

Study Protocol and Statistical Analysis Plan

Study Title:

Comparative Evaluation of SmartMouth Clinical DDS Advanced Oral Rinse and Chlorhexidine Mouth Rinse

Date:

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Introduction:

The primary etiology for dental caries, gingivitis, and periodontal disease is the formation of bacterial colonies on the tooth surface. An inflammatory process follows the accumulation of dental plaque resulting in gingivitis. Many studies have demonstrated that plaque-induced gingivitis can be reversed following complete plaque removal. Patients are instructed by oral health professionals to regularly and thoroughly remove plaque using a daily oral hygiene regimen. Many patients demonstrate poor compliance, possibly due to lack of motivation, dexterity, or skill. An antimicrobial mouth rinse, when used as an adjunct to daily oral hygiene routine, can be beneficial to patients by reducing plaque accumulation and gingival inflammation.

Mouth rinses with 0.2% chlorhexidine gluconate are used commonly in Europe. Numerous studies have demonstrated the antiplaque and anti-gingivitis effectiveness of this rinse. Side effects associated with this mouth rinse include tooth staining, calculus formation, and altered taste sensation. Studies have also been conducted with 0.12% chlorhexidine. These trials found the antiplaque and anti-gingivitis effectiveness is comparable to the 0.2% solution, but with diminished side effects. The 0.12% chlorhexidine mouth rinse (Peridex®) is widely used on a prescription basis in the United States.

Clinical trials have confirmed the efficacy of cetylpyridinium chloride (CPC) mouthrinses, but there is limited data comparing CPC to other antimicrobial ingredients. CPC rinses are not associated with side effects such as staining or taste alteration, and its use is well tolerated by patients. SmartMouth Clinical DDS Advanced Oral Rinse (a.k.a. SmartMouth Advanced Clinical Formula) (Triumph Pharmaceuticals, Inc., St. Louis, MO) is an over-the-counter mouth rinse formulated to reduce plaque and gingivitis and to provide long term fresh breath through the elimination and prevention of bad breath sulfur gases (Volatile Sulfur Compounds or VSC) utilizing zinc-ion technology. The active ingredient in SmartMouth Clinical DDS Advanced Oral Rinse is 0.05% cetylpyridinium chloride (CPC).

The aim of this six-week double-blind, placebo-controlled clinical trial was to provide evidence of the clinical efficacy of SmartMouth Clinical DDS Advanced Oral Rinse (SM) in comparison with the efficacy of 0.12% chlorhexidine rinse (CHX) and a placebo rinse (PL). The outcomes included measures of plaque, gingivitis, tooth staining, calculus formation, patient compliance, and taste perception.

Materials and Methods:

A six-week, double-blinded, clinical study will be conducted on 80 subjects diagnosed with plaque induced gingivitis and/or chronic periodontitis. The study will take place at two centers: Saint Louis University Center for Advanced Dental Education (Site 1) and Southern Illinois University School of Dental Medicine (Site 2). The study was approved by the Institutional Review Boards (IRB #25867) at Saint Louis University and Southern Illinois University Edwardsville.

Subjects who sign the informed consent and medical history forms and meet the preliminary entrance criteria will be examined at baseline (visit 1). All participants will be informed of the nature of the study, its purpose, and any possible risks. Subjects will be informed as to the length of the study and the specific procedures that would be performed. Subjects will be informed that this was a voluntary study and that they are free to withdraw from the study at any time.

Gingival index (GI) will be measured using the Loe and Silness method.²⁴ The gingiva will be scored at six sites of each tooth (distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual) and given a score from 0 – 3. By adding the scores and dividing by the total number of sites, the GI for the patient will be obtained.

Sites with GI scores of 2 or 3 will be counted as bleeding. A whole mouth total Bleeding Score (BS) will be calculated by summing the number of bleeding sites and dividing by the number of teeth.

Extrinsic tooth stain will be scored on the facial surfaces of the eight incisor teeth using a modification of the Lobene tooth stain index (TSI).²³ Each incisor will be scored for stain area on a scale of 0-3. The sum of the stain scores for the subject will be used as that subject's stain score.

Supragingival calculus on the lingual surfaces of the six mandibular anterior teeth will be scored using the Volpe-Manhold calculus index (CI).³⁶ Supragingival calculus height in three defined planes on the lingual surface of each tooth will be measured using a periodontal probe. The measurements will be added and a mean subject score calculated.

Plaque will be scored based on the Turesky modification of the Quigley—Hein Plaque Index (PI).³³ A score of 0 – 5 will be assigned to six sites of each tooth (distobuccal, buccal, mesiobuccal, distolingual, lingual, mesiolingual) following the use of a disclosing solution. The Plaque Index for the entire mouth will be determined by dividing the total score by the number of surfaces examined.

Teeth with gross caries and third molars will not be scored. A complete periodontal examination will be performed for all subjects that meet the inclusion criteria. A panoramic radiograph will be taken for all subjects who do not have full mouth radiographs within the last year. Safety assessments will be made at each measurement visit.

