J Clin Periodontol 2010; 37: 840–847 doi: 10.1111/j.1600-051X.2010.01599.x

Journal of Clinical Periodontology

An investigation of the effect of an essential oil mouthrinse on induced bacteraemia: a pilot study

Daniel H. Fine[1], David Furgang[1], Marie McKiernan[1], Debra Tereski-Bischio[1], Danette Ricci-Nittel[2], Paul Zhang[3] and Marcelo W. B. Araujo[2]

[1]Department of Oral Biology, University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Newark, NJ, USA; [2]Department of Oral Care Research, Development and Engineering; [3]Department of Biometric and Clinical Data System, Johnson & Johnson Consumer Companies Inc., Morris Plains, NJ, USA

Fine DH, Furgang D, McKiernan M, Tereski-Bischio D, Ricci-Nittel D, Zhang P, Araujo MWB. An investigation of the effect of an essential oil mouthrinse on induced bacteraemia: a pilot study. J Clin Periodontol 2010; 37: 840–847. doi: 10.1111/j.1600-051X.2010.01590.x.

Abstract

Aim: This pilot study was designed to assess the effect of an essential oil antiseptic mouthrinse (EOM) in reducing bloodstream bacteria after chewing an apple.

Material and Methods: From a panel of 200, we screened 62 individuals with mild-to-moderate gingivitis. Twenty-two individuals who showed a bacteraemia after chewing an apple were enroled. Subjects were recalled, instructed to chew an apple, had blood drawn (first baseline), and were randomly assigned EOM or a control (C) treatment for 2 weeks. Subjects were recalled, given an apple, and had blood taken for bacterial counts. Following a 1-week fluoride dentifrice wash-out, subjects were recalled, given the apple challenge, had blood drawn (second baseline), assigned the alternate treatment, and recalled for testing. Differences between baseline and 2-week post-treatment (EOM *versus* C) in blood-borne bacteria were assessed by analysis of covariance.

Results: Mean aerobic blood-borne bacteria decreased by 68.5% (17.7 viable counts from baseline; $p < 0.001$), while anaerobic counts decreased by 70.7% (14.5 mean viable counts from baseline; $p < 0.001$) for the EOM treatment. No reduction was seen for the C treatment.

Conclusions: This double-blind, placebo-controlled, randomized, 2-week cross-over study showed that rinsing with essential oils reduced the level of bloodstream bacteria in subjects with mild-to-moderate gingivitis.

Key words: bacteraemia from daily activity; essential oil mouthrinse; mastication; systemic disease

Accepted for publication 27 May 2010

Conflict of interest and source of funding statement

The statement below is intended to disclose any perceived conflict of interest with respect to the conduct of the study supported by the Johnson and Johnson Consumer Companies. Dr. Daniel Fine of UMDNJ/NJDS designed the study under consideration for review. Dr. Fine provided the oversight for the study and wrote the manuscript. David Furgang performed all the microbiology. Marie McKiernan was the study coordinator, helped with recruitment of all subjects, and with scheduling and supervision of mouthrinsing. Ms. Debra Tereski-Bischio was the hygienist and carried out all the examinations. Mr. Michael Grabow is a licensed phlebotomist and carried out all blood draws. All the individuals mentioned above are employees of the University of Medicine and Dentistry of New Jersey and have no financial interest or obligation to the Johnson and Johnson Company.

Danette Ricci-Nitell, Dr. Paul Zhang and Dr. Marcelo Araujo are employees of the Johnson and Johnson Consumer Companies. Danette Ricci-Nitell was in charge of all documents and provided the protocol oversight. Paul Zhang was involved in the statistical planning and analysis of data gathered. Dr. Marcelo Araujo assisted in study planning and management.

The study was conducted at UMDNJ by employees of UMDNJ and with no involvement by members of the Johnson and Johnson group. The manuscript and research are original in content and were written by Dr. Daniel Fine and reviewed by each of the authors. Comments and suggestions were received and incorporated into the manuscript where deemed appropriate but the final decision was made by Dr. Fine.

The study was funded by a grant from the Johnson and Johnson Consumer Companies.

In the past decade, the link between periodontal diseases and systemic diseases and disorders has received a great deal of attention (D'Aiuto et al. 2004). The association between periodontal disease and an increased risk for heart disease shows both biological and epidemiological plausibility; however, a causal relationship has yet to be established (Lamster et al. 2008). Two theories have emerged in efforts to explain the biological plausibility of the potential link between periodontal disease and an increased risk for heart disease. One theory implicates dental plaque microorganisms in this link, while the other proposes that host-derived inflammatory factors initiated and perpetuated by periodontal disease are related to this link (Offenbacher et al. 2008).

