Clin Oral Invest (2009) 13:189–194
DOI 10.1007/s00784-008-0219-3

ORIGINAL ARTICLE

Clinical and microbiological effects of an essential-oil-containing mouth rinse applied in the "one-stage full-mouth disinfection" protocol—a randomized doubled-blinded preliminary study

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Received: 25 February 2008 / Accepted: 25 July 2008 / Published online: 21 August 2008

Abstract The aim of this randomized double-blinded preliminary study was to evaluate the clinical and microbiological long-term effects of an essential-oil-containing mouth rinse as the active agent utilized in the "one-stage full-mouth disinfection protocol." Probing pocket depth and plaque and gingival indices were evaluated by the same calibrated examiner in all teeth of 20 moderate chronic periodontitis subjects. Presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* were determined by polymerase chain reaction in nonstimulated saliva, tongue dorsum, and pooled subgingival samples. The subjects were randomized into two groups: full-mouth disinfection plus essential oils (Listerine®) or full-mouth disinfection plus placebo. Clinical and microbial parameters were evaluated at baseline (T0), 45 (T1) and 180 (T2) days after therapy and analyzed using analysis of variance, Student *t*, and Wilcoxon tests ($p<0.05$). No significant differences were observed between groups regarding clinical measurements at baseline. However, in the later examinations, T1 and T2, the test group always presented higher reductions of pocket depth, plaque index, and gingival index compared to the control group. The essential-oils group revealed significant reduction on occurrence of *P. gingivalis* in saliva comparing baseline and 45 days; this difference still remain at 180 days. The essential-oil-containing mouth rinse demonstrated beneficial effects on clinical parameters. Microbiological findings were less consistent. The results of this preliminary study suggest further investigations.

Keywords Essential oils · Clinical trials · Periodontitis · Therapy · Full-mouth disinfection

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Introduction

In their review, Umeda et al. [33] reported that subgingival scaling and root planing clinically induces a reduction in periodontal pocket depth, a decrease in the number of gingival sites with bleeding on probing, attachment level gain, and a shift from a predominantly gram-negative to a gram-positive subgingival microbiota. However, the existence of intraoral translocation, from one oral site to another, of periodontal pathogens has been demonstrated [22] and reviewed [25]. Probably, this fact contributes for the commonly observed fast recolonization by pathogenic bacteria from remaining untreated sites, especially periodontal pockets, before a new and less pathogenic ecosystem has been established after conventional periodontal treatment, i.e., quadrant-by-quadrant treatment with 1-week interval.

Based on the notion that in quadrant or sextant-wise therapy a recently debrided pocket might be recolonized by pathogenic bacteria from remaining untreated pockets or from other ecologic sites, Quirynen et al. [21] in 1995 introduced the "one-stage full-mouth disinfection protocol." The proposed therapeutic protocol focused at reducing the bacterial load in periodontal pockets and intraoral sites to eliminate the risk of reinfection to assure a less pathogenic environment accompanied with prolonged periodontal health. In 2000, Quirynen et al. [24] claimed that the main benefits reached by the "one-stage full-mouth disinfection" protocol probably arise rather from mechanical therapy conducted within 24 h than from chlorhexidine usage. Later, this same group found contrasting results suggesting that the benefits allowed by the "one-stage full-mouth disinfection protocol" are related to both antiseptics and completion of the therapy in a short time [27].

Some authors reported comparable results of essential oils and chlorhexidine but with minimal or no side effects [5, 12] despite long-term usage. Overall, essential oils have demonstrated antiplaque and antigingivitis effectiveness in different clinical conditions [30] as postsurgery periods [2], implant dentistry [7], interdental cleaning, [5, 34], halitosis [28], and gingivitis control [31]. Furthermore, Quirynen et al. [23] showed that essential oils applied to "one-stage full-mouth disinfection" reduced halitosis by 90%.

The aim of this randomized double-blinded preliminary study was to evaluate the clinical and microbiological long-term effects of an essential-oil-containing mouth rinse as the active agent utilized on the "one-stage full-mouth disinfection protocol".

Materials and methods

Study population

Participants included in the present study were recruited from the University of Taubaté, SP, Brazil and diagnosed as carrying moderate generalized chronic periodontitis.

Exclusion criteria were: subjects requiring antibiotics prophylaxis; subjects with any furcation lesions; current and former smokers; diabetic and immunocompromised subjects; pregnant and breast-feeding women; subjects with orthodontic devices, extend prosthetic devices, or iatrogenic restorations; subjects ongoing periodontal treatment 12 months before the beginning of the study or who had taken systemic or local antibiotics within 6 months prior to the clinical examination. Subjects who used mouth rinse routinely in the previous 6 months were also excluded. Data and personal information related to the medical and dental histories were obtained by questionnaire. All subjects signed an Informed Consent which was previously approved by the Institutional Committee on Research Involving Human Subjects (protocol number 328/06).