Visit 2 will occur within four weeks of Visit 1. For this relatively small clinical study for which there are two centers and three treatments, subjects will be assigned treatments through the process of minimization to assure a balance in the number of subjects in each treatment group: Group 1 SM, Group 2 CHX or Group 3 PL. PL will be identical to SM, except it will not contain cetylpyridinium chloride, zinc chloride and sodium chlorite. Each group will consist of approximately 25 subjects. All subjects will be given a complete dental prophylaxis to remove plaque, calculus, and stain. Written and verbal oral hygiene instructions will be given that will includ a regular regimen of brushing twice daily and flossing daily. All subjects will be assigned a commercially available ADA accepted toothbrush and dental floss. They will be given a three-

week supply of the assigned mouth rinse with written and verbal instructions for use. Subjects will be instructed not to have their teeth professionally cleaned prior to study completion.

All mouth rinses will be in a plain package and dispensed by the faculty investigators. The package will be labeled with a code to indicate the product. Only the faculty investigators will have access to the code information. Group assignment will be performed by one of the investigators who will use the process of minimization and will not be aware of the periodontal health of subjects. Directions for use are contained in the package. The examiners will not see the product. The examiners will give instructions for mouth rinse use, but they will not be aware which rinse the subjects are using. Each product will contain two solutions which are mixed prior to use.

Subjects will be examined again at visit 3, three weeks after visit 2. All clinical measurements will be repeated. Each subject will return the unused rinse. Additional rinse will be given to each subject after visit 3 for use until the final visit. Compliance will be determined by regulating the amount of rinse dispensed on a 3 week basis and by the measurement of any unused rinse (compliance defined as percentage of product used).

Subjects will be examined again at visit 4, three weeks after visit 3. Clinical measurements will be repeated. Each subject will return the unused rinse. Compliance will be determined by the measurement of any unused rinse at the end of the study. Subjects will be given a Taste Perception Survey to answer questions about their experience with the mouth rinse.

Following the study, the subjects will be treated by their provider's discretion. Subjects can withdraw from the clinic trial at any time without providing reason. The investigators can also withdraw subjects at any time due to protocol irregularity. All instances of withdraw will be documented.

Intra-Examiner Calibration

Intra-examiner calibration will be completed prior to beginning the study. Three examiners (EB, DF, AS) will make original and repeat determinations of values for four teeth in ten subjects for each of four indices. A kappa value will be determined for each original and repeat determination, and the average kappa value will be determined for the four teeth for each index for each examiner. For an examiner to be considered calibrated, average kappa values for each index have to be between 0.81 and 1 (almost perfect agreement).

Data Analysis

The primary outcome measures are gingivitis and plaque. Secondary outcome measures are tooth discoloration, taste perception, malodor and calculus. For each outcome measure, a single value will be calculated for each subject—that is, the subject is the unit of measurement for analyses.

Analyses will be based on intention to treat (with randomized intervention). Sensitivity analyses will be based on as treated analyses. To test for differences among and between treatments, we will

use repeated measures analysis of variance (ANOVA) with treatment and location as the between-group factors and one within-subject (repeated-measures) factor with 2 levels: (1) measurements at visit 3 and (2) measurements at visit 4. For these analyses, we will use multivariate analysis of variance (MANOVA), which does not require the assumptions of compound symmetry (homogeneous pooled within-group variances and across-subjects covariances) and sphericity (orthogonal components). Post-hoc testing will be performed with the Tukey honestly significant difference ([HSD] test.

Sample-size calculation and power analysis (treatment effect of Smart Mouth ACF): One goal of the proposed study is to test the null hypothesis that the mean gingival score for Smart Mouth ACF is equal to the mean gingival score for the placebo (control). With alpha (the criterion for significance) set at 0.05, a 2-tailed test (which means that an effect in either direction will be interpreted), and a sample size of 20 and 20 for the two groups, the study will have a power of 99.9% to yield a statistically significant result. This computation is based on data from Ayad et al (2011), with the assumption that the mean difference is 0.44 (corresponding to means of 2.03 versus 1.59) and a common within-group standard deviation of 0.27 (based on SD estimates of 0.22 and 0.31). We assume that this effect size is reasonable, in the sense that an effect of this magnitude is anticipated in this field of research. We could report an observed difference of 0.44 with a 95.0% confidence interval of 0.27 to 0.61. Based on plaque scores given in Ayad et al (2011) and a sample size of 20 in each group, we would have a power >99.9% to demonstrate a treatment effect for Smart Mouth ACF. Our intent is to enroll 25 subjects into each group. Our sample-size calculations assume a 20% dropout rate. We, however, anticipate that the dropout rate will be less than 10%.

Equivalence of Smart Mouth ACF with 0.12% chlorhexidine. We will have power of 82% to demonstrate that the mean gingivitis score for Smart Mouth ACF is the same (neither lower nor higher) than the mean for .12% chlorhexidine. This assumes that the mean values for the two treatments are equal, with a common within-group standard deviation of 0.25, that a difference of .20 points or less is unimportant, that the sample size in the two groups will be 21 and 21, and that alpha (1 tailed) is set at .05. For this calculation, we used standard deviations from Ayad et al (2011). Formally, the null hypothesis is that the mean for Smart Mouth ACF is 0.20 points lower or higher than the mean for .12% chlorhexidine, and the study has power of 81.7% to reject this null. Equivalently, the likelihood is 81.7% that the 95.0% confidence interval for the mean difference will exclude a difference of 0.20 points in either direction.