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ORIGINAL ARTICLE

Photodynamic therapy in endodontic treatment of deciduous teeth

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Abstract The purpose of this study was to evaluate photodynamic therapy in deciduous teeth with necrotic pulp by means of fully quantifying viable bacteria, before and after instrumentation and after the use of photodynamic therapy. Radicular canal cultures were conducted (n=10): the first one was performed right after access and location of the radicular canal; the second was performed after the conclusion of chemical–mechanical instrumentation, and the last one after photodynamic therapy. The photodynamic therapy was performed with 4 J/cm energy low-intensity diode together with toluidine blue. The results ($\log_{10}$) were submitted to a descriptive analysis and Wilcoxon test. The percentage of reduction was submitted to the Mann–Whitney test. The instrumentation resulted in a reduction of 82.59% of viable bacteria, and, after photodynamic therapy, the microbial reduction observed was 98.37% (P=0.0126). Photodynamic therapy is recommended as adjunct therapy for microbial reduction in deciduous teeth with necrotic pulp.

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Introduction

According to most of the claims in the literature, one of the main requirements for attaining successful endodontic therapy in deciduous teeth is the achievement of significant microbial reduction after the chemical–mechanical instrumentation [1, 2].

The prevailing microbiota in deciduous teeth with necrotic pulp is present in a great number of the cases of anaerobic microorganisms, aerobic microorganisms in 60% of the cases, streptococcus in 85% and gram-negative bacilli in 15% of the cases; the gram-positive *Streptococcus* and *Enterococcus faecalis* are responsible for most of the endodontic failures, mainly due to microbial resistance after conventional treatment [3].

After the canal has been prepared and sealed, selected anaerobic bacteria can reproduce again and invade dentinal tubules near cement areas [4, 5]. Therefore, it is important that studies be conducted about alternative methods to reduce microbes in endodontic treatment, taking into consideration difficulties such as: possibility of microbial resistance/survival after endodontic treatment, presence of accessory foramina in the deciduous molar furcation area, ectopic root reabsorption, lower tolerance towards long treatment, and children's movement during canal access, instrumentation and obturation. The use of photodynamic therapy may be an alternative as an adjunctive therapy to reduce microbial bacteria in the endodontic treatment of deciduous teeth with necrotic pulp.

In this therapy, the light emitted by a low-power laser activates a specific photosensitizer that has a lethal effect on microorganisms [6–11]. The therapy consists of a photosensitizer agent, which is usually exogenous, and a light source, the purpose of which is to prompt cell necrosis or microbial death. The mechanism of the action occurs when the photosensitizer agent absorbs the light source photons and its electrons go into an excited state. In the presence of a substrate (such as oxygen), the photosensitizer transfers its energy to the substrate, forming short-lived and highly reactive species, such as the singlet oxygen, causing serious damage to microorganisms through irreversible oxidation of cell components [12].

The wavelengths of the laser source may vary according to the dye used. However, today, the high-power red-emission laser diodes and simpler optical systems are used. The typical band of a red-emission diode laser is around 660 nm. Light emitting diodes (LEDs) are alternative light sources for photodynamic therapy, which can also be used successfully [7, 8, 13].

Over the past few decades, the literature has been investigating the elimination of microorganisms by photodynamic therapy, and several dyes have been tested, such as blue, purple, brown and green dyes, among other complementary to the laser wavelength [6–14].

Blue dyes, especially toluidine blue and methylene blue, used with a 632.8 nm wavelength laser have shown significant results in the reduction of several in vivo bacterial and fungi cultures [15]. The chemical reactions occur among the photosensitizer, light and substrate. By maintaining the same dose (power density or fluency), but varying the intensity or exposure time, different results can be obtained, and the effects can also depend on the photosensitizer's absorption and concentration. The efficacy of this therapy depends on: the photosensitizer selectivity and its retention, the electromagnetic radiation intensity that reaches the treatment region, the optical tissue properties, the activator photons absorption efficiency, the molecular excitation energy transfer and the molecule oxidant effect [6, 7, 10, 12].

The purpose of our study was to evaluate the antimicrobial action of photodynamic therapy in deciduous teeth with necrotic pulp, using total viable bacteria before and after instrumentation and after the use of photodynamic therapy.

