus extract-containing chewing gum); ◆ = low-concentration group ($n = 32$; intake of 0.4% [60 mg/10 tablets] eucalyptus extract-containing chewing gum); ○ = placebo group ($n = 33$; intake of placebo chewing gum). *Significant differences among the groups were designated by $P < 0.05$ as determined by repeated-measures ANOVA and followed by the Games-Howell pairwise comparison test.

the present investigation. Subjects with oral pathologic halitosis and severe periodontitis may be suitable for periodontal treatment but not for the use of eucalyptus-extract chewing gum. Periodontally healthy subjects ($GI = 0$) who may not show malodor were excluded. Thus, some subjects may be classified as demonstrating physiologic halitosis in the absence of periodontitis. Furthermore, the protocol used in the present study was identical to the protocol used in a previous study¹² that examined the effect of eucalyptus-extract chewing gum on periodontal health. A justification for using parameters related to oral malodor was not performed in the present investigation. We did not find statistical differences with respect to malodor assessments and clinical parameters at baseline among the three groups. However, to clarify the clinical effects of eucalyptus extract on oral malodor, more extensive double-masked, randomized studies in subjects who display oral malodor are required.

CONCLUSIONS

Chewing gum that contains eucalyptus extract possesses some advantage as an antimicrobial agent. Eucalyptus extract consists of edible substances derived from food and is very safe. In addition, the slow dissolution of eucalyptus extract enables a sustained release of the active ingredients. An appealing advantage of this vehicle is its compatibility with daily activities.

ACKNOWLEDGMENTS

This study was supported by commissioned research from Lotte Central Laboratory at Osaka University (J050801012). Drs. Tanaka, Toe, Nagata, Ojima, Kuboniwa, and Shizukuishi report no financial relationships related to any products involved in this study. Drs. Osawa and Shimizu are employed full-time as researchers at Lotte Central Laboratory.

REFERENCES

1. Delanghe G, Ghyselen J, Bollen C, van Steenberghe D, Vandekerckhove BN, Feenstra L. An inventory of patients' response to treatment at a multidisciplinary breath odor clinic. *Quintessence Int* 1999;30:307-310.
2. Kleinberg I, Westbay G. Salivary and metabolic factors involved in oral malodor formation. *J Periodontol* 1992;63:768-775.
3. Loesche WJ, De Boever EH. Strategies to identify the main microbial contributors to oral malodor. In: Rosenberg M, ed. *Bad Breath: Research Perspectives*. Tel Aviv, Israel: Ramot Publishing; 1995:41-54.
4. Bosy A, Kulkarni GV, Rosenberg M, McCulloch CAG. Relationship of oral malodor to periodontitis: Evidence of independence in discrete subpopulations. *J Periodontol* 1994;65:37-46.
5. Kozlovsky A, Goldberg S, Natour I, Rogatky-Gat A, Gelernter I, Rosenberg M. Efficacy of a 2-phase oil: Water mouthrinse in controlling oral malodor, gingivitis, and plaque. *J Periodontol* 1996;67:577-582.

