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Effect of an Essential Oil Mouthrinse, with and without Fluoride, on Plaque Metabolic Acid Production and pH after a Sucrose Challenge

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Key Words
Essential oil mouthrinse · Fluoride · Metabolic acids · Plaque · pH

Abstract
This clinical study evaluated the effect of rinsing with an essential oil-containing antiseptic mouthrinse, with or without 100 mg/kg fluoride ion, on the plaque metabolic acid production and plaque pH response after a sucrose challenge. This observer-blind, randomized study used a three-way crossover design. Twenty-four subjects rinsed with 20 ml of one of the following rinses: (1) essential oil (EO) mouthrinse, (2) essential oil mouthrinse plus 100 mg/kg fluoride, or (3) negative control, for 30 s, twice daily for 16 days. On day 17, 1 h after the last mouthrinse, subjects rinsed with 20 ml of mass fraction 10% sucrose solution for 1 min. Seven minutes after the sucrose challenge, supragingival plaque was collected from molar and premolar teeth. Plaque pH and metabolic acid ions were analyzed using a micro pH electrode and capillary electrophoresis, respectively. The results showed that after EO mouthrinse dental plaque produced 36% less lactate, 36% less acetate and 44% less propionate than after the negative control rinse. The dental plaque also exhibited a pH 0.42 unit higher after EO rinse than after the negative control rinse. These results were not affected by the addition of 100 mg/kg fluoride to the EO mouthrinse. From these results we concluded that this EO antiseptic mouthrinse, with or without fluoride ion, is effective in reduction of plaque acidogenicity after a sucrose challenge.

Dental plaque contains oral bacteria that metabolize dietary fermentable carbohydrates to produce short-chain carboxylic acids such as lactate, acetate and propionate. These metabolic acids can diffuse through hard and soft tissues to produce a variety of biological effects in the pathogenesis of dental caries [Margolis and Moreno, 1992], gingivitis [Niederman et al., 1996], and periodontitis [Ohwaki, 1988]. Effective suppression of plaque metabolic acid production may be an important approach for prevention or reduction of dental caries and gingivitis. Although fluoride is an effective anticaries agent, the reported clinical effect by fluoride on plaque metabolic activity was somewhat contradictory [Giertsen and Scheie, 1995; Hamilton, 1990; Vogel et al., 2002]. Chlorhexidine-based antimicrobial mouthrinses, however, have been shown to reduce plaque acid production and to increase the postsucrose minimum pH [Giertsen and Scheie, 1995]. Essential oils, which are often used as 'over-the-counter' mouthrinses, have also been shown to reduce pathogenic bacteria [Fine et al., 2000] and control plaque accumulation [Charles et al., 2001; Overholser et

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al., 1990]. However, quantitative measurements of metabolic acid production and pH response after essential oil antimicrobial mouthrinsing have not been reported. The objective of this study was to determine the effect of an essential oil mouthrinse, with or without fluoride, on plaque metabolic acid production and plaque pH response following a sucrose challenge.

Materials and Methods

Study Design and Subject Information

This controlled, observer-blind, randomized study used a three-way crossover design. The study protocol was approved by the appropriate institutional review boards before subject enrollment. Each subject completed an informed consent form. All subjects were 18 years or older with good general and oral health, and had no history of significant adverse effects following use of oral hygiene products. Twenty-four qualified subjects, 18 males and 6 females, entered the study and all of them completed all three legs of the study.

Study Materials

Three treatment regimens were administered twice daily for 14 days with 20 ml for 30 s:

(1) An essential oil (EO) antiseptic mouthrinse, which contains 0.064% thymol, 0.092% eucalyptol, 0.060% methyl salicylate, and 0.042% menthol. Listerine (FreshBurst Listerine Antiseptic; Pfizer Inc., Morris Plains, N.J., USA) was chosen as the essential oil test rinse because its antimicrobial properties have been clinically proven [Charles et al., 2001; Overholser et al., 1990] and it has the American Dental Association Seal of Approval.

(2) Essential oil mouthrinse with mass fraction 0.022% NaF (100 mg/kg or ppm F, EOF), which contains the same essential oils as in the above rinse.

(3) A 5% mass fraction ethanol-water solution as a negative control.

Clinical Procedures

Prior to the study, the subjects were screened for the ability to produce 48-hour resting plaque samples of at least 3 mg in mass and a plaque pH <6.0, 7 min after a sucrose challenge. Of 31 subjects screened, 24 subjects were qualified and completed all legs of the experiment. For the first 14 days of each treatment leg, the subjects rinsed unsupervised with 20 ml of a randomly assigned test mouthrinse twice daily for 30 s. The treatment period of 14 days was based on previous results showing that Listerine mouthrinse was effective against plaque and gingivitis after 2 weeks of treatment. Subjects were provided with nonfluoride toothpaste to use through the entire test period and were instructed to maintain their usual diet and mechanical oral hygiene routine during this period. From the morning of day 15 through the morning of day 17 of each test leg, subjects continued to use their assigned mouthrinse but abstained from mechanical oral hygiene procedures to facilitate plaque accumulation. On the morning of day 17 and after overnight fasting, the subjects abstained from oral hygiene and rinsed with their assigned mouthrinse for the last time. Sixty minutes after mouth rinsing they rinsed for 1 min with 20 ml of mass fraction 10% sucrose, and 7 min later supragingival plaque was collected. A washout period of at least 2 weeks separated each leg of the experiment.

