EFFECT OF AN ESSENTIAL OIL-CONTAINING MOUTH RINSE ON VSC-PRODUCING BACTERIA ON THE TONGUE

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Abstract. The objective of the present study was to investigate the inhibitory effect of a commercially available essential oil-containing mouth rinse 12 hours after a single rinse and two weeks of twice daily rinsing, on volatile sulphur compounds (VSC) producing bacteria on the tongue. The study was a randomized, double-blind, controlled crossover design. Thirty-six healthy subjects, aged 20-48 years, volunteered to participate in the study. Subjects were randomly assigned to rinse twice daily with either an essential oil-containing mouth rinse (Cool Mint Listerine® Antiseptic) or a negative control rinse. Bacteria samples were taken from the dorsum of the tongue at baseline, after the first rinse and two weeks later. They were plated on OOPS medium to enumerate the VSC-producing bacteria. Intergroup comparisons of log10 transformed colony-forming units of the samples were made using analysis of covariance. Each comparison was performed at a 5% significance level. The mean VSC-producing bacteria in subjects using the essential oil mouth rinse were significantly lower than those using the control rinse twice daily. In healthy subjects, rinsing with an essential oil-containing mouth rinse can have a significant effect on VSC-producing bacteria on the tongue and may be useful for controlling intrinsic oral malodor over prolonged periods.

Keywords: essential oil, mouth rinse, VSC-producing bacteria

INTRODUCTION

Epidemiologic studies have revealed the prevalence of malodor among the general population ranges from 2.4% to 28% (Loesche and Kazor, 2002; Liu *et al*, 2006; Hughes and McNab, 2008). Although malodor can originate from the digestive and respiratory systems, up to 90% of breath odor arises locally in the mouth. Accumulation of bacteria, food residue and other debris such as shed epithelial cells at the posterior part and the furrows of the tongue, are considered major causes of malodor (Porter and Scully, 2006). This is understandable since the large surface area of the tongue is exposed to expired air and the available of substrates can be degraded to malodorous molecules by bacteria on the tongue. These bacteria are gram-negative anaerobes (Krespi *et al*, 2006). End products of bacterial metabolism include the volatile molecules that contribute to oral malodor, such as volatile sulphur compounds (VSC), short-chain fatty acids, diamines and phenyl compounds (Loesche and Kazor, 2002).

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These bacteria also degrade the sulphur-containing peptides and amino acids found in saliva, gingival crevicular fluid, blood, retained food and desquamated epithelial cells (van den Broek *et al*, 2008).

Treatment strategies for malodor include interventions aimed at reducing or eliminating intra-oral debris and the associated microbial load by mechanical debridement (*ie*, brushing, flossing and tongue scraping) and the use of antimicrobial mouth rinses (Loesche and Kazor, 2002). In a recent review, it was found mechanical tongue cleaning appeared to have very limited, short acting benefits in controlling oral malodor (Fedorowicz *et al*, 2008). The limitations of mechanical methods to effectively reach and remove VSC-producing bacteria from all oral ecological sites are recognized. The possibility that mouth rinses may be more effective in reaching the less accessible parts of the oral cavity, their greater social acceptance and ease of use has led to the development of a large number and range of over the counter mouth rinses (van den Broek *et al*, 2008).

The clinical use of essential oil-containing mouthrinse has been reported to reduce oral malodor by changes in the organoleptic score and halimeter ratings (Borden *et al*, 2002). However, this reduction may be the result of the masking effect of the mouth rinse. To prove the effectiveness of this mouth rinse, this study aimed to evaluate the inhibitory effect of a commercially available essential oil-containing mouth rinse on VSC-producing bacterial counts on the tongue in a group of healthy Thai subjects.

