Antibacterial and antifungal activity of intraoral products containing phthalocyanine: in vitro study

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Abstract

The present study aimed to evaluate *the in vitro* antiseptic efficacy of a mouthwash and a dental gel containing phthalocyanine derivatives (Pc) against bacteria and fungi frequently found in patients with ventilator-associated pneumonia. The experiment in this study was conducted following Good Laboratory Practices. The product was tested at concentrations of 0.015% (mouthwash) and 0.100% (dental gel). The contact time of the suspension test (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella sp.*, *Candida albicans*, and *Aspergillus niger*) was 60 s (1 min). In this analysis, the Phtalox® Mouthwash and Dental Gel resulted in a 99.99% reduction against the tested microorganisms after 1 min of contact time in both products. The Pc-containing mouthwash and dental gel were effective against bacteria and fungi found in patients with ventilator-associated pneumonia.

Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs at least 48 hours after endotracheal intubation or tracheostomy for mechanical recovery, including pneumonia occurring within 48 hours after extubation. This pneumonia usually occurs in patients admitted to the intensive care unit (ICU). The risk of VAP increases during mechanical ventilation and hospitalization, and can lead to death. VAP is responsible for most antibiotic prescriptions in the ICU (1).

The most important mechanism in the development of VAP is the continuous microaspiration of microorganisms present in the oropharynx. The most frequent microorganisms found in oropharyngeal samples are *Acinetobacter*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Staphylococcus aureus*, *Candida albicans*, and *Escherichia coli*. The last three are found most frequently in patients with VAP (2, 3). The normal oropharyngeal flora is overwhelmed by gram-negative pathogens approximately one day after hospitalization. This causes an increase in dental plaque, which is suitable environment for the growth and accumulation of pathogens. The tracheal tube can also act as a conduit for pathogens from the oral cavity to the lungs. The treatment of VAP is mainly antibiotics. However, evidence suggests that its use has generated bacterial resistance and increased the development of resistant bacteria (4, 5).

The incidence of VAP is reduced by identifying the risk factors and enhancing prevention. Oral hygiene procedures such as combining toothbrushing and mouthwash, are efficient methods for preventing VAP (6). Chlorhexidine is a broad-spectrum antiseptic agent widely used in patients because of its ease, safety, and slow-release properties that maintain its antimicrobial activity for up to 12 hours. Studies have confirmed that chlorhexidine reduces the incidence of VAP, but there is no consensus on the best concentration, the frequency of use, or the optimal application technique in the oral cavity (2).

Phthalocyanine derivatives (Pc) have been shown to be important antimicrobial agents (7, 8). Pc are non-cytotoxic and have no known side effects (8, 9, 10). When incorporated into dental products, Pc have improved clinical symptoms and reduced the length of hospital stay (8, 10, 11).

Thus, the present study aimed to evaluate the *in vitro* antiseptic efficacy of a Pc-containing mouthwash and dental gel against bacteria and fungi frequently found in patients with VAP.

Material And Methods

For microbiological tests on non-sterile products, aseptic techniques were used for sampling and testing. The test was conducted in a laminar flow hood, and the membrane filtration technique was employed. When a sample showed antimicrobial activity, it was conveniently removed or neutralized. The efficacy of the inactivating agent for the considered microorganisms, and the absence of toxicity were demonstrated. When surfactant substances were used during sample preparation, the absence of microorganism toxicity and compatibility with the inactivating agent were also evaluated by counting the total number of mesophilic microorganisms. With this test, it is possible to determine the total number of mesophilic bacteria and fungi in non-sterile products and raw materials to determine whether the product meets pharmacopeia microbiological requirements. When used for this purpose, the instructions must be followed strictly, including the number of samples and interpretation of the results. The test was not applied to products containing viable microorganisms as an active ingredient.

The experiment in this study was conducted following Good Laboratory Practices. In the absence test, homogenization of the A dilution was performed and the volume corresponding to 1 g or 1 mL of the product was transferred to the enterobacteria enrichment broth mossel (*Aeromonas* and *Pseudomonas* can also grow in this medium, as well as other types of bacteria) and then incubated at $32.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ for 24 - 48 h. The subculture was prepared on plates containing neutral bile glucose red-violet agar and incubated at $32.5^{\circ}\text{C} \pm 2.5^{\circ}\text{C}$ for 18 - 24 h. The product passed the test if there was no growth of colonies.

The dilution-neutralization method was used, in which the neutralizer corresponded to a mixture of Tween, saponin, L-histidine, sodium thiosulfate, and lecithin. The tested product was kept at concentrations of 0.015% (mouthwash) and 0.100% (dental gel) ready to use. The contact time of the suspension test (bacteria/fungus/yeast) was 60 s (1 min) and the interfering substance for cleaning was 1.5 g/l.

The substance identification test was evaluated according to the concentration indicated in its use. A sample of the product, either ready to use or diluted with water, was added to a suspension of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella sp.*, *Candida albicans*, and *Aspergillus niger* prepared in a solution of interfering substances. The mixture was maintained at a specified contact temperature and time under mandatory conditions for "hand rub" products. At the end of the contact time, an aliquot was removed, and the bactericidal and/or bacteriostatic action of the portion was immediately neutralized by a validated method. The same procedure was adopted for the control, in which hard water was used. The viable bacteria/fungus/yeast in each sample were counted, and the reduction in the number of viable cells was calculated for relation control.