Plaque Sample Collection and Analysis

A plastic strip held by a hemostat was used to collect pooled supragingival plaque from the buccal-interproximal areas of all premolars, first and second molar teeth. The plaque was scraped into the cap of a preweighed Microcon centrifuge filter (Millipore YM-100). The plaque mass was determined and the sample mixed with 100 µl of 80 mmol/l NaCl solution and centrifuged (Eppendorf model 5402, approximately 15,000 *g*, at 4°C for 10 min). The pH of the filtrate was measured using a combination micro pH electrode (Microelectrode Inc., model 401, Londonderry, N.H., USA).

Total plaque lactate, acetate, and propionate concentrations, including ionized and unionized acids, in the plaque supernatant were measured using capillary electrophoresis after appropriate dilution. The experimental conditions of the capillary electrophoresis method have been described previously [Vogel et al., 2000]. A Beckman MDQ (Beckman Coulter, Palo Alto, Calif., USA) was used for capillary electrophoresis analysis. The running buffer contained 5 mmol/l sorbic acid as the displaced ion, 0.6 mmol/l tetra-decyltrimethyl-ammonium bromide to reverse the electro-osmotic flow, and 3.0 mmol/l MES (2-(N-morpholino)ethanesulfonic acid) to control pH. Samples and standards were diluted into deionized water containing 0.1 mmol/l pentane sulfonic acid as an internal standard which was used to compensate for small shifts in peak location. The standard uncertainty of the pH measurements was 0.01 unit. The standard uncertainty of the capillary electrophoresis measurements was 1–5% depending on the ion detected and its concentration.

Statistical Analysis

The efficacy response variables were postsucrose challenge plaque lactate, acetate, and propionate ion concentrations and plaque pH. Between-treatment comparisons for each efficacy response variable were performed using an ANOVA model with subject, treatment, and period as factors. Each comparison was performed at the 0.05 level of significance and was two-sided. No multiple comparison adjustment was used. The error bars in graphs 1, 2 and 3 refer to the standard error which, in these experiments, was used as a measure of the standard uncertainty.

Results

The mean plaque lactate, acetate, and propionate concentrations for all three treatment groups are presented in figure 1.

The plaque pH after EO rinse, EOF rinse or negative control rinse, followed by sucrose rinse, were 5.95 ± 0.09, 5.90 ± 0.10, and 5.48 ± 0.10, respectively. The average pH was calculated as the average of the individual pH values rather than the negative log of the average hydrogen ion concentration [Margolis and Moreno, 1992].

Average harvested plaque mass after essential oil rinse, essential oil fluoride rinse and negative control rinse were 3.53 ± 0.36, 4.12 ± 0.36, and 8.82 ± 0.35 mg, respectively.

For plaque lactate, acetate, and propionate concentrations and plaque mass, the values for the negative control

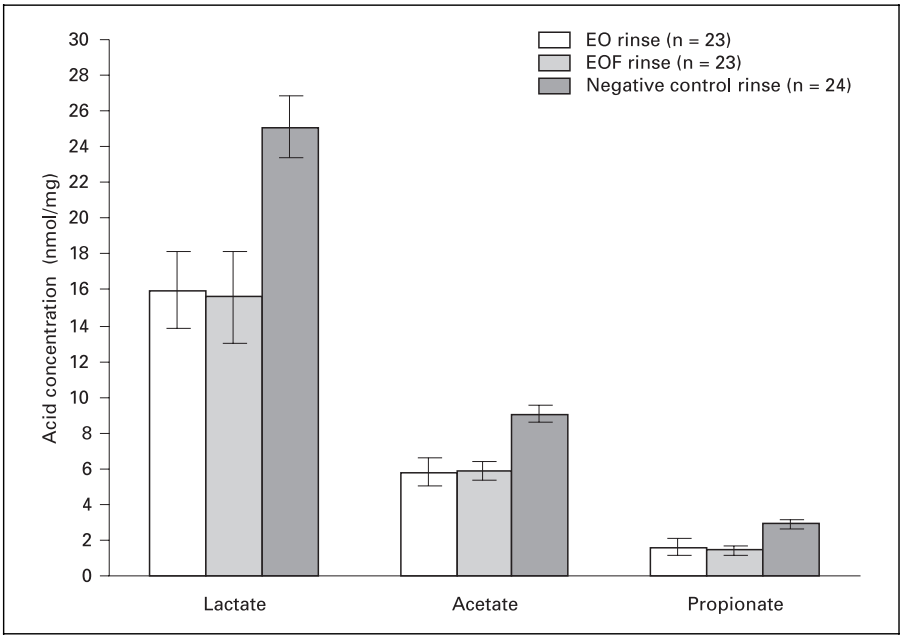

Fig. 1. Average of the plaque metabolic acid concentrations (nmol/mg) after using different mouthrinses and a sucrose challenge.

rinse were statistically higher ($p < 0.005$) than that for the EO or EOF rinses. With respect to the plaque pH, the value for the negative control rinse was significantly lower ($p < 0.005$) than those for EO or EOF rinses. No significant difference was observed between the EO and EOF rinses for all five measured parameters.