MATERIALS AND METHODS

Subjects

The study was conducted at Mahidol University, Thailand during July 2008 to April 2009. Thirty-six subjects in good general health (aged 20-48 years) volunteered to participate in this study and signed an informed consent form. The study protocol was reviewed and approved by the Institutional Review Board of Mahidol University (MU-IRB 2008/053-2207). The exclusion criteria were: subjects with medical disorders taking antibiotics or undergoing other antimicrobial therapy four weeks prior to the beginning of the study, smokers and pregnant women. They were also required to have a minimal plaque accumulation equivalent to plaque index score of 1.5 (Turesky *et al*, 1970), no more than mild gingivitis, equivalent to a modified gingival index of 1.5 (Lobene *et al*, 1986) and no pockets greater than 4 mm. Exclusion criteria included the presence of active caries, removable dentures, implants and xerostomia.

Study design

This study was a randomized, double blind, placebo-controlled 2x2 crossover clinical trial. It investigated the effect of mouth rinse 12 hours after a single rinse and after two weeks of twice daily rinsing. Since label directions specify rinsing in the morning and at night, two studies were conducted using the same protocol, one of which included a 12 hour overnight period and the second included a 12 hour period during the daytime to determine the effect of rinsing over a 24 hour cycle.

The sample size of the study was estimated using an expected mean VSC-producing bacterial counts ($\log_{10}$ CFU) difference of 0.36, a within-subject variance around the mean VSC-producing bacterial count difference of 0.41, a significance level of 5% and a power of 80%. The results showed the required sample size should be at least 11 subjects for each crossover design.

Mouth rinse

Essential oil-containing mouth rinse (Cool Mint Listerine Antiseptic®, Warner-Lambert, Thailand) was provided by Johnson & Johnson (Thailand). Five percent hydroalcohol was used as a negative control.

Pre-experimental phase

Subjects were given a standard fluoride toothpaste and soft textured toothbrush for use one week prior to baseline bacterial sampling and for the duration of the study. Baseline data on plaque and gingival indices were recorded in order to exclude volunteers with periodontal disease, following the exclusion criteria.

Experimental phase

The method performed in the experimental phase was modified from Fine *et al* (2005). In brief, for the daytime study, qualifying subjects reported to the clinical site for baseline sampling in the evening after refraining from eating, drinking, or any oral hygiene procedures for 2 hours prior to sampling. For the overnight study, subjects reported to the clinical site in the morning for baseline sampling after refraining from eating, drinking, or oral hygiene procedures that morning. Bacteria were taken from the right and left halves of the dorsum of the tongue using sterile cotton swabs. These tongue samples were collected by placing the cotton swab at the midline of the posterior region of the tongue and then rolling the swab toward the lateral boarder of the tongue approximately four times from posterior to anterior. The swabs from the right and left sides of the tongue were placed in separate tubes containing 1 ml phosphate buffer saline solution.

Subjects were randomly assigned to either the essential oil-containing mouth rinse group or the control group. For the daytime study, subjects were instructed to rinse with 20 ml of their assigned rinse for 30 seconds after brushing their teeth at approximately 5:00 AM the next morning. They reported to the study site at approximately 5:00 PM for bacterial sampling. For the overnight study, subjects were instructed to use their assigned rinse at approximately 10:00 PM the same day. They reported back to the study site at approximately 10:00 AM the next morning for bacterial sampling.

For single-use 12 hour sampling, the tongue sample was harvested from the right half of the tongue in the same manner as at baseline. The subjects continued their usual oral hygiene with the provided toothpaste and rinsed twice daily for 30 seconds with 20 ml of their assigned mouth rinse for the next 13 days. For the 2-week use, the tongue sample was harvested from the left side of the tongue.

Following a 1-week wash-out period, the entire procedure was repeated with subjects using the alternative rinse.

Microbiological evaluation

The PBS with the bacterial sample was sonicated for 30 seconds and then serially diluted to 10^{-4}. Dilutions of 10^{-2} and 10^{-4} were plated in duplicate on OOPS medium (Paryavi-Gholami *et al*, 1999) supplemented with 5% blood to enumerate the VSC-producing bacteria. The plates were incubated in an anaerobic chamber (Forma Scientific, Marietta, OH) at 37°C for 5 to 7 days. Colony-forming units (CFU) were calculated and $\log_{10}$ transformed.