The bacterial theory suggests that endogenous plaque microorganisms residing in a periodontal pocket can gain entrance into the blood circulation and attach to organs distant from the oral cavity (Herzberg & Meyer 1998, Van Dyke & Kornman 2008), act as exogenous and deleterious agents, and contribute to an increased risk for diseases such as, coronary heart disease (Haraszthy et al. 2000, Tonetti et al. 2007), stroke (Beck et al. 1996, Elter et al. 2003), pre-term delivery (Offenbacher et al. 1996), and renal disease (Fisher et al. 2008).

It has been shown that trauma to dental tissues can induce bacteraemias resulting from the manipulation of dental tissues by procedures that include, extraction (Lockhart 1996), scaling and root planing (Fine et al. 1996), and patient oral hygiene methods such as, toothbrushing (Silver et al. 1977, Lockhart et al. 2008) and flossing (Crasta et al. 2009). While bacteraemia induced by patient oral hygiene-derived trauma has been shown repeatedly (Sconyers et al. 1973, Lockhart et al. 2008), only a limited number of studies have shown that chewing hard food can induce bacteraemia (Cobe 1954, Forner et al. 2006).

It has been shown that dental plaque bacteria thought to reside subgingivally can be found in the bloodstream after trauma to dental tissues (Fiehn et al. 1995). Many studies have shown that antimicrobial mouthrinses can have a significant impact on the levels of supragingival plaque microorganisms and the gingivitis that ensues (Grossman et al. 1989, Ross et al. 1989, Overholser et al. 1990). Moreover, reduction in supragingival plaque by either mechanical or chemical means can have an impact on the viability of the subgingival plaque flora several millimetres below the gingival margin (Smulow et al. 1983, Dahlen et al. 1992, Hellstrom et al. 1996). Reports indicating that antimicrobial mouthrinses can have an impact on induced bacteraemias are inconsistent (Lockhart 1996). The study described herein was designed as a follow-up to previous reports, which indicated that an essential oil antiseptic mouthrinse (EOM) could be efficacious in reducing subgingival bacteria either directly by subgingival irrigation (Pitts et al. 1981, 1983, Fine et al. 1996), or, indirectly by affecting supragingival plaque (Fine et al. 2007). This study was designed to question whether an EOM could affect members of the microbial flora sufficiently to result in a reduction in bloodborne bacteria caused by a traumatic challenge to oral tissues induced by chewing an apple.

More specifically, the objective of this randomized, single-centre, double-blind, placebo-controlled, 2-week, cross-over design clinical trial was to determine if consistent use of an antimicrobial mouthrinse could reduce the bacteraemia induced by eating an apple in vulnerable individuals with mild-to-moderate gingivitis.

Material and Methods

Subjects

Volunteers for the study were selected from a panel of 200 subjects drawn from a database developed in the Clinical Research Center at New Jersey Dental School (see flow diagram in Fig. 1). Potential subjects from this panel were excluded from consideration if they had periodontal disease, defined as one or more pockets of 6 mm or greater. Sixty-two subjects who had mild-to-moderate gingivitis and plaque levels with the following inclusion criteria were screened for the study.

Inclusion criteria

Subjects were included if they were male or female at least 18 years or older and in good general and oral health. Individuals taking medications for the treatment of chronic conditions (e.g. hypertension, diabetes, depression, etc.) were required to be well controlled and stable for at least 3 months before study participation. A minimum of 20 natural teeth with facial and lingual surfaces that could be scored were required. Volunteers were required to agree to all study regulations and subjects were required to have had a gingival index score $\geqslant 1.50$ according to the modified gingival index (MGI) (Lobene et al. 1986) and a plaque index (PI) $\geqslant 1.5$ using the Turesky modification of the Quigley Hein Index (Turesky et al. 1970) as part of their database record.

Exclusion criteria

Subjects were excluded if they had teeth that were grossly carious, undergoing orthodontic treatment, or had crowned surfaces or partial dentures. They were excluded if they had a history of rheumatic fever, heart murmur or defect, orthopaedic implants, or any other condition requiring prophylactic antibiotic therapy; or if they had any history of illness requiring antibiotic or anticoagulant or steroidal therapy. Potential participants were also excluded if they had a history of any medical complication such as blood dyscrasias, renal disease, or immunosuppression. If subjects had participated in a dental plaque study within the last 30 days, or if they were using any antimicrobial dentifrice, mouthrinse, and chewing gum products on a regular basis they were also excluded. Subjects could not participate if they reported any adverse events resulting from either the use of oral hygiene products, or as a result of venipucture.

Initial screening

Sixty-two subjects from our database who met these initial entry criteria and read and signed a consent form approved by The Institutional Review Board of the University of Medicine and Dentistry of New Jersey were screened for possible entry into the study (Table 1).

