CLINICAL STUDY PROTOCOL

Evaluation of the Efficacy of a Mouthwash Formulation for the Treatment of Minor Oral Ulcers: A Triple-blind Randomized Controlled Trial

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1. INTRODUCTION

Recurrent aphthosis is a disease with a multifactorial and immune-mediated inflammatory etiology. Clinically it is often associated with the manifestation of ulcers affecting the soft tissues and oral mucosa [1,2]. Recurrent aphthosis is epidemiologically the pathology of the oral district with the highest incidence around and occurs most frequently in subjects between 20 and 30 years of age [3,4].. The clinical course is often associated with periods of quiescence followed by phases of recrudescence and tends to occur less frequently and severely in the late phase of the adult subject [5,6]. In the case of an individual predisposition, this pathology can produce the appearance of recurrent lesions in adulthood, often also in association with systemic diseases or immunodepression [2,5,7]. The main individual and environmental predisposing factors are:

- Familiarity and heredity
- Microbiological factors
- Hypersensitivity
- Local traumas
- Hormonal disorders
- Immunological disorders
- States of stress and psychological disorders
- Smoking

An association with vitamin deficiencies (zinc, iron, vitamin B1, B2, B6, B12, folic acid) as well as gastrointestinal diseases, celiac disease and chronic inflammatory bowel diseases (Crohn's disease) is also reported in the literature [4,5,8,9]. In addition, recurrent aphosis has been associated with Behcet's syndrome, Reiter's disease and AIDS. In addition, neutropenia and administration of beta-blockers, bisphosphonates, NSAIDs, protease inhibitors, sulfonamides are also predisposing factors [4–6,8–11].

In the literature, several protocols have been proposed for the treatment of recurrent aphthosis with symptomatic therapies and active ingredients such as chlorhexidine 0.2% in mouthwash, benzidamine, zinc chloride and polycresulene, where, however, many ulcerative lesions can sometimes recur and be refractory to such administrations [5,6,12–15]. Among the possible correlations to this failure is the effect of dilution and *Wash-out* of saliva. Bio-adhesion to oral tissues affected by canker sores is also a key factor in determining the effectiveness of the treatment [6,8–11].

2. PURPOSE OF THE RESEARCH

The aim of the present research is to evaluate the efficacy of a mouthwash formulation for a total treatment of 14 days for the treatment of oral ulcers of traumatic or aphthous nature.
The study groups are as follows:

- Group I (Test): Curasept Canker Sores Rapid Mouthwash: Dicaprylyl Ether, Coco-Caprylate/Caprate, Hydrolyzed RNA/DNA, Hyaluronic Acid, Cetyl Palmitate, Beta-Glucan.

Versus

- Group II (Control): Placebo mouthwash

Null hypothesis (H0): There is no difference at 14 days in the reduction of the mean pain sensation and ulcer area score, thermography [16,17] and Ulcer Severity Score (USS) [18] in the two groups, against the alternative hypothesis of a difference between them.

3. STUDY DESIGN

The present study is a 2-week double-arm randomized controlled trial.
The study will be carried out in the following ways:

- Randomization: Using a computer-generated, centralized random number with random codes (A, B) placed in sequentially numbered sealed opaque envelopes provided by the study consultant. A simple randomization procedure will be used.
- Concealment of assignment: Doctors involved in patient treatment will never know which mouthwash will be delivered to patients. Identical and indistinguishable bottles with mouthwash will be prepared by a person designated by the sponsor. The mouthwash will be poured into identical vials labeled with only one letter (A, B), and only the person designated by the sponsor will be of which product matches the letters. The random assignment will be made by opening the sequentially numbered sealed envelope just before the surgery.
- Blinded assessment: A single, independent, blinded assessor will perform all clinical measurements. Patients will not be aware of the mouthwash they will use. In addition, the operator evaluators and statistician will remain blinded until statistical analysis (blinded study) is performed. The codices (A, B) will be broken at the time of writing the manuscript.