For the substance test to be considered satisfactory, the conditions of the validated test of the "hand rub" products must reduce the number of viable cells to at least 10×5 (≥ 5 logs or $\geq 99.999\%$) at 20°C .

Results

Table 1 demonstrates the results of the positive control for the antiseptic efficacy of the Phtalox® Mouthwash and Dental Gel with a 99.99% reduction against the tested microorganisms after 1 min of contact time.

Discussion

In discussing VAP is necessary to understand that they are hospital-acquired pneumonia (HAP), and is the main cause of death from hospital infections in critically ill patients and the second most common cause of nosocomial infections (12). As an aggravating factor in the pandemic, it is known that about 33% of hospitalized patients with COVID-19 tend to require ICU care. In addition, up to 20% of these patients may require the use of invasive mechanical ventilation (13). This reaffirms the need for intraoral topical antiseptic measures for preventing infections of those under mechanical ventilation and to act against the imbalance of the intraoral biome (14,15,16).

Some studies have demonstrated the application of topical products in patients on mechanical ventilation, such as chlorhexidine and povidone-iodine (17,18,19). Chlorhexidine is the gold standard. However, the reduction in the incidence of VAP and chlorhexidine use remains controversial. There is also insufficient evidence regarding its benefits in decreasing mortality, duration of mechanical ventilation, and reduction in the length of ICU stay (18,20). Moreover, chlorhexidine has side effects that affect patients who use it for long periods, such as dental pigmentation, changes in taste, irritation, dryness, and oral mucosal lesions, teratological effects, allergy, increased bacterial accumulation after its use, pH changes, and burning sensations in the oral mucosa and on the tongue (21,22,23,24,25,26). Due to concerns relating to the side effects of chlorhexidine, particularly reports of anaphylaxis, Japan does not allow its use in the oral mucosa of patients under mechanical ventilation (19). Regarding povidone-iodine, its effectiveness in preventing VAP remains unclear due to the low number of available studies (27). Moreover, povidone-iodine use has been associated with cytotoxicity to the oral mucosal membranes and tooth pigmentation (19).

In clinical studies, 0.12% chlorhexidine antiseptic action against gram-negative bacteria such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter spp.* and *Escherichia coli* was not effective in intubated children (17). In contrast, povidone-iodine showed a reduction in microorganisms such as *streptococci*, *MRSA*, *S. pneumoniae*, *P. aeruginosa*, *P. gingivalis*, and *C. albicans* for up to three hours (19).

As potential alternatives, a mouthwash and a toothpaste containing Pc were effective in destroying 99.99% of bacteria and fungi *in vitro*. There is already evidence supporting the use of Pc-containing mouthwash as a complementary therapy against COVID-19, for example, in reducing signs of the disease, reducing the length of hospital stay, as well as avoiding the need for ICU admission (8,10,11). All of these

findings combined with no reports of adverse effects in clinical studies, according to the tolerability questionnaires applied support the use of Pc-containing products in patients with VAP (8,10,11).

The promising *in vitro* results of dental gel and mouthwash containing Pc demonstrate the need for further *in vivo* studies to determine whether oral care using these products can prevent VAP (27).

In this *in vitro* analysis, both Phtalox® Mouthwash and Phtalox® Dental Gel showed a 99.99% reduction of the tested microorganisms, demonstrating the potency of these antiseptic products. Although this study presents promising results, randomized clinical trials are needed to clarify the specific mechanism of action of these products against the microorganisms found in patients with VAP.

Declarations

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DISCLOSURE

Dr Vilhena reports personal fees from TRIALS Inc, during the conduct of the study; in addition, Dr Vilhena has a patent classified pending. Dr da Silva Santos reports grants from CNPq process nº. 309525/2018-7. The other authors claim there are no conflicts of interest.

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Tables

Table 1 - Antiseptic efficacy of Phtalox® mouthwash and Phtalox® dental gel

Strains	Positive control CFU/ml	Mouthwash Phtalox®	Positive control CFU/ml	Dental Gel Phtalox®
<i>E. coli</i>	6,0 x 10 ⁸	1,9 x 10 ³	8,3 x 10 ⁸	2,0 x 10 ²
<i>P. aeruginosa</i>	6,0 x 10 ⁸	1,9 x 10 ³	8,3 x 10 ⁸	2,0 x 10 ²
<i>S. aureus</i>	6,0 x 10 ⁸	1,9 x 10 ³	8,3 x 10 ⁸	2,0 x 10 ²
<i>Salmonella sp</i>	6,0 x 10 ⁸	1,9 x 10 ³	8,3 x 10 ⁸	2,0 x 10 ²
<i>C. albicans</i>	3,9 x 10 ⁸	3,2 x 10 ³	7,2 x 10 ⁸	1,5 x 10 ²
<i>A. niger</i>	3,9 x 10 ⁸	3,2 x 10 ³	7,2 x 10 ⁸	1,5 x 10 ²

CFU (colony-forming unit)