In order to examine the influence of plaque quantity on antiacid effectiveness from Listerine with or without fluoride treatment, the correlation coefficient between plaque weight after negative control rinse and lactate reduction was –0.27 and –0.15 for EO rinse and EOF rinse, respectively.

Discussion

Essential oil-containing mouthrinses have been shown to reduce plaque accumulation [Charles et al., 2001]. This reduction was reflected in the significant plaque mass reductions seen in the current study between the negative control and the essential oil mouthrinse groups. The acid concentrations were normalized to plaque mass in order to adjust for these differences. Heavy-plaque (>3 mg after 48-hour accumulation in negative control group) subjects were selected in this study for the purpose that sufficient plaque could be collected for chemical analysis. However, the low correlation coefficient (–0.27 and –0.15) between plaque weight and lactate level reduction, after either EO or EOF rinse, respectively, indicated that the quantity of plaque did not have any influence on the reduction of plaque metabolic acids after essential oil treatment.

The results for plaque acids are in agreement with the previously reported range of these acids (17–20.5 nmol/mg for lactate, 6–12.3 nmol/mg for acetate, and 3–11.0 nmol/mg for propionate) [Distler and Kröncke, 1983; Oliveby et al., 1990; Simone et al., 1992]. The slightly higher concentrations for lactate in the current study are probably caused by the differences in subject population, and the plaque sampling technique.

The approximately 36% reduction in the amount of lactate, acetate, and propionate in the current experiment after the EOF and EO rinses can be interpreted as a reduction in the total acidogenic activity of the dental plaque microflora. Whether this reduction of plaque acidogenicity is attributable to a plaque viability reduction, or to shifts in the microflora to less acidogenic populations, or to an effect on plaque metabolism is not known. Since previous studies indicated that viable bacterial populations in plaque were reduced to a highly significant extent [Pan et al., 2000], the reductions in acid production in the plaques treated with the EO or EOF mouthrinses are probably at least partially related to lower populations of viable plaque bacteria.

Lactate is known to be the main carboxylic acid generated by dental plaque immediately after fermentable carbohydrate challenge. The observation in this study that

lactate accounted for about 76% of metabolic acid production agrees with this point of view. Acetate and propionate accounted for only 18 and 6% of the total metabolic acid production. However, since these acids have been associated with gingival irritation [Niederman et al., 1996, 1997; Stehle et al., 2001] and the ratio of acetate to lactate increases with time in the resting starved plaque, the observed reduction in acetate and propionate levels might contribute to the reported reduction of gingivitis by the EO mouthrinses [Charles et al., 2001].

As expected, the plaque pH fell after sucrose rinsing. The minimum pH achieved after a challenge is an important determinant of demineralization potential and therefore a factor in the pathogenesis of dental caries. The mean plaque pH from resting plaque after the negative control rinse and sucrose challenge was similar to previously reported means of 5.10–5.75 [Margolis and Moreno, 1992; Tanaka and Margolis, 1999; Vogel et al., 1998]. This value is within the range of the so-called 'plaque critical pH, 5.0–5.5', below which tooth enamel is thought to dissolve [Larsen and Pearce, 1997]. In comparison, after EO or EOF rinses, the plaque pH was not only significantly higher than that of negative control rinse, but also above the critical plaque pH. An increase in postsucrose minimum pH after antimicrobial mouthrinsing has been reported previously [Giertsen and Scheie, 1995]. The significantly higher plaque pH may imply a decrease of plaque acidogenicity and possibly cariogenicity after EO or EOF rinses.

Listerine mouthrinse contains a fixed ratio of four essential oils: thymol, eucalyptol, methyl salicylate, and menthol. The suppression of acid production after rinsing with Listerine mouthrinse was due to the combination of these essential oils.

All experimental variables (plaque mass, pH and plaque metabolic acids) were very similar between the EO rinse and EOF rinse, suggesting that the addition of 100 mg/kg fluoride to the formulation did not affect the antimetabolic activity of the essential oil mouthrinse. Though a previous study has reported that an experimental 228 mg/kg F 'controlled release' F rinse given, as in the current study, 1 h before sucrose rinse significantly reduced lactate production and increased plaque pH [Vogel et al., 2002], no additional suppression of metabolic acids was observed from the 100 mg/kg fluoride in the current study. Previous studies have also found that fluoride did not increase the antiglycolytic effects of antimicrobial rinses [Giertsen and Scheie, 1995].

In conclusion, this study has demonstrated that an essential oil mouthrinse reduces the production of plaque acids associated with a sucrose challenge and reduces the postsucrose fall in plaque pH. No additional effects were observed when 100 mg/kg fluoride was added to the mouthrinse. The current study did not examine the persistence of these effects at periods longer than 1 h after rinsing and further studies to examine how long the antiseptic rinses can exert an inhibitory effect upon plaque acidogenesis are warranted.

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