At the screening, participants were subjected to an oral examination consisting of a hard and soft tissue evaluation and plaque (PI) and gingivitis (MGI) assessments. Subjects who were screened were then asked to refrain from oral hygiene, eating, drinking (except for water), and smoking for at least 12 h, but no more than 24 h before returning to the clinic for an assessment

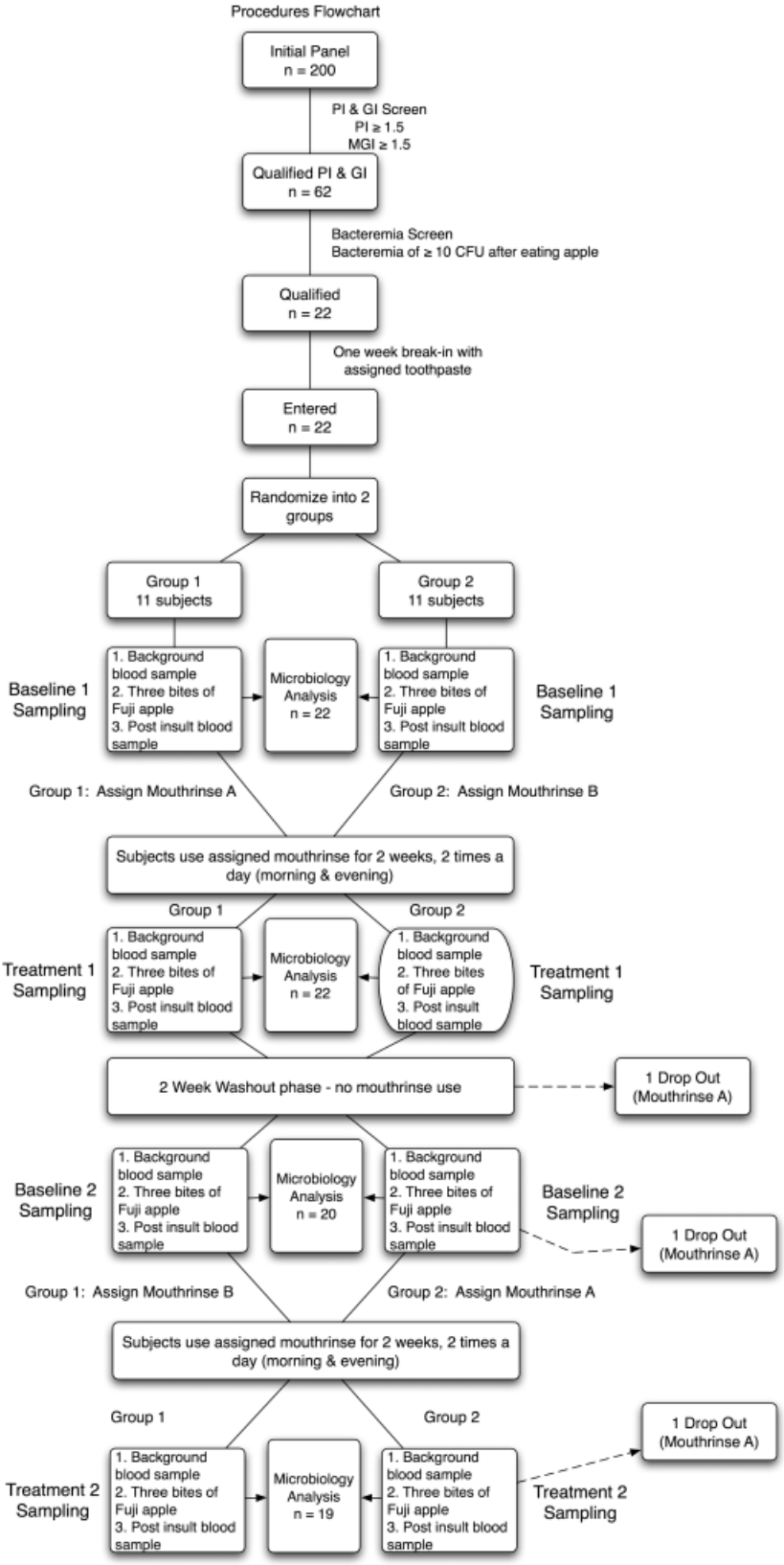

Fig. 1. Flow diagram illustrating the study design. Two hundred subjects formed the initial panel from which 62 were qualified based on the absence of periodontal disease and the presence of a gingival and plaque index of 1.5 or greater. Of the 62 subjected to a screen for susceptibility to a bacteraemia as a result of eating an apple, 22 qualified and were entered into the study.