Clinical examinations

A complete periodontal examination was carried out. Measurements of probing pocket depth (PPD) and plaque [32] and gingival indices [17] were obtained by the same blinded and calibrated examiner (for review, see Araujo et al. [1]) in six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) using a manual periodontal probe.[1]

Microbiological examinations

Microbial samples were obtained as previously reported [8]. Briefly, eight periodontal sites, two in each quadrant (PPD≥ 5 mm associated with bleeding on probing and clinical attachment loss), were selected in each subject. Each selected tooth was isolated with sterile cotton rolls and the supragingival plaque was removed with sterile cotton pellets. A sterilized paper point (number 30) was carefully inserted to the depth of the periodontal pocket and kept in position for 60 s. The pooled subgingival samples were stored at −80°C in microtubes containing 1 mL of reduced Ringer's solution.

Microbial samples of the left-side cheek and the dorsal of the tongue were obtained from areas of approximately 1 cm^2, using a swab with reduced Ringer's solution, rotated six times. Each swab was placed in a microtube containing 1 mL of reduced Ringer's solution. Samples of nonstimulated saliva were also collected in sterile tubes. Immediately after collection, 0.1 mL of whole saliva was diluted in 1 mL of reduced Ringer's solution.

The presence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythensis* were established by polymerase chain reaction (PCR) using specific primers under standard conditions [9]. The DNA was extracted using InstaGene Matrix.[2] The reaction mixture consisted of 100 μl of 10 m M Tris–HCl pH8.3, 50 mM KCl, 1.5 mM MgCl2, 100 μM each of dATP, dCTP, dGTP, dTTP, 0.001% (*w*/*v*) gelatin, 100 ng of each primer,[3] 0.5U *Taq* polymerase, and 100 ng of genomic DNA. After electrophoresis in 2% agarose gel, the DNA fragments were stained with ethidium bromide and visualized by UV illumination. The PCR amplificates were compared with both positive and negative controls.

[1] PCPUNC 15 Hu-friedy Mfg. Co. Inc., Chigago, IL, USA

[2] BioRad Laboratories, Hercules, CA, USA

[3] Invitrogen, USA

Periodontal therapy

The participants were randomly allocated to a given experimental group (test group: essential oils–Listerine® cool mint; control group: placebo) based on the original protocol according to Quirynen et al. [21]. The periodontal therapy was conducted as described in Table 1.

Scaling and root planing was done with Gracey and McCall curettes and Hirschfield periodontal files (a new kit of scalers every six patients), by the same trained periodontist. The time required for each quadrant was approximately 1 h and the quadrants I–IV were treated clockwise. Every month, each subject received a standard kit for mechanical supragingival plaque control containing fluoride dentifrice, toothbrush, interdental toothbrushes, and dental floss.

To assure participant's blindness, the placebo was packaged on Listerine® bottles (240 mL), previously washed with distilled water three times a day for 3 days consecutively. After 6 days, each participant received a new bottle with the same previous volume. All participants also received cups with a mark at 20-ml volume. During experimental time, dental treatment was extended to volunteer's family members to guarantee visits to the University of Taubate everyday, except Saturdays and Sundays, which allow verification of patients' compliance. At baseline pretreatment (T0), 45 (T1), and 180 (T2) days after periodontal therapy, the examiners collected the clinical and microbial data. Because active periodontal therapy was performed within 15 days, these examinations corresponded to days 0, 60, and 195 of the study, respectively.

The pharmacy Byofórmula (São José dos Campos, SP, Brazil) produced the placebo solution (sorbitol solution 15%; ethanol USP 21.6%; sodium saccharin 0.05%; benzoic acid 0.1%; mint flavoring QS; sodium benzoate; dye green QS and water QSF 1 L) according to Listerine® cool mint formula except for active agents (thymol 0.064%; menthol 0.042%; eucalyptol 0.092% and methyl salicylate 0.06%).

Statistical analysis

Clinical parameters were analyzed with the software Biostat 5.0 using analysis of variance and Student t tests while microbiological data were analyzed applying Wilcoxon test. Differences between test and control groups in each time point were considered statistically significant with $p<0.05$.

