In vitro antimicrobial effect of chloroaluminum phthalocyanine nanoemulsion on periodontal bacteria

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Abstract:
Context: Nowadays, complementary therapies are necessary for a major removal of microbial subgingival biofilm in the conventional treatment of periodontitis. Research has suggested the use of photodynamic therapy (PDT) as a promising therapy to manage oral cavity infections. This project proposes a new combination of photosensitizer chloroaluminum phthalocyanine and nanoemulsion as a strategy for improving bioactivity. The main purpose of this in vitro study was to evaluate the antimicrobial activity of nanoemulsion CIAI<PcAlCl (CIAI<PcAlCl-NE) on relevant periodontal bacteria before and after PDT. Materials and Methods: The phototoxic and antibacterial effect of CIAI<PcAlCl-NE was evaluated against epithelial cells derived from an African green monkey kidney using the colorimetric method with salt tetrazolium 3-(4.5-dimethylthiazolyl-2)-2.5-Diphenyltetrazolium bromide (Merck) and periodontopathogen bacteria (Porphyromonas gingivalis (ATCC 33277), Aggregatibacter actinomyctecomitans (ATCC 33384), and Prevotella intermedia (ATCC 25611) using the plate microdilution method according to Tavares et al., 2018, respectively. The light source used for the PDT was a LED laser (400–700 nm); the cells were irradiated for 2 min using 4.83 joules/cm². Results: Antibacterial effect of NE‑PcAlCl against P. intermedia with minimum inhibitory concentration (MIC) 0.63 μM after TFD was determined. In the case of P. gingivalis and A. actinomyctecomitans, no biological activity was found after PDT (MIC > 20 μM) under-evaluated experimental conditions. On the other hand, the CIAI<PcAlCl-free and CIAI<PcAlCl-NE cells were phototoxic on epithelial cells. Conclusion: The results helped to identify the potential use of CIAI<PcAlCl to inhibit the periodontal bacterial and additional studies are being developed.

Key words:
Antimicrobial activity, nanoemulsion, periodontal bacteria, periodontitis, photodynamic therapy, phthalocyanine

INTRODUCTION

Periodontitis is a global health problem that includes a complex multifactorial group of diseases associated with subgingival biofilm, it also causes the destruction of the tooth-supporting structure and can lead to tooth loss.[1] In the subgingival microbial community, bacteria such as Porphyromonas gingivalis, Prevotella intermedia, and Aggregatibacter actinomyctecomitans are frequent. They have been called “key pathogens” and have an important role in the dysbiosis of periodontal tissues.[1]

Scaling technique and root planning are conventional periodontitis treatments that could be combined with local or systemic antibiotics. However, the effectiveness of this kind of procedure to completely control this pathology are variable; therefore, adjuvant therapy as a treatment of periodontal diseases including antimicrobial photodynamic therapy (aPDT) has been introduced as a therapeutic alternative since clinical studies are in progress.[2,3] The antimicrobial PDT mechanism is based on cellular toxicity through singlet oxygen and free radicals generated by a light-activated photosensitizer (PS). In dentistry, aPDT with a conventional PS such as toluidine blue (TBO) and methylene blue are emerging therapies in oral infectious treatments including chronic periodontitis, however further studies are necessary.[3] Aluminum-phthalocyanine is a second-generation PS with high absorption of light at longer wavelengths that allows penetration to deeper tissues, which is important.

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for the activation and antimicrobial effect. This PS is highly potent for PDT, but it is a hydrophobic molecule that aggregates in aqueous media, an event that leads to quenching phenomena. This extinguishes any photodynamic activity of this PS.\[3\]

This project proposes to dissolve this PS in the oil nanodroplets of the nanoemulsion maintaining the photodynamic activity in biological fluids. AlClPc has shown promising results in other biological model.\[3\] Therefore, the objective of this in vitro study was to evaluate the antimicrobial activity of nanoemulsion ClAlPc (ClAlPc-NE) before and after PDT on relevant periodontal bacteria.

MATERIALS AND METHODS

The phototoxic and antibacterial effect of ClAlPc-NE prepared by Muelhmann et al.\[3\] was evaluated against epithelial cells derived from an African green monkey kidney (VERO, ATCC CCL-81) using the colorimetric method with salt tetrzolium 3-(4, 5-dimethylthiazolyl-2)-2. 5-Diphenyltetrazolium bromide (Merck) and periodontopathogen bacteria (P gingivalis (ATCC 33277), A. actinomycetemcomitans (ATCC 33384), and P. intermedia (ATCC 25611)) using the plate microdilution method according to Tavares et al., 2018, respectively.\[3\] The nanoemulsion vehicle, CAlPc (Sigma-Aldrich, St. Louis, MO, USA) dissolved in DMSO and 2%-chlorhexidine gluconate from Ultra dent were evaluated as a reference. The light source used for PDT was a LED laser (660 nm), the cells were irradiated for 2 min using 4.83 joules/cm\(^2\). The treatment with the formulation was carried out for 24 h before and after irradiation. Finally, the results were expressed as the cytotoxic concentration 50 (CC\(_{50}\)) calculated using the XLIii5 (IBM) for phototoxicity and as a minimum inhibitory concentration (MIC) and bactericidal (MBC) for antibacterial activity assays. Experiments were performed by triplicate and controls were evaluated without irradiation.