of their susceptibility to bacteraemia to complete their screening examination. In the clinic, the next day (Visit 1) blood was drawn from the antecubital fossa to establish unchallenged background levels of bacteria. A licensed phlebotomist swabbed the area with alcohol and then used a butterfly procedure to perform the blood draw. Following the first blood draw to check for contamination, subjects were provided with an apple as a means of inducing a bacteraemia. Subjects were instructed to take three bites of the apple, which was used as a standardized method for the induction of a bacteraemia. It took about 2 min. to chew and swallow the three bites of the apple ingested. Blood samples were obtained to determine whether or not the apple challenge caused a bacteraemia in screened subjects. The blood was drawn approximately 2 min. ± 30 s after the first bite was taken. As mentioned, the butterfly was inserted before the apple challenge and was not withdrawn until after the apple challenge when the second and final blood draw were completed.

Microbiological assessment

Blood handling

Each 2 ml of blood sample that was drawn was collected in a citrated vacutainer tube. One millilitre was withdrawn from the tube and pipetted into a second tube containing 1 ml of a 1% sterile solution of sodium polyanethol sulphonate (Sigma Chemical Co., St. Louis, MI, USA) to deactivate complement.

Media

Schaedler blood agar (SBA) was obtained from Fisher Scientific (New York, NY, USA; Catalog R01800) and used to determine total anaerobic counts. Trypic soy agar (TSA) with 5% sheep blood was obtained from Fisher Scientific (Catalog B21261X) and used to determine total aerobic counts. Anaerobic media was pre-reduced in an anaerobic chamber overnight at 37°C.

Direct plating

To determine total cell counts, 0.5 ml of the blood/polyanethol sulphonate mixture was plated in duplicate on SBA for total anaerobic bacteria, and on TSA for total aerobic bacteria. All plates were

identified using the subject's random number, the assay date, and the sampling time. All plates were incubated at 35 ± 2°C. Anaerobic plates were incubated for 5–7 days, while plates grown aerobically were incubated for 1–2 days. The colonies were counted and recorded as colony-forming units (CFUs) per millilitre.

Enumeration

Replicate plates with less than 200 colonies were counted. If a plate had more than 200 colonies, the plate was designated ''too numerous to count''. Plates displaying no colonies were assigned a raw count of 0.5 to account for the counts below the limit of detection.

Definition

Bacteraemia was defined as a minimum increase of 10 CFU/ml after the apple challenge above the background seen in the bloodstream before the challenge (Fine et al. 1996). These values were considered the screening bacteraemia levels.

Study design

The 22 subjects, who met the inclusion/exclusion criteria and thus showed a bacteraemia at screening after chewing an apple, were randomly assigned to one of two treatment groups (Table 2). The study coordinator performed the assignment. All other study staff and subjects were blinded. The study statistician, utilizing a true random number generation model, developed the randomization of group assignments. Treatment I consisted of an EOM (Listerine Antiseptic®, Johnson & Johnson Inc., Morris Plains, NJ, USA). Treatment II consisted of a 5% hydroalcohol control rinse. Early clinical studies that examined the effect of the essential oils on plaque and gingivitis used a vehicle control (containing 26% alcohol), which was shown to be no more effective than water in its antimicrobial activity (Fine et al. 1985, Gordon et al. 1985). Subsequently, 5% hydroalcohol was approved for use as the control rinse (Federal Register, 2003). Subjects were required to rinse forcefully for 30 s twice a day for a 2-week period with 20 ml of the assigned treatment, followed by a 1-week wash-out period, after which they were instructed to rinse with the alternate treatment.

Before the start of the mouthrinse or treatment phase of the study, all 22 subjects were given a fluoride dentifrice and soft-bristled toothbrush to use twice daily for a 1-week wash-out or ''normalization'' period. After the normalization period, subjects were required to return to the clinical site for the baseline values before the Phase I treatment portion of the cross-over study (Visit 2). As described above, before returning to the clinic, these subjects were required to refrain from oral hygiene, eating, drinking, and smoking for at least 12 h, but no more than 24 h before the blood draw. In the clinic for Visit 2, a pre-challenge background blood draw was performed described as a check for contamination. Following this blood draw, subjects took three bites of an apple, chewed, and swallowed and the post-challenge blood draw was taken as described. The Baseline 1 value for the bacteraemia levels induced by eating an apple was calculated as the bacterial counts found in the blood after chewing the apple (post-apple challenge) minus the bacterial counts found in the blood before the apple challenge (pre-apple challenge-background levels, see Fig. 1). Subjects were then provided with their assigned mouthrinse as well as a toothbrush, commercial fluoride dentifrice, and a diary to keep a detailed record of their use of the mouthrinse and toothpaste on weekends. Following this, subjects began their regimen of rinsing with 20 ml of their assigned test product for 30 s, twice daily. Twice daily rinsing was supervised in the clinic from Monday through Friday. Unsupervised rinsing occurred on the weekends and was documented in the diary completed by each subject. This protocol was followed for 2 weeks.

