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In vitro antimicrobial activity of CatDex against *Porphyromonas endodontalis*

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An achievable goal in root canal therapy is to reduce the bacterial population to the lowest level. Irrigation and instrumentation play an important role in achieving this objective. Sodium hypochlorite (NaClO) is the most frequently used irrigation solution; however, its main disadvantage that it is cytoxic to soft tissues following inadvertent extrusion. CatDex is a substance that promises to match the antimicrobial effect of NaClO without being toxic. The aim of the study is to evaluate the *in vitro* antimicrobial activity of CatDex in doses ranging from 0.17 to 0.05% and compare it with NaClO 5.25, 2.5 and 1.25%, against *Porphyromonas endodontalis*. *P. endodontalis* sensitivity to NaClO and CatDex was performed using the disk diffusion method and the minimum inhibitory concentration (MIC). The percentage of *P. endodontalis* inhibition was 97.8% at 5.25% NaClO and 55% at 0.145% CatDex. CatDex could be considered as a possible alternative for irrigation in root canal therapy. However, more studies are needed to verify its effect and to determine its correct clinical use.

Key words: *Porphyromonas endodontalis,* root canal, antimicrobial, efficacy, dental disinfectants.

INTRODUCTION

Pulpal and periapical diseases are the result of bacterial contamination that includes microorganisms that normally inhabit the oral cavity (Fouad, 2017). Bacterial infections are a major cause of pulp necrosis and periapical lesions and the main cause of endodontic failure (Shabahang, 2005). Successful endodontic therapy involves the elimination of bacteria and their products from root canals. Together with instrumentation, irrigation is essential for cleansing and shaping the root canal system before three-dimensional sealing. Endodontic infections are polymicrobial and include strict anaerobes, facultative anaerobes, and microaerophiles (Rocas et al., 2008; Shabahang, 2005; Haapasalo et al., 2005). Bacteria located in the apical portion of the root canals potentially

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participate in the pathogenesis of periapical lesions. The microbiota is usually complex and includes different bacterial species *Porphyromonas endodontalis* in 65% (Rôças et al., 2010).

CatDex is a guanidine conjugate prepared by reacting aminoguanidine or agmatine with periodate oxidized dextran followed by reductive amination. It has a cationic charge, a wide pH range and it is hydrophilic. Also, CatDex has shown effect on tumor cell growth in prostate, breast, bladder and renal cancer cell lines (Meurling et al., 2009; Escamilla-Garcia et al., 2017). There are similarities in the proliferation, growth and development of tumor cells and bacteria as well as in the electrostatic condition of the cell-membrane wall relationship. A recent study demonstrated the antimicrobial activity of CatDex against oral cariogenic (*Streptococcus mutans*) and periodontal (*Porphyromonas gingivalis*) pathogens (Escamilla-Garcia et al., 2017). The proposed method of action in tumour cells is an electrostatic interaction with an anionic cell membrane, internalisation by the polyamine uptake system, and electrostatic binding of anionic structures in the cytoplasm, which kills the cell (Deka et al., 2015; Marquez et al., 2002). Similar to CatDex, but as a hydrogel, cationic synthetic dextran demonstrated antimicrobial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (O'Connoret al., 2015). Therefore, this study was performed to determine if CatDex has a similar effect against *P. endodontalis* compared to sodium hypochlorite (NaClO).

MATERIALS AND METHODS

Bacterial culture

P. endodontalis ATCC35406 strain was used in this study and was obtained from the American Type Culture Collection (ATCC). The microorganism was activated and cultured under the growth conditions recommended in the technical specifications of ATCC. *P. endodontalis* was subcultured at 37°C for 48 h on agar plates with a brain-heart infusion medium (BHI) (BD Bioxon, Becton Dickinson, Mexico City) in anaerobic atmosphere using an anaerobic gas chamber (Plas-Labs, Lansing, MI). The preculture was washed with 0.9% NaCl (w/v), then inoculated at an optical density at a wavelength of 600 nm (OD_{600nm}) between 0.08 to 0.1 corresponding to 0.5 McFarland (Genesys 10S UV-Vis Spectrophotometer, Thermo Fisher Scientific Inc., Waltham MA) in a 250 ml Erlenmeyer flask with 100 ml of BHI broth. The bacterium was incubated at 37°C for 18 h until it reached the logarithmic phase of bacterial growth.