RESULTS

Biological activity results about formulations on periodontal pathogens and Vero cells are presented below [Table 1]. The nonencapsulated ClAlPc and nanoemulsion showed to be phototoxic for Vero cells without significant differences (P = 0.100) after PDT. Chlorhexidine, used as a reference drug, also showed a toxic effect independent of PDT. In addition, a biological effect of the formulations (ClAlPc-DMSO, ClAlPc-NE, and vehicle) without PDT was not demonstrated.

On the other hand, no antibacterial effect was observed after PDT against A. actinomycetemcomitans with any of the formulations evaluated. In the case of P. gingivalis, inhibition was observed after the exposure with ClAlPc-DMSO and PDT (MIC 1.66 µM). In contrast, P. intermedia was six times more susceptible to treatment with ClAlPc-NE compared to ClAlPc-DMSO after PDT. Chlorhexidine showed antibacterial effect against the three strains evaluated with MIC between 330.78 and 2646.25 µM independent of PDT [Table 1].

DISCUSSION

The search on aPDT as a complementary therapy to conventional periodontitis treatments seems to be therapeutically useful.\[3\] Therefore, the incorporation of a new PS and nanotechnologies are a promising option.

The in vitro effectiveness of NE-CIAIPc against P. intermedia was demonstrated, after PDT. However, this same effect was not observed against P. gingivalis and A. actinomycetemcomitans. The bioactivity of PS conveyed in NE has not been previously reported for oral microorganisms; therefore, this project innovates and develops a new alternative of TDF for EP.

Moreover, PDT with ClAlPc-DMSO slightly inhibited with MIC and MBC of 1.66 µM but without effect in NE, endogenous porphyrins in black-pigmented oral bacteria could interact with PS and explain different susceptibilities to PDT.\[5\]

The effect to PDT against A. actinomycetemcomitans was contradicted with previous reports with PS as a TBO, MB, and Safranine O, among others.\[2\] However, some studies suggest that this effect depends on system irradiation parameters, internalization, and subsequent activity of the PS.\[4\]

Similarly, NE-CIAIPc showed cytotoxicity in vitro in vero cells after PDT. These results indicate the internalization of the PS and determine the optimization of the irradiation scheme to determine the selectivity of the Nano system. Similar effects have been reported with this same NE system on lung carcinoma cells (MCF-7). In contrast, CIAIPc nanoparticles demonstrated variability of the cell line-dependent cytotoxic effect (4T1, MCF-7, NIH/3T3, and MCF-10A) being the nontumor cells most susceptible to PDT by AICIPc.\[5\]

In this survey, we evaluated the effects in planktonic cells, which can be referred to as one of the restrictions in our study. Furthermore, we did not measure a wide range of concentrations for CIAIPc-NE, nor radiations with different wavelengths or times. However, the general results of this

| Compounds | Porphyromonas gingivalis | Aggregatibacter actinomycetemcomitans | Prevotella intermedia | Vero cells |
|-----------|--------------------------|--------------------------------------|----------------------|-----------|
| ClAlPc-DMSO | 1.66 | 1.66 | >20 | >20 | 6.63 | 6.63 | 0.15±0.08 |
| ClAlPc-NE | >20 | >20 | >20 | >20 | 0.63 | 0.63 | 0.09±0.03 |
| Vehicle | >20 | >20 | >20 | >20 | >20 | >20 | >0.49 |
| GCX | 330.78 | 330.78 | 2646.25 | 2646.25 | 330.78 | 330.78 | 3.20±0.6 |

The results are expressed in µM. ClAlPc – Chloroaluminum phthalocyanine; GCX – Chlorhexidine gluconate; MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; CC\(_{50}\) – Cytotoxic concentration 50; NE – Nanoemulsion; DMSO – Dimethyl sulfoxide
study showed the need to complement the experimentation with TDF + ClAlPC-NE in future, probably in biofilm models.

This study concluded that ClAlPC associated with nanoemulsion showed significant antibacterial effect against *P. intermedia*, after PDT. Complementary studies in oral cell lines would be important to measure the selectivity of the nanoemulsion.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Caton GC, Armitage G, Berglundh T, Chapple IL, Jepsen S, Kornman KS *et al.* A new classification scheme for periodontal and peri-implant diseases, conditions-Introduction, and key changes from the 1999 classification. *J Clin Periodontol* 2018;45:S1-8.

2. Kikuchi T, Mogi M, Okabe I, Okada K, Goto H, Sasaki Y, *et al.* Adjunctive application of antimicrobial photodynamic therapy in nonsurgical periodontal treatment: A review of literature. *Int J Mol Sci* 2015;16:24111-26.

3. Muehlmann LA, Rodrigues MC, Longo JP, Garcia MP, Py-Daniel KR, Veloso AB, *et al.* Aluminium-phthalocyanine chloride nanoemulsions for anticancer photodynamic therapy: Development and *in vitro* activity against monolayers and spheroids of human mammary adenocarcinoma MCF-7 cells. *J Nanobiotechnology* 2015;13:36.

4. Tavares LJ, de Avila ED, Klein MI, Panariello BHD, Spolidório DMP, Pavarina AC. Antimicrobial photodynamic therapy alone or in combination with antibiotic local administration against biofilms of Fusobacterium nucleatum and Porphyromonas gingivalis. *J Photochem Photobiol B* 2018;188:135-45.

5. Moslemi N, Rouzmeh N, Shakerinia F, Bahador A, Soleimanzadeh Azar P, Karazifard MJ, *et al.* Photodynamic inactivation of *Porphyromonas gingivalis* utilizing radachlorin and toluidine blue o as photosensitizers: An *in vitro* study. *J Lasers Med Sci* 2018;9:107-12.