On day 15 (the next day following the 14-day rinsing protocol), subjects were required to report to the clinical site for evaluation. As described above, subjects were required to refrain from oral hygiene, including use of the test product, eating, drinking, and smoking on the night before they re-visited the clinic for the blood collection. At this the post-rinsing Phase I treatment evaluation visit (Visit 3), the pre-challenge background evaluation was performed as described previously and the induced bacteraemia levels were calculated (as mentioned, induced bacteraemia levels were considered as the post-apple challenge bacteraemia minus the pre-apple challenge; pre-apple challenge is considered as the background level).

Following Visit 3, study subjects participated in a 1-week wash-out period when they used the fluoride dentifrice (in preparation for the Phase II treatment phase of the study). For Phase II, following the wash-out period, subjects repeated the baseline exams (Baseline 2 evaluation; Visit 4), and were then provided with the alternate mouthrinse (control or active) and the new treatment regimen began (Phase II treatment), which was completed 2 weeks later with the final examinations as described above (Phase II treatment evaluation on Visit 5).

Five blood draws occurred as follows: one at screening (*Screening*), two at baseline for each of the 2-week mouthrinse use periods (*Baselines 1 and 2*), and at each of the post-rinse

Table 1. Demographics, gingival, and plaque indices of bacteraemia-negative and bacteraemia-positive subjects at screening

Number (*n*) (gender, female/male)	Bacteraemia negative (*n* = 40) (28/12)	Bacteraemia positive (*n* = 22) (16/6)
Age (years); mean ± SD	32.3 ± 1.40	34.3 ± 1.89
Modified gingival index; mean ± SD	1.72 ± 1.72	1.75 ± 0.21
Plaque index; mean ± SD	1.88 ± 0.06	2.00 ± 0.08

Table 2. Bacterial counts in subjects at screening and two separate baseline sampling time points

	Screen sampling	Baseline 1 sampling	Baseline 2 sampling
Susceptible subjects (*n* = 16); mean ± SD	16.0 ± 4.50	13.4 ± 3.14	15.5 ± 4.06
Highly susceptible subjects (*n* = 3); mean ± SD	133.0 ± 45.9	53.2 ± 14.1	89.0 ± 24.1

treatment phases (*Phases I and II*). Excluding the mouthrinse visits, there were three time points (screening and two baselines) to evaluate subjects for an unhindered bacteraemia challenge. The full study design is depicted in the flow diagram as seen in Fig. 1.

Study compliance

As described, all weekday rinses were supervised and documented by the research coordinator in the daily rinsing log. The subject documented his or her weekend rinsing routine in their daily diary. Compliance for rinse usage was assessed by reviewing the diary report, rinsing logs, and weighing test product bottles at final Phase I and on the final Phase II assessment visits. Subjects were provided with take-home rinse bottles and paste; while bottles and dentifrice for clinic use were stored in a locked cabinet in the clinic. Both the weekend take home bottles and the subjects supervised rinsing bottles were weighed. In order to determine compliance, it was estimated that each subject would complete approximately 28 rinses using 20 ml of each rinse by the final visit in each phase of the study.

For the duration of the study, subjects were not permitted to use any unassigned oral care product. Subjects were allowed to use an inter-dental cleaning device only to remove impacted food between the teeth. Moreover, subjects were not permitted to have their teeth cleaned or to have any dental procedures during the study period.

Both the subjects and the evaluators were blinded to the treatment. Neither the person assessing the indices, taking the blood, dispensing, and supervising the mouthrinse usage, or plating the bacteria was aware of the treatment assigned to the subject. All blood and blood plates were given a subject number that was coded so that they could not be identified by treatment assignment.

Statistical analysis

Sample size calculation

Based on a pilot study carried out previously, the common standard deviation was estimated as $\sigma = 4$ CFU/ml. Based on a two-sided test with a significance level 0.05, it was estimated that 16 subjects were needed to complete both Phases I and II treatment. This sample size provided at least 90% power to detect a 5 CFU/ml treatment difference between the essential oil-containing mouthrinse and 5% hydroalcohol control. Considering possible attrition rate, a total of 22 subjects were entered into the study.