The principles outlined in the Declaration of Helsinki on clinical research involving human subjects will be respected. Written informed consent will be obtained for each patient.

The study will be reported according to the CONSORT statement to improve the quality of reports of randomized parallel-group trials (http://www.consort-statement.org/).
A total of 3 visits will therefore be carried out:
• A screening/baseline visit will help establish patients' eligibility for inclusion in the study, followed by baseline data recording and first supervised mouthwash application
• A 1-week experimental visit
• A 2-week experimental visit and end of study

3. CRITERIA FOR THE SELECTION OF STUDY SUBJECTS

3.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for study enrollment:

1. Patients with at least one well-defined ulcer
2. Any patient who is at least 18 years old and able to sign an informed consent.

3.2 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment:

1. Intolerance or allergy to the product.
2. Patients with hematologic deficiency such as anemia, iron, vitamin B12 and/or folic acid deficiency; systemic diseases such as ulcerative colitis, Crohn's disease, Behcet's syndrome in which RAS is part of their clinical presentation; alcohol consumption and smoking; history of allergy; treatment of ulcers with systemic steroids, vitamins, antibiotics, antihistamines, oral retinoids, or immunomodulatory agents within three months prior to study entry; and the use of nonsteroidal anti-inflammatory drugs or mouthwash for the treatment of ulcer prior to 72 hours of study entry.
3. Patients who are pregnant or breastfeeding.

4. Procedure

4.1 Treatment

The local trial manager will open the sequentially numbered sealed pouch corresponding to the patient's enrollment number and provide the patient with the randomly assigned coded vial (A, B) instructing the patient to rinse the ulcer with mouthwash, twice a day, after oral hygiene.

The treatments assigned are:
• Curasept Afte Rapid Mouthwash: Dicaprylyl Ether, Coco-Caprylate/Caprate, Hydrolyzed RNA/DNA, Hyaluronic Acid, Cetyl Palmitate, Beta-Glucan.

Versus

• Placebo mouthwash

4.2 Treatment compliance

Compliance will be assessed based on the information recorded at the last visit by the subject and the information recorded in the CRF during the treatment visits.

4.3 Product Storage

Store in a cool place (< 30°C) and keep away from light sources in an unopened original vial
STUDY PLAN

4.4 Study program

Screening and baseline visit (V1)
Potentially eligible patients are screened to determine their eligibility for the study. Complete (Patient Eligibility CRF) and record the number and reason for patients not included.
Prior to enrollment, all patients will be asked to sign an informed consent form to document that they understand the purpose of the study (including procedures, follow-up assessments, and any potential risks involved), have been given the opportunity to ask questions related to this study, and have been informed of treatment alternatives.
At the recruitment visit, the clinician should assess the presence of an oral ulcer, rule out lesions of neoplastic, infectious, or allergic origin, and remove, if present, any causative agents (such as sharp edges of teeth, fillings, crowns, dentures, or braces).

Primary Results:

• Pain intensity. It is measured using the VAS consisting of a 10 cm line [13], where 0 indicates no pain and 10 indicates severe pain.

• Size of each ulcer. A sheet of clear plastic will be applied directly to the ulcer and using a permanent waterproof marker the circumference of the ulcer will be traced and then placed on a graph paper and the number of mm2 units included within the drawn area will be counted.

Secondary Results:

•Thermography
• Ulcer Severity Score (USS)

Treatment will be started on the same day with strict infection control measures.
The assessor will open the sequentially sealed envelope corresponding to the patient's recruitment number and write on the CRF the progressive number of the patient contained in the envelope and to which group (A or B) the patient will be assigned.

Patients will receive three anonymous bottles, coded with A, B containing the mouthwash and a scoop. Patients will apply mouthwash. This procedure will be performed under the supervision of the outcome measure which will provide further explanations to patients if necessary.

Participants will be asked to flush with 10mL of mouthwash twice daily ~~directly onto the ulcer~~. Patients were prevented from eating, drinking, or rinsing their mouths for at least one hour after each application.