6. Quirynen M, Mongardini C, van Steenberghe D. The effect of a 1-stage full-mouth disinfection on oral malodor and microbial colonization of the tongue in periodontitis. A pilot study. *J Periodontol* 1998;69:374-382.
7. Young A, Jonski G, Rölla GA. A study of triclosan and its solubilizers as inhibitors of oral malodour. *J Clin Periodontol* 2002;29:1078-1081.
8. 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;111:400-404.
9. Roldán S, Winkel EG, Herrera D, Sanz M, Van Winkelhoff AJ. The effects 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.
10. Nagata H, Inagaki Y, Yamamoto Y, et al. Inhibitory effects of macrocarpals on the biological activity of *Porphyromonas gingivalis* and other periodontopathic bacteria. *Oral Microbiol Immunol* 2006;21:159-163.
11. Tanaka M, Yamamoto Y, Kuboniwa M, et al. Contribution of periodontal pathogens on tongue dorsa analyzed with real-time PCR to oral malodor. *Microbes Infect* 2004;6:1078-1083.
12. Nagata H, Inagaki Y, Tanaka M, et al. Effect of eucalyptus extract chewing gum on periodontal health: A double-masked, randomized trial. *J Periodontol* 2008;79:1378-1385.
13. Miyazaki H, Sakao S, Katoh Y, Takehara T. Correlation between volatile sulphur compounds and certain oral health measurements in the general population. *J Periodontol* 1995;66:679-684.
14. Saito H, Kawaguchi Y. Halitosis prevention campaign: A report of oral health promotion activities in Japan. *Int Dent J* 2002;52(Suppl. 3):197-200.
15. Bornstein MM, Stocker BL, Seemann R, Bürgin WB, Lussi A. Prevalence of halitosis in young male adults: A study in Swiss army recruits comparing self-reported and clinical data. *J Periodontol* 2009;80:24-31.
16. Coil J, Tonzetich J. Characterization of volatile sulfur compounds production at individual gingival crevicular sites in humans. *J Clin Dent* 1992;3:97-103.
17. Delanghe G, Ghyselen J, van Steenberghe D, Feenstra L. Multidisciplinary breath-odor clinic. *Lancet* 1997;350:187.
18. Rosenberg M. Bad breath and periodontal disease: How related are they? *J Clin Periodontol* 2006;33:29-30.
19. Greenberg M, Urnezis P, Tian M. Compressed mints and chewing gum containing magnolia bark extract are effective against bacteria responsible for oral malodor. *J Agric Food Chem* 2007;55:9465-9469.
20. Lodhia P, Yaegaki K, Khakbaznejad A, et al. Effect of green tea on volatile sulfur compounds in mouth air. *J Nutr Sci Vitaminol (Tokyo)* 2008;54:89-94.
21. Løe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;21:533-551.
22. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;31:103-115.
23. Oho T, Yoshida Y, Shimazaki Y, Yamashita Y, Koga T. Characteristics of patients complaining of halitosis and the usefulness of gas chromatography for diagnosing halitosis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001;91:531-534.
24. Shimura M, Watanabe S, Iwakura M, et al. Correlation between measurements using a new halitosis monitor and organoleptic assessment. *J Periodontol* 1997;68:1182-1185.
25. Kieser M, Wassmer G. On the use of the upper confidence limit for the variance from a pilot sample for sample size determination. *Biometrical J* 1996;38:941-949.
26. Rosenberg M, Kulkarni GV, Bosy A, McCulloch CA. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. *J Dent Res* 1991;70:1436-1440.
27. Rosenberg M, Gelernter I, Barki M, Bar-Ness R. Day-long reduction of oral malodor by a two-phase oil: Water mouthrinse as compared to chlorhexidine and placebo rinses. *J Periodontol* 1992;63:39-43.
28. van Steenberghe D, Avontroodt P, Peeters W, et al. Effect of different mouthrinses on morning breath. *J Periodontol* 2001;72:1183-1191.
29. Brading MG, Cromwell VJ, Green AK, DeBrabander S, Beasley T, Marsh PD. The role of Triclosan in dentifrice formulations, with particular reference to a new 0.3% Triclosan calcium carbonate-based system. *Int Dent J* 2004;54(Suppl. 5):291-298.
30. Paraskevas S, Rosema NA, Versteeg P, Van der Velden U, Van der Weijden GA. Chlorine dioxide and chlorhexidine mouthrinses compared in a 3-day plaque accumulation model. *J Periodontol* 2008;79:1395-1400.
31. Saito MV, Nagata H, Maeda K, et al. Antibacterial activity of extracts from eucalyptus leaves on periodontopathic bacteria. *J Dent Health (Tokyo)* 2003;53:585-591.
32. Shinada K, Ueno M, Konishi C, et al. Effects of a mouthwash with chlorine dioxide on oral malodor and salivary bacteria: A randomized placebo-controlled 7-day trial. *Trials* 2010;11:14.

Correspondence: Dr. Muneo Tanaka, Department of Preventive Dentistry, Osaka University Graduate School of Dentistry, 1-8, Yamadaoka, Suita, Osaka 565-0871, Japan. Fax: 81-6-6879-2925; e-mail: tanakam@dent.osaka-u.ac.jp.

Submitted April 28, 2010; accepted for publication June 9, 2010.