Data analysis

Eighteen subjects were selected for each of the two studies. Baseline demographic variables were summarized by treatment sequence. The treatment sequences were compared with respect

Table 1
Demographic characteristics of subjects.

	Treatment sequence	
	Essential oil/ Control	Control/ Essential oil
Daytime study		
Number	9	9
Age (years)		
Range	20-48	20-45
Mean	27.44	28
SD	8.56	7.39
Median	25	26
Gender		
Male	3	3
Female	6	6
Overnight study		
Number	9	9
Age (years)		
Range	20-40	20-39
Mean	26.77	26.55
SD	6.68	6.1
Median	25	24
Gender		
Male	4	5
Female	5	4

to age using the Mann-Whitney *U* test, and with respect to sex by means of the chi-square test.

Inter-group comparisons of VSC-producing bacterial counts were made using an analysis of covariance (ANCOVA) with the baseline counts for the corresponding period as a covariate and period and treatment as factors. Each comparison was performed at a 5% significance level.

RESULTS

Demographic data of the subjects in the studies are shown in Table 1. No significant differences were noted by age or sex for the two treatment sequences. The mean number and inter-group comparisons of VSC-producing bacterial counts ($\log_{10}$) at baseline, 12 hours after a single rinse and after 2 weeks of using the essential oil or control rinse are presented in Table 2 and Table 3. The mean bacterial counts after a single rinse and after 2 weeks of using the mouth rinse were significantly lower than counts after using the negative control for both the daytime and overnight studies. The percent reduction in bacteria after a single rinse in the daytime study was 47.5% and after 2 weeks of using the mouth rinse was 98.0%. In the overnight study, the percent reduction in bacteria after a single rinse was 90.8% and after 2 weeks of using the mouth rinse was 96.7%.

DISCUSSION

Oral malodor is an oral health problem ranked behind dental caries and periodontal disease as a cause for patients to visit dentists (Loesche and Kazor, 2002). Most malodor is caused by microbial degradation of oral organic substrate (Krespi *et al*, 2006). The basic management method for malodor is mechanically reducing the amount of microorganisms and substrates in the oral cavity. However, even after implementing good oral hygiene, many patients continue to have malodor of oral origin. In such instances, rinsing or gargling with an efficacious antimicrobial mouth rinse is advised (Fedorowicz *et al*, 2008). The purpose of the present study was to determine the inhibitory effect of a commercially available essential oil-containing mouth rinse on VSC-producing bacteria on the tongue of healthy subjects. The results show the mouth rinse has an inhibitory effect on these bacteria when compared with a negative control.

Essential oils have been used for

Table 2
VSC-producing bacterial counts (log_{10}) at baseline, 12 hours after a single rinse and after 2 weeks use.

	Baseline	12 hours after a single rinse	After 2 weeks use
Daytime study			
Essential oil rinse	6.71±0.51	6.22±0.63	4.77±0.40
Control	6.72±0.58	6.50±0.75	6.47±0.56
Overnight study			
Essential oil rinse	6.69±0.61	4.69±0.82	5.73±0.55
Control	6.70±0.67	5.22±0.65	6.71±0.68

Data expressed as mean±SD

Table 3
Comparison of VSC-producing bacterial counts (log_{10}).

