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Analysis of The Bacterial Inhibition Capacity of The Topical Anesthetics Pliaglis: *In Vitro* Study

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Abstract

The expansion and recognition of the Orofacial Harmonization (HOF) within dental field, made the search for aesthetic and functional procedures increase. This study proposes to evaluate an *In Vitro* antimicrobial action of the topical anesthetic Pliaglis for the bacteria *S. aureus*, *S. epidermidis* and *E. coli*, microorganisms that are commonly related to skin diseases arising from HOF procedures. And even if it is an *In Vitro* test, the results obtained are in accordance with Pliaglis for use in the injectables procedures.

Keywords: Anesthesia; Bacteria; Infection Control and Skin

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Introduction

The search for aesthetic and functional procedures in the field of dentistry brought the expansion and recognition of the dental specialty Orofacial Harmonization (HOF), which has been gaining prominence and adherents every day, increasing the therapeutic arsenal of dentists, with the use botulinum toxin, dermal fillers and collagen biostimulators, among others [1].

HOF techniques require minimally invasive procedures and for that, the precepts of antisepsis and asepsis have become essential items for the good result. However, with the increasing number of procedures, as well as the associated adverse effects, some complications have been reported such as infections, acute, chronic inflammation and nodule formation [2, 3, 6, 8].

Although it is a controversial subject, studies have been carried out to assess the causes of infections and adverse events, as well as to evaluate the substances used [1-5]. Some professionals and opinion leaders suggest that after performing the injection through anesthetic and / or marking point, skin tattooing and / or infection of the application point may occur due to the possibility of the bacteria be taken into the tissue layers [21].

In addition, factors related to patients should also be considered, such as the immune response and other comorbidities. Changes in the immune system, its impairment due to some disease, as well as a transient bacteremia at the time of injection can facilitate the infection [7,8] corroborating the contraindications in patients with active infection at the application site or with some decompensated underlying disease [12]. The microorganisms most commonly involved in infections of the skin are bacterias that belong to the microbiota of the skin such as Staphylococcus epidermides (*S. epidermidis*), and Staphylococcus aureus (*S. aureus*), which can be associated with various infections when the skin is ruptured. In addition to these microorganisms, Escherichia coli (*E. coli*) and Pseudomonas aeruginosa (*P. aeruginosa*), also have great potential to cause these infections. In addition, when the skin is ruptured in a dermal filling procedure, the bacterial biofilm that resides on the surface of the skin can penetrate into deeper layers, releasing bacteria that can cause a local infection, systemic infection or granulomatous reaction [9-15]. Careful skin preparation, aseptic technique and the use of products in appropriate conditions in HOF procedures are necessary to prevent infection and adverse events. Disinfection of the skin is consensual in the literature, following protocols for injectable procedures based on a research carried out in 2007 in the United Kingdom, a comparative study that replaced Isopropanol 70% with Chlorhexidine 2% for catheter antisepsis, obtained an important decrease of the infection. Thus, it is recommended to wash the face with soap and water and perform antisepsis of the skin surface with 2% chlorhexidine [29].

There is a need to prevent infection when performing HOF, that's why antisepsis is so essential. In addition, any additional factor related to the technique may contribute to the genesis of the infection, such as topical anesthetic, quality of the antiseptic and injection point markers.

Local anesthetics (LAs) are drugs that provide analgesia in different regions of the body by blocking sodium channels [16]. In procedures for orofacial harmonization, local and topical anesthetics are used to minimize pain at the injectable site. They are placed on the markings and left for the time recommended by the manufacturer in order to promote local analgesia. Therefore, the aseptic condition of anesthetics is very important [1].

Razavi, *et al.* 19 in a literature review showed that, in addition to pain control, local anesthetics (LAs) can be used as antimicrobial agents against a wide variety of microorganisms. In this review, the antibacterial effect of several LAs were compared and the differences found can be attributed to the variable conditions of the tests, such as type of microorganism used, concentrations of anesthetics or microorganisms studied, time of exposure and others [18,26]. Although not fully understood, the antimicrobial or microbial growth inhibition mechanisms proposed for these ALs are related to cell membrane disruption, inhibition of cell wall synthesis, dysfunction of the respiratory system, alteration of DNA synthesis, alteration of membrane permeability and others [22,24,26].