Efficacy evaluations

The primary efficacy endpoints were the total induced level of microorganisms found in the bloodstream calculated as the post-apple challenge bacteria level minus the pre-apple challenge bacteria level (background level) at each apple challenge test (Baselines 1 and 2 and Phase I treatment and Phase II treatment) when baseline and post-treatment challenge-induced bacteraemia counts were evaluated. Descriptive statistics and graphics by treatment group were used to summarize the study results and were presented as the total number of microorganisms in counts (CFUs/ml) derived from the induced bacteraemic event. The reduction at Day 15 from baseline in the induced level of total microorganisms in each phase of treatment was analysed using an analysis of covariance (ANCOVA). The model included treatment sequence, phase, and treatment as the factor. The total of blood-borne bacteria at baseline of each phase was used as a covariate and the subject within the treatment sequence was considered as a random effect.

Results

Demographics

Sixty-two subjects were selected from a panel of 200 who had no periodontal disease and an MGI and PI of $\geqslant 1.5$. These subjects were then subjected to an apple challenge to assess bacteraemia levels. Of the 62 screened, the majority of volunteers were females (44; see Table 1). Of the 44 females, 23 were of Asian and 21 were of Caucasian descent (data not shown). Of those screened, 22 showed positive bacteraemia (10 or more CFUs per millilitre of blood); while 40 were negative. Of those 22 positive, 16 were females (16), while six were males (6) (Table 1). The mean age of bacteraemia-positive subjects was 34.3 ± 1.89; while the age for those who were bacteraemia-negative subjects was 32.3 ± 1.40 (Table 1). The mean MGI of the positive subjects was 1.75 ± 0.21 and the PI was 2.0 ± 0.08; while the bacteraemia-negative subjects had an MGI of 1.72 ± 1.72 and a PI of 1.88 ± 0.06 (Table 1).

Study population

Each of the three times that blood was drawn (screening and the two baselines), each of the 19 subjects challenged by eating an apple showed a positive bacteraemia (see Table 2). Thus, positive bacteraemia levels were seen in each of the 57 separate challenges (each challenge showed more than 10 CFU/ml of bacteria in the blood sampled; data not shown). Moreover, the two positive baseline bacteraemia levels were not shown to be significantly different when compared with the screening challenge, showing reasonable reproducibility (see Table 2).

Treatment efficacy results

Twenty-two subjects were randomized into the study and all of those were included in the intent-to-treat statistical analysis. All 22 completed the first phase of the study. Nineteen subjects completed the second phase of the study. Of the three subjects with incomplete data sets, one subject discontinued the study due to a scheduling conflict during the wash-out period between the first and second treatments. One subject had the Baseline 2 blood draw but did not have the Phase II treatment blood draw. The third subject had the Phase II treatment blood draw but blood could not be drawn at Baseline 2. Intent-to-treat analysis was performed without imputing missing data and thus included all 22 subjects who had complete data sets for Phase I treatment and all data points available for Phase II treatment, which included the 19 who had complete data sets (both baseline and treatment as seen in the figure) as well as the three with partial data sets. Adverse events were found in three other individuals. One event was seen in the control rinse group, one in the wash-out group, and one was in the essential oil group. Adverse events were reported as a backache and two headaches.

Background blood-borne bacteria were calculated at each blood draw, which included screening, two baselines, and two treatments. Blood was drawn by inserting a butterfly needle to take blood before the apple challenge

for analysis of background blood-borne bacteria, followed by chewing the apple. The butterfly was then opened for the second blood draw for the analysis of induced bacteraemia after the apple challenge to complete the process. Thirty-one of 62 had background blood-borne bacteria at screening. Of the 31, 24 had one bacterium per plate while seven had two. Forty-two blood draws were taken at Baselines 1 and 2 and 22 of these had background blood counts. None of the 22 had more than two bacteria per plate. Forty-one had successful blood draws at Phases I and II treatments and 23 of those had background counts. Of these, one subject had three bacteria and all others had no more than two bacteria per plate. Induced bacteraemia levels, either at baseline or in the treatment phase, were calculated by subtracting background levels from the levels induced by chewing an apple.

The study was designed to assess the efficacy of twice a day usage of an antiseptic mouthrinse for 2 weeks in reducing blood-borne bacteria from a daily activity such as eating an apple. The ANCOVA used to assess these differences adjusted the baseline value in the model.

In this respect, following the use of EOM as directed for a 2-week period, the levels of induced total aerobic bacteria in the blood changed from 25.7 CFU/ml at baseline to 8.0 CFU/ml, a 17.7 CFU/ml reduction (Table 3). Meanwhile, the levels of aerobic bacteria for the negative-control rinse treatment increased (Table 3). The levels of total induced anaerobic bacteria changed from 20.5 CFU/ml at baseline to 6.0 CFU/ml, a 14.5 CFU/ml reduction (Table 3). Meanwhile, the levels of total induced anaerobic bacteria for the negative-control rinse treatment increased ($p < 0.001$; Table 3). When these data were analysed to determine the mean percentage reduction, the use of an antimicrobial mouthrinse for a 2-week period caused a 68.5% and 70.7% reduction in total induced aerobic and anaerobic bacteria, respectively (data not shown as percentages in Table 3).