Comparison (Essential oil rinse *versus* control)	Differences in mean	Percent reduction[a]	Standard error of difference	*p*-value
Daytime study				
12 hours after a single rinse (6.22 *vs* 6.50)	-0.28	47.5	0.13	<0.05
After 2 weeks use (4.77 *vs* 6.47)	-1.70	98.0	0.16	<0.001
Overnight study				
12 hours after a single rinse (4.69 *vs* 5.73)	-1.04	90.8	0.17	<0.001
After 2 weeks use (5.22 *vs* 6.71)	-1.49	96.7	0.14	<0.001

[a]Percent reduction = $(1\text{-}10^{diff}) \times 100$, where diff is the difference in means in log_{10} scale

cosmetics and medicinal purposes for many years. The medicinal properties of essential oils have been used to treat several health problems. Essential oils are odorous, volatile products of plants secondary metabolism, many of them possessing strong antimicrobial properties (Kalemba and Kunicka, 2003). In this study, the tested mouth rinse contained a combination of four essential oils: thymol, eucalyptol, methyl salicylate and menthol. The mixture of these oils has antibacterial properties. The mechanism of action involves bacterial cell wall destruction, bacterial enzymatic inhibition and extraction of bacterial lipopolysaccharides (Mandel, 1994). The short-term effect of essential oil-containing mouth rinse has been found to be more effective against oral malodor than placebo at 0.5 hours. Greater effectiveness was maintained by a sustained reduction in bacterial load for up to 3 hours (Loesche and Kazor, 2002; van den Broek *et al*, 2008). The results obtained from the present study reveal rinsing with an essential oil-containing mouth

rinse has long-lasting effects on reducing VSC-producing bacteria on the tongue (approximately 1 log reduction) for up to 12 hours. Significant reductions were seen 12 hours after a single rinse, with a trend to higher reductions after 2 weeks of use. The effect observed during the daytime was similar to that in the overnight study. These results are somewhat different from a previous study by Fine *et al* (2005), who demonstrated a 0.5 log reduction in VSC-producing bacteria on the tongue after rinsing. The difference may be due to different patient characteristics (age, race and smoking habits) of the subjects enrolled in the study. Due to intra-examiner reproducibility, using a single examiner in the present study may be a limitation for performing an oral examination at the time of subject recruitment.

The percent bacterial count reduction found in the daytime study was 47% at 12 hours after a single rinse and 98% after 2-weeks of use. In the case of the overnight study, the percent reductions were 91% and 97% at 12 hours after a single rinse and after 2 weeks use, respectively. After a single rinse, a greater reduction was observed in the overnight study than in the daytime study. According to Krespi *et al* (2006), oral mouth rinses are recommended for use before bedtime since the residue of the mouth rinse may remain in the oral cavity longer due to the lower salivary flow rate during the night. This could prolong the efficacy of the mouth rinse.

Previous studies have attempted to define tongue flora in a healthy person and a person with malodorous breath. Malodor has been associated with an increased tongue bacterial load, especially with *Porphyromonas, Prevotella, Treponema, Actinobacillus* and *Fusobacterium* species (Krespi *et al*, 2006; Kishi *et al*, 2010). These VSC-producing bacteria have been implicated as the main cause for malodor and were found to be significantly associated with the intensity of oral malodor. However, there is a lack of clear correlation between any specific bacterial genus and malodor (Donaldson *et al*, 2005). Instead, malodor is the result of complex interactions between multiple species of bacteria. Only 50% of these species are cultivable (Paster *et al*, 2006). Based on the method used to evaluate the efficacy of an essential oil-containing mouth rinse, it is worth noting that the method employed in the present study could be beneficial to detect only cultivable bacteria. Some bacterial species that are not yet cultivable may contribute to oral malodor. Further studies are planned to investigate the impact of using this essential oil mouth rinse on other non-cultivable bacteria using molecular techniques to evaluate the efficacy of this mouth rinse in patients with malodor.

In summary, the results of the present study indicate in healthy subjects, an essential oil-containing mouth rinse has a significant effect on VSC-producing bacteria on the tongue when compared to controls. The finding confirms the effectiveness of this mouth rinse in controlling intrinsic oral malodor over prolonged periods. However, a clinical study in patients with malodor is needed to further clarify and broaden our understanding of the role of this essential oil-containing mouth rinse in the management of bad breath.

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