Growth kinetics

An absorbance of 0.5 McFarland was used for this study at a wavelength of 600 nm. The growth curve was performed in duplicate in 250 ml Erlenmeyer flasks with a volume of 100 ml of culture medium. Bacterial growth was monitored at time intervals of 2 h to reach the most representative kinetic points. The culture pH was monitored using an Ultrabasic UB-5 PH Meter (Denver Instrument Co., Bohemia, NY); also, the bacterial concentration (CFU/ml) and OD_{600nm} were determined.

Minimum inhibitory concentration (MIC)

The MIC was used to determine the lowest concentration of CatDex which inhibits growth. For this, CatDex was diluted in BHI broth. Next, 500 µl of each concentration 0.05% were transferred to 5 ml assay tubes. The positive control for this study was NaClO (Cloralex®). A concentration of 5.25, 2.5 and 1.25% was used. The negative control was saline solution. 500 µl of *P. endodontalis* inoculum was cultured for 18 h at a final bacterial concentration of 1.5×10^8 CFU/ml with a 0.5 McFarland turbidity for a final volume of 1 ml, as previously described by Wanger et al. (1995). *P. endodontalis* was incubated in an anaerobic atmosphere of H_2 (10%), CO_2 (5%), and N_2 (85%) (Praxair Mexico, Monterrey, Mexico) at 37°C for 24 h. After the incubation, each tube was analyzed qualitatively by observing the presence or absence of turbidity and its optical density was measured.

Disk diffusion method

The sensitivity of *P. endodontalis* to CatDex was determined by the Kirby-Bauer method. An aliquot of 100 µl of each bacteria on agar plates was seeded in BHI agar. A no. 40 Whatman filter paper disc with a diameter of 6 mm (GE Healthcare, Boston, MA) was placed in 20 ml of CatDex at different concentrations. Saline solution was the negative control. Petri dishes were marked with external dividing lines. The seeded plates with discs were incubated at 37°C for 24 h. The inhibition or lack of inhibition around the discs was measured and the results are given in millimeters. Each concentration was done in triplicate. Culture media and material were pre-sterilized for 15 min at 120°C (ALL-AMERICAN® Sterilizer, Hillsville, VA). All procedures were performed under sterile conditions in class II, type A2 biosafety cabinets (Thermo Fisher Scientific Inc.). The results represent the mean of three repetitions of three independent experiments.

Statistical analysis

Three independent replicates of each experiment were performed, and their results were expressed as mean diameter ± standard deviation (SD). We used data analysis, and the significances of the inhibitory effect of CatDex against *P. endodontalis* were evaluated with unequal variance analysis based on the *t* test and the Mann–Whitney ($P<0.05$) test and calculated with statistical software SPSS v22.0.

RESULTS

Growth kinetics

The culture of *P. endodontalis* presented an exponential bacterial growth phase from 12 h until reaching a maximum peak at 17 h, during which time OD_{600} was 2.16±0.14, corresponding to 10.8×10^8±7.07 CFU/mL and pH 7.0 ±0.0 (Figure 1).

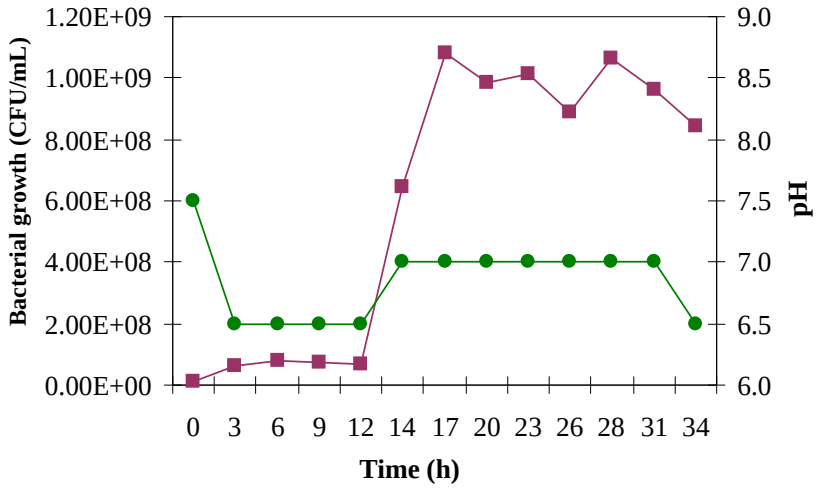

Figure 1. Growth kinetics and pH of *P. endodontalis.*

Table 1. Bacterial growth values of CatDex and NaClO against *P. endodontalis*.