Safety results

Three minor adverse effects were reported as mentioned above and thus no serious adverse events were reported during the study.

Discussion

This pilot study was designed to determine the effect of twice daily rinsing with an antimicrobial mouthrinse on the viability of blood-borne bacteria resulting from eating an apple in a susceptible population with mild-to-moderate gingivitis. This study was not intended to establish a link between plaque levels and/or plaque reduction and induced bacteraemia nor was it intended to establish any link between gingivitis reduction and induced bacteraemia levels. The study was not intended to demonstrate that reductions in the frequency or severity of the induced bacteraemias were related to reductions in plaque and/or gingivitis. The study was also not designed to take into account the individual microbes found in the bloodstream, the magnitude, and/or the duration of the planned bacteraemic event. These variables will be addressed in future studies. Moreover, the study was not designed to evaluate the efficacy of an EOM in reducing plaque and gingivitis because many studies have documented these results in the past (Ross et al. 1989, Overholser et al. 1990, Gunsolley 2006).

The focus of this study centred on subjects susceptible to bacteraemia associated with viable microbial species thought to enter the circulation through the gingival/periodontal pocket epithelial barrier at the site of trauma. It has been shown that this form of transient bacteraemia is relatively common when ulcerated periodontal tissue is manipulated by dental procedures (Lockhart 1996). It has also been suggested that daily activity can result in trauma to local tissues and thus can induce this form of transient bacteraemia (Forner et al. 2006, Lockhart et al. 2008, Crasta et al. 2009). It has also been suggested that patient susceptibility to bacteraemia resulting from the manipulation of dental tissues varies widely (Kinane et al. 2005).

With regard to subject susceptibility, it should be mentioned that in this study, 22 of 62 subjects with mild-to-moderate gingivitis showed a positive bacteraemia. We chose to limit our population to those who were susceptible to bacteraemia because our goal was to assess treatment differences. Therefore, in our study, 64.5% of the population screened (40 of 62) were not enroled. Undoubtedly, if we had enroled all 62 subjects in the study, our results would have been overwhelmed and the reduction in blood-borne bacteria after rinsing with the antiseptic mouthrinse would have been missed. Not all studies have shown antimicrobial agents to be effective in reducing bacteraemias derived from dental trauma but these discrepancies may be due to variables in experimental design that could include but are not limited to subject selection, study design, methods of evaluation, and the difference in mechanism of action of antimicrobial agents evaluated (Witzenberger et al. 1982, Waki et al. 1990, Lockhart 1996). As mentioned, our study limited inclusion to subjects susceptible to bacteraemia, which could in part explain the differences in our results as compared with those seen in other studies where all subjects were evaluated.

In our studies and those of Lockhart et al. (2008) and Crasta et al. (2009), blood was drawn immediately after the challenge. The 34.5% incidence of bacteraemia seen in this study was similar to that found in the studies by Lockhart

Table 3. Comparison of bacterial counts in subjects using control and active mouthrinse

CFU/ml bacteria in blood	Control rinse ($n = 22$)	Essential oil rinse ($n = 19$)	p-Value**
Aerobic counts (mean ± SD)			
Baseline	25.0 ± 25.14	25.7 ± 26.75	
2-week post use	35.1 ± 36.29	8.0 ± 11.12	
Change*	+10.1 ± 23.77	− 17.6 ± 17.96	<0.001
95% CI for change	(− 1.4, 21.6)	(− 25.6, − 9.7)	
Anaerobic counts (mean ± SD)			
Baseline	19.5 ± 16.61	20.5 ± 20.21	
2-week post-use	30.3 ± 34.74	6.0 ± 7.92	
Change	+10.7 ± 23.90	− 14.5 ± 13.72	<0.001
95% CI for change	(− 0.8, 22.3)	(− 20.6, − 8.4)	

*Change in counts was calculated by subtracting 2-week counts from baseline. Negative values indicate reduction.

**p-Value determined by ANCOVA comparing change in EOM treatment *versus* control.

et al. (2008) and Crasta et al. (2009) where the initiating event was toothbrushing and flossing, respectively (see Table 1). In other studies, where lower levels of bacteraemia were seen, blood was drawn 10 min. following the insult (Bender & Barkan 1989). The current study did, however, show a higher level of bacteraemia when compared with some other more recent studies (Kinane et al. 2005, Forner et al. 2006). In our study, individuals were asked to abstain from oral hygiene for a 12-h period, which allowed plaque to accumulate, perhaps adding to the level of the bacterial challenge. In some cases, where blood samples were collected immediately after the challenge, analysis of bacterial levels was carried out using DNA methods, which would not be appropriate for studies designed to assess bacterial viability (Kinane et al. 2005).