Results

Clinical results

Table 2 shows the comparisons of clinical parameters between test and control groups at the three time points T0, T1, and T2. No statistically significant differences were observed between test and control groups regarding clinical measurements at baseline. Both therapies led to beneficial clinical effects. However, essential oils always presented higher reductions of PPD, plaque index (PI), and gingival index (GI) compared to placebo, which, in any case, reached the level of significance.

Microbial results

Table 3 shows the comparisons of the microbial parameters between test and control groups at the three time points T0, T1, and T2. The essential-oils group revealed significant reduction on occurrence of *P. gingivalis* in saliva samples comparing baseline and 45 days (T2) after the end of the periodontal therapy. This difference remained at T3. However, no differences were found between groups regarding *T. forsythensis* from saliva samples.

Considering both subgingival and dorsum of the tongue samples, no statistically significant differences were observed between test and control groups regarding the detection of study bacterial species.

Table 1 Modified protocol of "one-stage full-mouth disinfection" as conducted in the present study

Modified protocol of "one-stage full-mouth disinfection"
Full-mouth scaling and root planing (the entire dentition in two visits within 24 h, i.e., two consecutives mornings) under local anesthesia
Polish of the treated quadrants with abrasive paste
Friction twice (at the beginning and at the end of each visit) of the dorsum of the tongue with a sterilized cotton swab absorbed with 0.2 mL of essential oils or placebo for 1 min
Mouth rinsing twice (at the beginning and at the end of each visit) with 20 mL of essential oils or placebo mouth rinses for 30 s (during the last 10 s, the subject had to gargle)
Subgingival irrigation of all pockets (PPD≥4 mm) three times within 10 min with essential oils or placebo mouth rinses (5 mL per irrigation per pocket) after both sessions of scaling and root planing and repeated on day 8
Mouth rinsing at home with 20 mL of essential oils or placebo mouth rinses twice daily for 30 s for the following 2 weeks
Oral hygiene instructions including tooth brushing, flossing, or interdental cleaning with interdental brushes and tongue brushing

Table 2 Comparisons of clinical parameters between test and control groups at baseline (T0) and 45 (T1) and 180 (T2) days after periodontal therapy

Clinical parameter	Time point	Baseline (T0)	45 days (T1)	180 days (T2)
PPD, mean±SD	Test	4.90±0.47	3.16[a]±0.56	3.19[a]±0.54
	Control	5.02±0.51	4.15±0.94	4.13±0.88
p value		0.679	0.002	0.003
PI, mean±SD	Test	2.70±0.27	0.65[a]±0.60	0.75[a]±0.60
	Control	2.42±0.70	1.38±0.93	1.41±0.98
p value		0.367	0.024	0.026
GI, mean±SD	Test	2.87±0.21	0.46[a]±0.50	0.46[a]±0.50
	Control	2.31±0.89	1.12±0.88	1.24±0.88
p value		0.075	0.036	0.038

PPD Periodontal pocket depth, *PI* plaque index, *GI* gingival index, *SD* standard deviation, *Test group* full-mouth disinfection protocol plus essential oils (Listerine® cool mint), *Control group* full-mouth disinfection protocol plus placebo
[a] Statistically significant differences between test and control group

Concerning *A. actinomycetemcomitans*, only one patient showed a positive sample at baseline. Thus, no differences could be verified.

Table 3 Number of positive subjects regarding three bacterial species related to sampling site and therapeutic protocol at baseline and 45 and 180 days after treatment

		Time point		
		Baseline (T0)	45 days (T1)	180 days (T2)
A. actinomycetemcomitans				
Periodontal pocket	Test	0	0	0
	Control	1	1	1
Saliva	Test	0	0	0
	Control	0	0	0
Tongue	Test	0	0	0
	Control	0	0	0
P. gingivalis				
Periodontal pocket	Test	9	6	7
	Control	8	5	5
Saliva	Test	6	0[a]	0[b]
	Control	6	5	5
Tongue	Test	10	3	3
	Control	8	4	6
T. forsythensis				
Periodontal pocket	Test	5	3	4
	Control	5	3	4
Saliva	Test	5	3	5
	Control	4	3	4
Tongue	Test	7	3	4
	Control	8	3	4

Test group Full-mouth disinfection protocol plus essential oils (Listerine® cool mint), *Control group* full-mouth disinfection protocol plus placebo
[a] Statistically significant difference between T0 and T1 (Wilcoxon test, $p<0.05$)
[b] Statistically significant difference between T0 and T3 (Wilcoxon test, $p<0.05$)

Discussion

Based on the hypothesis that untreated periodontal sites could infect treated periodontal sites, Quirynen et al. [21] introduced a new conservative therapeutic protocol named "one-stage full-mouth disinfection." This mechanical–chemical protocol includes scaling and root planing within 24 h associated with extensive use of chlorhexidine.