Observation at 1 week (V2) and at 2 weeks (V3):

- Pain intensity using the VAS scale. The following outcome measures will be recorded by the blinded outcome assessor:
- Size of each ulcer using a clear plastic sheet

- Thermography/ Ulcer severity score (USS)

In the event of any complication observed during any of the scheduled visits or during an emergency visit, protocol deviation and drop out should be filled out CRF.

Unscheduled visits may be made during the study at the discretion of the Investigator.
Relevant information will be collected in the CRF.
The study plan and the scheduled items are summarized in the following flowchart:

Visit	**Screening and Baseline (V1)**	**1 week (V2)**	**2 week (V3)**
	Day 0	**Day 7**	**Day 14**
Evaluation			
Inclusion and Exclusion Criteria	✓		
Informed consent	✓		
First mouthwash application	✓		
Pain Assessment (VAS)	✓	✓	✓
Ulcer measurement	✓	✓	✓
Thermography	✓	✓	✓
Ulcer severity Score (USS)	✓	✓	✓
Complications		✓	✓

6 CLINICAL PROCEDURES

6.1.1 Summary of procedures and follow-up

Day 0 Patient Recruitment, Informed Consent.
Pain Intensity (VAS), Lesion Measurement, Thermography, USS and Mouthwash Application
Day 7 Pain Intensity (VAS), Lesion Measurement, Thermography, USS, and Recording of Complications and Adverse Effects
Day 14 Pain Intensity (VAS), Lesion Measurement, Thermography, USS and Recording of Complications and Adverse Effects

6.1.2 Drop-out

All drop outs will need to be reported by recording the study group's reason for drop out. No included patient can be excluded from the final evaluation, for any reason, by the clinical investigators.

6.1 Evaluation of the primary objectives

- Pain intensity. It will be measured using the VAS consisting of a 10 cm line [13], where 0 indicates no pain and 10 indicates severe pain.
- Size of each ulcer. A sheet of transparent plastic will be applied directly to the ulcer and using a permanent waterproof marker the circumference of the ulcer has been traced and then placed on a graph paper counting them units of mm2 included within the drawn area. Treatment will begin on the same day with strict infection control measures.

6.2 Evaluation of secondary objectives

a. Thermography [16,17,19]
b. Ulcer severity Score (USS) [18]:

Ulcer severity Score (USS)

	Characteristics of the lesion	**Score**	**Description of the USS**
Average number of injuries			Score = average number of ulcers in a culture Maximum score = 20
Average lesion size (in mm)			Score = mean size of ulcers in mm Maximum score = 20
Average duration of lesions (in weeks)			Score = number of 1/2 weeks i.e. Half a week (3 days) scores 1, one and

			a half weeks (10 days) scores 3. Maximum score = 10
Injury-free period (in weeks)			Score = 10 minus the average ulcer-free period in weeks Maximum score = 10 (never ulcer-free)
Patient-perceived pain (scaled from 0–10)			1 for slight discomfort in the presence of ulcers.10 for excruciating ulcers that interfere with eating and speaking Maximum score = 10
Site of injury	**Group 1** Labial mucosa Buccal mucosa Buccal sulcus Soft palate Belly of the tongue Floor of the mouth **Group 2** Hard palate Adherent gum Alveolar ridge Dore of tongue Tonsils Tonsil pillars Uvula Oropharynx		Score = total of sites involved 1 for each site of group 1 (non-keratinized mucosa) 2 for each site of group 2 (keratinized, specialized) Maximum score = 10
Scarring	Yes/No		**USS Total**

Complications and adverse events: Any post-operative complications and adverse events (mucosal irritation, allergic reactions, etc.) will be recorded and reported by study group by blinded evaluators in the Complications, protocol deviation, and drop out.

7 ANALYSIS

7.1 Sample size

A sample size of 29 subjects for each group (power 80%) to detect a significant difference in means of 0.820 assuming the common standard deviation is 1.80 using a two-group t Student test with a significance level for $p<0.05$. Considering a 3% drop out, a sample of 30 subjects per study group will be enrolled.