Treatment	Bacterial growth (10^8 CFU/mL)	P value*
CatDex (%)		
0.170	3.6±0.52	0.0049
0.145	2.6±0.38	0.0021
0.120	4.01±0.25	0.0134
0.075	3.5±0.19	0.0050
0.050	3.7±0.79	0.0259
NaClO (%)		
5.25	0.14±0.092	0.0001
2.50	2.4±1.8	0.0383
1.25	0.12±1.0	0.0013
Saline solution (%)		
0.9	5.78±0.68	-

NaClO, sodium hypochlorite.
Values are mean ± standard deviation.
**P* values obtained from *t* test compared to saline solution control.

Minimal inhibitory concentration

The greatest reduction in growth was observed with 5.25% NaClO, 0.145±0.092×10^8 CFU/ml, OD_{600} 0.029±0.0, pH 8±0.0. The growth reduction of 2.6±4.38×10^8 CFU/ml, OD_{600nm} 1.1 ± 0.376 and pH 7±0.6 was achieved at concentration of 0.145% CatDex (Table 1). For the control of *P. endodontalis*, a bacterial concentration of 5.78±0.685×10^8 CFU/ml, OD_{600} 1.36±0.42 and pH 8.0±0.0 was obtained.

In summary, the percentage of bacterial growth reduction of *P. endodontalis* using 5.25, 2.5 and 1.25% of NaClO was 97.8, 78.73 and 57.9%, respectively. The MIC of CatDex was 0.145% with a 55±0.2% cell reduction. For other CatDex concentrations (0.17, 0.12, 0.08 and 0.05%), cell reduction reached ~35.26 ± 2.62% (Table 1).

Inhibitory effect

NaClO had a greater inhibitory effect against *P. endodontalis*. The diameter of the inhibition halo for 5.25, 2.5 and 1.5% of NaClO was 9.5±0.70 mm, 7.5±0 mm

and 6.5±0 mm, respectively. In the case of CatDex, the dose with the greatest antimicrobial effect was 0.17% with a diameter of 9 ± 0.7 mm. The inhibition halo of 0.145% CatDex was 7.5±0.58 mm, 0.12% was 6.75±0.96 mm and 0.075% was 6.25±0.50 mm.

DISCUSSION

Based on our results of CatDex and NaClO against *P. endodontalis*, the antimicrobial effect of NaClO was greater than CatDex at all the concentrations tested except for 0.145% (P<0.05). CatDex is a molecule that has not been previously reported in irrigation studies in endodontics. However, Escamilla-Garcia et al. (2017) showed that CatDex is effective against oral bacteria such as *S. mutans* and *P. gingivalis*. The MIC for *P. endodontalis* with CatDex was 0.145%. This is in contrast with Escamilla-Garcia et al. (2017) who mention that for *S. mutans* and *P. gingivalis*, the MIC was 0.05 mM with CatDex. In this study, *P. endodontalis* showed sensitivity to the positive control (NaClO). NaClO has been used as an irrigant at different concentrations (from 5.25 to 1.5%) in other studies (Haapasaloet al., 2014; Yamashita et al., 2003).

The most effective concentration of NaClO to inhibit the bacterial growth was 5.25%. The antimicrobial potential of 2.5% NaClO has also been analyzed at different concentrations against *P. endodontalis* where its ability to significantly reduce the presence of bacterial colonies in root canals is notable (Rocas and Siqueira, 2011). In this investigation, 2.5% NaClO achieved a 60% cell reduction of *P. endodontalis*, unlike the study by Sena et al. (2006) where 100% of bacterial colonies of *P. endodontalis* were eliminated with NaClO.

The third NaClO concentration tested was 1.25% where results for *P. endodontalis* showed a reduction of 80%. Results that are similar to those recently published in which the mean number of bacterial cells recovered from 1% NaClO group was significantly higher than that of 4% NaClO (Christo et al., 2016). The logarithmic growth phase identified by growth kinetics for *P. endodontalis* was 17 h. It was possible to observe an antimicrobial effect on *P. endodontalis* with CatDex using the disc susceptibility technique by the Kirby-Bauer method at a concentration of 0.17%. The percentage of *P. endodontalis* inhibition for 5.25% NaClO was 97.8%, while for CatDex was 55%.

Conclusions

NaClO is a frequently used irrigant and disinfectant fluid. CatDex showed an antimicrobial efficacy against oral pathogenic *P. endodontalis* similar to NaClO. Consequently, CatDex could be considered as a possible alternative for irrigation in root canal therapy. However, more studies are needed to verify its effect and to determine its correct clinical use.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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