According to Moshrefi [19], chlorhexidine is named the gold-standard agent; however, undesirable side effects could be found after 15 or more days of continuous use of a mouth rinse containing 0.12% of chlorhexidine [5, 10, 13, 14, 16, 26] which may limit patient compliance.

Besides chlorhexidine, only essential oils have been approved by the *American Dental Association* as antiseptic due to its antiplaque and antigingivitis properties [6].

In spite of previous seemingly good results [4, 18], after evaluation of the effects of chlorhexidine on "one-stage full-mouth disinfection" protocol, Quirynen et al. [21] concluded that the main benefits probably are more related to mechanical therapy conducted within 24 h than to chlorhexidine. Similar results were reported by Zanata et al. [35] who tested povidone–iodine in the "one-stage full-mouth disinfection" in comparison with the quadrant periodontal therapy at 1-week intervals. Controversially, in 2006, Quirynen et al. [27] compared the "one-stage full-mouth disinfection" followed by different regimes of chlorhexidine and/or amino fluoride–stannous fluoride in patients with moderate periodontitis. This paper indicated that "one-stage full-mouth disinfection" results in additional clinical improvements when compared with a standard quadrant-by-quadrant therapeutic approach. Furthermore, these authors suggested that the additional benefits are

related to both the extensive antiseptics usage and the completion of the therapy in a short time. Quirynen et al. [24] suggested to combine this type of therapeutic protocol with systemic antibiotics for treatment of low-compliance patients. Finally, Koshy et al. [15] had yet speculated that other active chemical agents could be tested on the one-stage full-mouth disinfection protocol.

Supported by these previous findings, we proposed to evaluate the clinical and microbiological long-term effects of a periodontal therapeutic protocol, which combines the extensive use of an essential-oil-containing mouth rinse with mechanical procedures, regarding clinical parameters as well as the presence of *A. actinomycetemcomitans*, *P. gingivalis*, and *T. forsythensis* on subgingival, dorsum of the tongue, and saliva samples.

Regarding clinical findings, Bollen et al. [3] compared partial- and full-mouth disinfection protocols in a randomized clinical trial conducted with 16 advanced periodontitis subjects. After 4 months, these authors demonstrated probing depth reduction and gain in attachment level for both single-rooted and multirooted teeth. Additional benefits were also observed by Mongardini et al. [18] after an 8-month evaluation of chronic (n=24) and aggressive (n=16) periodontitis subjects treated by the original "one-stage full-mouth disinfection" protocol.

By our study, test and control groups showed similar conditions at baseline regarding PPD, PI, and GI (Table 2). Our results corroborate those preliminary clinical reports since after periodontal therapy the test group always presented higher reductions in PPD, PI, and GI compared to the control group. Especially concerning PPD changes, it is interesting to mention that our study population did not present a too severe disease that usually lead to higher reduction regarding this clinical parameter [20]. In fact, essential oils have demonstrated antiplaque and antigingivitis effectiveness in different clinical conditions [2, 5, 7, 28, 31, 34]. However, regarding essential oils in the "one-stage full-mouth disinfection protocol," we found only limited data and these especially concerned on reduction of halitosis [23].

In 1989, Ross et al. [29] have yet demonstrated the efficacy of essential oils (eucalyptol, thymol, menthol, and methyl salicylate) against *A. actinomycetemcomitans*, *Actinomyces viscosus*, *Streptococcus mutans*, and *Streptococcus sanguis*. Essential oils present antimicrobial activity against planktonic bacteria cell and more relevant against bacteria colonizing clinical samples from teeth, tongue, and saliva [11, 12]. In our study, essential oils reduced *P. gingivalis* from saliva samples.

It is interesting to observe better clinical results in the test group considering our pattern of periodontal disease, i.e., subjects presenting periodontal pockets in all quadrants. Although, in general, we did not observe significant reductions on subgingival bacterial prevalence, we can ask if in our study some chemical application may contribute to the decrease of subgingival biofilm pathogenicity which supports our clinical findings.

In summary, our present study indicated that the essential-oil-containing mouth rinse demonstrated beneficial effects on clinical parameters. Microbiological findings were less clear. Like any other preliminary study, our findings should be interpreted with caution and further investigations are required to evaluate the true effects of essential oils utilized in the "one-stage full-mouth disinfection protocol."

Conflict of interests statement The authors declare that they have no conflict of interest.

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