One of the topical anesthetic preparations used in the HOF is 7% lidocaine and 7% tetracaine, Pliaglis®. It is a 70mg / g lidocaine-based cream 70mg / g and tetracaine indicated to numb the skin before performing painful procedures, such as the insertion of needles. It has been shown to have the best anesthetic result when compared to other anesthetic preparations in injectable procedures, showing greater comfort for the patient [27-30].

Among its components, the lidocaine is the most studied drug 20,21. It has fast onset of action and intermediate duration. An antimicrobial effect has been demonstrated for Gram negative microorganisms such as *E. coli* and Pseudomonas aeruginosa, and Gram-positive ones such as Staphylococcus aureus and Staphylococcus epidermidis. Tetracaine, on the other hand, showed greater antimicrobial activity in studies comparing other Als. These anes-

thetics can play an important role in the prophylaxis of surgical site infections, thus, dentists use it for this purpose and as a pre-puncture topical anesthetic to prevent pain [17,19].

Marking the puncture points is a common and necessary practice for orofacial harmonization procedures. In practice, most professionals carry out these markings with a white dermatographic pencil and apply topical anesthetic on them, but it is cleared before the injection. This removal makes it more difficult for the professional to perform a safe application avoiding risks such as asymmetries, injections in unwanted places and noble structures [1].

Although studies have demonstrated the antimicrobial effect of lidocaine and tetracaine, there are still precautions regarding to the risk of contamination that the topical anesthetic and marking points may cause at the time of the injection, therefore, it is suggested to disinfect the skin and remove the mark and topical anesthetic before performing the puncture, or in some cases, professionals prefer to deviate and apply outside the mark and anesthetic [28].

Considering the importance of skin antisepsis for HOF procedures and that Pliaglis produces an efficient local analgesic effect, the present study proposes to evaluate the *In Vitro* antimicrobial action of Pliaglis for microorganisms commonly related to skin infections associated with HOF procedures.

Materials and Methods

In Vitro study

Bacteria used

Standard strains of Staphylococcus aureus (ATCC 29213), Escherichia coli (ATCC 25922) and Staphylococcus epidermidis (ATCC 12228) were used. These bacteria were prepared in concentrations of 10-6 UFC / mL and 10-8 UFC / mL for use in the tests.

Topical Anesthetic

The anesthetic Pliaglis® topical cream (Galderma Brasil Ltda, São Paulo, Brazil) was used as a commercial preparation of Lidocaine 70mg / g and Tetracaine 70mg / g.

Procedure

The methodology described by Kirby Bauer31 was used. The bacteria were previously grown in nutrient broth (TSB - Difco) and incubated for 24h at 37 °C for later adjustment of the concentrations to be used, by reading the optical density (OD). Petri dishes containing Mueller Hinton's medium (Difco) were seeded with the prepared bacterial inoculum, using Drigaski's loop, to obtain homogeneous growth. Then, sterile filter paper discs (6mm), soaked with the test product (Pliaglis), were deposited on the plates containing the seeded culture medium. The plates were incubated for 24 to 48 hours at 37 °C for subsequent observation of the formation of microbial growth inhibition halos. The tests were performed in triplicate.

Results

The *In Vitro* study, growth inhibition halos of all microorganisms used were observed.

The plates containing *S. aureus* 10^6 and 10^8 UFC / mL showed halos of inhibition formed around the disks containing the anesthetic in both concentrations (Figure 1). The halos formed were the same size in the two concentrations used (Table 1).

Tests with *S. epidermidis* showed the formation of bacterial growth inhibition halos in the two concentrations used (Figure 1), presenting a larger halo in the concentration of 10^6 CFU / mL (Table 1).

The tests performed with *E. coli* showed the presence of inhibition halos for the different concentrations used, 10^6 and 10^8 (Figure 1), also showing a greater halo for the concentration of 10^6 CFU / mL (Table 1).