7.2 Population

- **Intention-to-Treat population (ITT):** All randomized subjects who complete the clinical procedure and use mouthwash at least one day and with at least one available assessment of variables after baseline will be considered in this population.

- **Per-protocol population (PP):** All subjects in the ITT population with primary outcome measures available and without major protocol violations (e.g., misinclusions, poor compliance).

7.3 Statistical analysis

Descriptive statistics will be provided in summary tables by group based on the type of outcome measure summarized.
General descriptive statistics for continuous outcome measures will include n (number of observed values), mean with its 95% CI, standard deviation, median, minimum and maximum values. For categorical variables, the number and percentage of subjects with a specific level of the variable will be presented.
This primary efficacy analysis will be performed on ITT, using the null hypothesis of no between-group difference in overall VAS change from baseline between both groups:

H0: μAfteRapid~~DNA~~ = μ Placebo
H1: μAfteRapid~~DNA~~ μ Placebo

Where μ is the mean change from baseline on Day 7 within the given product.
This hypothesis will be tested with an Analysis of Variance (ANOVA) with baseline values of VAS as a covariate. The groups will be compared to the bilateral significance level of 5%.
The assumption of normality will be verified by means of QQ graphs and by plotting the studentized residuals with respect to the expected values of the variable. If there are strong indications of non-normality of the data, the Wilcoxon Rank-Sum Test will be used to test the hypothesis of no median difference between buccacollutorio and placebo at Day 7 in a nonparametric manner. Summaries for each treatment group will include sample size, mean, median, standard deviation, minimum, maximum, Wilcoxon Rank-Sum test p-value, Hodges-Lehman point estimates of median treatment difference, and its 95% confidence interval.
From the Day 7 endpoint ANOVA model, a least squares estimate (LSMEANS) of the group effect and group difference (both with 95% confidence intervals) will be displayed along with the p-value of the difference between the groups.
Analysis will also be performed for the PP Analysis Set to test the robustness of the results.
For secondary quantitative efficacy variables, cross-treatment group comparison of change from baseline will be done by the same ANOVA model used in the primary efficacy analysis. Categorical derived variables (complications/adverse events) will be compared via a chi-square test or Fisher's exact test, as appropriate (chi-square test will be used if all groups have sufficient observations,

otherwise Fisher's exact test will be used). All statistical comparisons will be conducted at the significance level 0.05. SAS version 9.4 will be used for scanning.

8 Data management

An electronic CRF (eCRF) will be completed by the Investigator and/or his/her delegate.
All patients who sign the informed consent will be archived. A minimum set of information is required for patients who are screened but not randomized: date of signed informed consent, demographics, assessment of inclusion/exclusion criteria where applicable, primary reason for non-continuation, adverse events, and any concomitant medications.
Front-end change checks will be performed at the time of data collection, and back-end change checks will be used by the Data Manager to check for discrepancies and ensure data consistency and completeness.

Administrative practices and data management

Medical record forms (CRFs) must be completed at the time of data collection. The complete CRF memory device containing the digitized clinical images should be attached to each patient's study file.
All data required for this study will be collected using paper-based CRFs. CRFs will be kept in a secure, locked location that will only be accessed by the Principal Investigator and the data collector. The data will also be accessible to the sponsor for monitoring and evaluation purposes at its discretion. After completion of the 1-week follow-up, copies of all CRFs will be sent to the investigator who will check the data and store them in a protected computer with a password known only to him. The data will be processed by an expert statistician. The patient's identity will be protected and known only to the study participants listed in this protocol.

Case Report Forms (CRFs)

- Patient informed consent form
- CRF Patient Eligibility
- CRF Rating
- Complications, protocol deviation and CRF drop-out

Changes and violations of the Protocol

Changes to this protocol may be necessary, and protocol violations are likely to occur. Any protocol violation should be reported in the "Complications, Protocol Deviation, and CRF Dropout" section. If you experience any problems, contact your studio monitor immediately.

Data Ownership

The data belongs to the sponsor.

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