It is also worth noting that the 19 volunteers, who enroled and completed the study, received three separate unhindered bacteraemia challenges, which amounted to a total of 57 separate challenges (screening challenge and Baselines 1 and 2 challenge). This calculation excluded an evaluation of the challenges where subjects used either the control or active mouthrinse treatment. Each of the 19 subjects was positive for bacteraemia at each challenge over the 7-week period of the study. These findings indicate that individuals susceptible to bacteraemia at any one particular time were susceptible to bacteraemia repeatedly in this study protocol. Sixteen of 19 subjects had similar levels of blood-borne bacteria at each of the three blood draws, while three subjects had high levels of bacteria in their bloodstream (Table 2). Nevertheless, despite these discrepancies, reductions in blood-borne bacteria in the subjects using the EOM treatment were similar in all subjects (see Tables 2 and 3). In spite of the variability in these highly susceptible individuals, the differences between the EOM treatment and the control treatment were highly significant. The large confidence intervals support this conclusion (Table 3). While the magnitude of the challenge, in this case the eating of an apple, may not have been as great as that seen from more invasive procedures derived from visits to the dentist, our data support the concept that frequent routine daily challenge can result in repeated bacteraemic events.

The results obtained from this study supported our initial hypothesis suggesting that the consistent use of an antiseptic mouthrinse could reduce the level of blood-borne bacteria provoked by a standardized challenge, i.e. chewing an apple. Moreover, the incidental findings of this study can provide two additional pieces of data that could lead to a better understanding of induced bacteraemias. First, our data reinforce the concept that routine daily activities such as toothbrushing, flossing, or in this case chewing, can provoke a bacteraemic event (O'Leary et al. 1970, Sconyers et al. 1973, Forner et al. 2006). Second, these results although limited (by virtue of the number of subjects studied) may represent the first data set that supports suggestions by other investigators implying that daily activity can repeatedly result in bacteraemias in individuals who are susceptible (Roberts 1999, Lockhart et al. 2008).

To better illustrate this last point, Roberts (1999) estimated that there was a 5.6 million times greater chance of inducing bacteraemia by toothbrushing as compared with tooth extraction, although Lockhart et al. (2008) in a well-controlled experiment estimated that the risk from daily activity was 200 times greater than that derived from tooth extraction (Roberts 1999). As a result, these authors conclude that maintenance of good oral hygiene and the eradication of dental disease is the best approach to reduce the frequency of bacteraemia resulting from routine daily activities.

Our study supports these conclusions and suggests that the role of antimicrobial mouthrinses known to assist in the maintenance of good health can perhaps reduce levels of bacteraemia in susceptible individuals. These results provide a strong rationale for examining this area in more depth. In summary, after 2 weeks of twice-daily rinsing, total induced aerobic and anaerobic counts in the blood were significantly lowered in those subjects who used an EOM as compared with those subjects who used the control rinse. This placebo-controlled, randomized, double-blinded, 2-week cross-over study showed that the use of an EOM reduced the amount of blood-borne bacteria resulting from chewing an apple in a susceptible group of subjects that had mild-to-moderate gingivitis.

Acknowledgements

Mr. Michael Grabow, a member of the UMDNJ staff and a licensed phlebotomist, is thanked for his help with the blood draws. The study could not have been carried out without his assistance. The authors also wish to extend their gratitude to members of the Clinical Research Center at NJDS for their assistance in scheduling sessions so efficiently. We would also like to thank all the participants for their cooperation in the study and for volunteering to have multiple blood draws for the study.

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Address:
Daniel H. Fine
Department of Oral Biology
University of Medicine and Dentistry of New Jersey
Room C-636 MSB
185 South Orange Avenue
Newark
NJ 07103
USA
E-mail: finedh@umdnj.edu

Clinical Relevance

Scientific rationale for the study: To determine whether the use of an EOM can reduce the level of bacterial viability found in the bloodstream resulting from eating an apple.
Principal findings: The study showed that eating an apple can produce sufficient trauma to induce repeated and consistent levels of blood-borne bacteria in individuals susceptible to bacteraemia and that rinsing with an essential oil antiseptic significantly reduced the bacterial levels found in the blood.
Practical implications: Viability of bacteria found in the bloodstream due to an induced bacteraemic event could be reduced in susceptible individuals with mild-to-moderate gingivitis by the consistent use of an EOM.