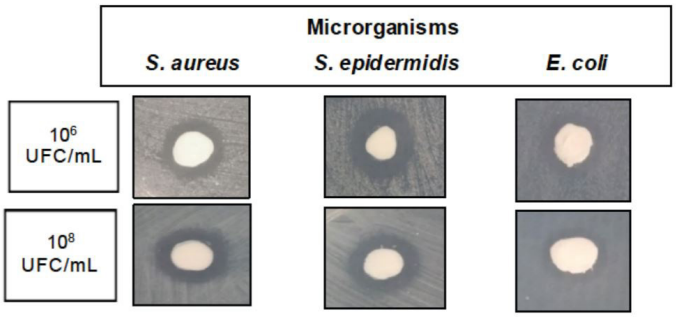

Figure 1: Images of the bacterial growth inhibition halos at concentrations of 10^6 and 10^8 CFU/mL.

Microrganisms	10^6 UFC/ mL	10^8 UFC/ mL
S.aureus	12mm	10mm
S. epidermidis	14mm	12mm
E.coli	12mm	10mm

Table 1: Measures related to microbial growth inhibition halos

Discussion

Orofacial harmonization procedures, even if considered minimally invasive, must follow safety protocols to prevent adverse events 23, infections and to provide comfort to the patient. For this, washing the face with soap and water, adequate disinfection with 2% chlorhexidine, precise markings to avoid asymmetries and the use of local topical anesthetics are important factors for safety 29, providing comfort to the patient.

The markings are made with pencils or dermatographic pens that allow the orientation of the application sites, both should be disinfected with 70% alcohol or, if possible, with a plastic barrier, in order to avoid cross contamination. At the time of the injection, the pencil mark and anesthetics are commonly removed because of the fear of infiltration of the marking pencil and anesthetics together with the injectable materials that could cause infection and / or tattooing. However, so far, there is no evidence to show that these elements can induce infection, nor does the tattoo. In addition, studies have demonstrated the antimicrobial capacity of several Las [10-19].

The present study showed that the association of local anesthetics 7% lidocaine and 7% tetracaine in combination (Pliaglis) [27,28] have the ability to inhibit bacterial growth of Gram positive and Gram negative bacterial samples, presenting a significant inhibition halo even for high concentrations of the evaluated microorganisms. The cutaneous concentration of healthy individuals varies between 10 to 10^6 CFU / cm^2 [32]. Thus, the tested AL showed a capacity to inhibit bacterial growth *In Vitro* 100 times greater than the maximum usual condition. In addition, the use of the modified Kirby Bauer test allowed the observation of the size of the halos of inhibition of bacterial growth formed for all bacterial concentrations used, allowing to qualitatively evaluate the result against the concentrations of lidocaine and tetracaine present in this product evaluated [31].

Differently from what was observed in other studies, which used different concentrations of LAs to verify its antimicrobial effect, this study aimed to test the variation of the innocuous bacterial, in order to observe the antimicrobial capacity of this association of LAs, in fixed concentration, in front at different bacterial concentrations.

It was found that for S. aureus the results regarding the size of the bacterial growth inhibition halos obtained for both concentrations of the bacteria was the same, for *S. epidermidis* and *E. coli* the size of the halos was higher the lower the bacterial concentration, showing that the antimicrobial effect of this LA may be related to bacterial concentration. However, further studies should be carried out with other bacteria to evaluate the spectrum of antimicrobial action of this LA.

The antimicrobial action of lidocaine and tetracaine appears to be associated with its structure and to be dose-dependent [33]. Its antimicrobial effects for Gram positive and Gram negative have been shown in several studies and appear to be related to changes in the membrane permeability of the microorganism [25,26,34-37].

Considering that pain reduction in procedures is a relevant factor in inhibiting the post-operative pro-inflammatory response by reducing the stimulation of nociceptors, a local anesthetic with the dual function of analgesia and antimicrobial action, represents an important condition for a good recovery clinical with low inflammatory response, as well as preventing the infection of the surgical site.

Even though it is an *In Vitro* test, the results presented in this study suggest the reliability of Pliaglis for use in injectable procedures in HOF. However, clinical studies must be carried out to better assess and validate this hypothesis.

Conclusions

This study showed that the Pliaglis formulation has an *In Vitro* antimicrobial action for bacteria *S.aureus*, S.epidermidis and E.coli, microorganisms commonly associated with infections on the procedures in HOF.

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