Materials and methods

This study was approved by the PUC-Campinas Ethics Committee (protocol no. 347/05). Ten deciduous teeth with necrotic pulp, from children of both genders aged between 4 years and 7 years, were selected for this study, and the children were treated at the Pediatric Clinic of the Catholic University of Campinas (PUC-Campinas).

Inclusion criteria

The inclusion criteria were: caries lesions of deciduous molars, affecting the pulp and diagnosed as necrotic pulp, evidenced by the presence of a radiolucid area in the furcation, observed by peri-apical radiography on children or adult radiographic film (Kodak, São Paulo, Brazil), with the children positioned by a parallelism technique in the XRM apparatus (70 kV×12 mA, Ribeirão Preto, Brazil); children were not taking any medication. The children selected to participate in the study were only those whose parents or legal guardians were knowledgeable of the research and had signed the free and informed consent form.

Exclusion criteria

The exclusion criteria were: children undergoing medical treatment and taking antibiotics; necrotic pulp in the teeth, with a radio-lucid area in the furcation area affecting the permanent successor recommended for exodonties; deciduous teeth with radicular reabsorption of more than half of the total root length.

Clinical procedure and sample culture

The root length was measured with a millimeter rule on an intrabuccal peri-apical radiograph, and the area length for chemical–mechanical instrumentation was obtained by the subtraction of 2 mm from the root length.

After anesthesia with lidocaine (Novocol, Toronto, Canada) and rubber dam isolation (Madeitex, São José dos Campos, Brazil), the carious tissue and pulp chamber ceiling were removed with a spherical rotary bur no. 2 at high rotation and cooling with sterile saline drip. Endodontic access was achieved with a Batt bur no. 2 (Maillefer, Ballaigues, Switzerland). The radicular canals of each deciduous molar received a sterile paper cone with an anatomic diameter compatible to the canal for 30 s. The first samples of bacterial contamination were immediately placed in the same brain heart infusion (BHI). Thereafter, the chemical–mechanical instrumentation was performed with Kerr files (Maillefer), with anatomic diameter compatible to the radicular canal, together with 0.5% sodium hypochlorite irrigation (Fórmula & Ação, São Paulo, Brazil) and Endo PTC (urea peroxide, Tween-80 and Carbowax; Fórmula & Ação). When the instrumentation had been concluded, the radicular canals of each deciduous molar received a sterile paper cone with an anatomic diameter compatible to the canal for 30 s

The second samples of deciduous teeth canals with necrotic pulp were immediately transferred to the same BHI. After the conclusion of the chemical–mechanical instrumentation, a sterile paper cone with anatomic diameter compatible to the canal and soaked with 0.005% mg/l toluidine blue aqueous solution (Fórmula & Ação) was inserted into the canals and kept there for 3 min. After the removal of the cone soaked with a photosensitizing agent, the "Flash Lase III" model with 4J/cm^2, 100 mW power output and 660 nm (DMC, São Carlos, Brazil) was applied for 40 s, leaving the laser's active tip in contact with the canal. After that, a sterile cotton cloth was soaked with 0.005% mg/l toluidine blue aqueous solution (Fórmula & Ação) and applied to the internal furcation area for 3 min. Following the removal of the photosensitizer, the "Flash Lase III" laser with 4J/cm^2, 100 mW power output and 660 nm (DMC) was used for 40 s. Subsequent to the conclusion of photodynamic therapy, the radicular canals of each deciduous molar received a sterile paper cone with an anatomic diameter compatible to the canal for 30 s, and the sample was immediately transferred to the same BHI.

Microbiological processing

All samples (before instrumentation, after instrumentation and after laser application) were cultivated so that we could detect the total number of viable bacteria.

Homogenization, dilution and seeding

The samples were homogenized in a tube shaker (Vortex-Wizard, Porto Alegre, Brazil) for 3 min, and decimal dilutions were performed in 4.5 ml of peptoned water up to 10^{-6}. Three 25 μl aliquots of these decimal solutions were seeded on to the plate surfaces containing blood agar, with a micropipette [16].

Cultivation conditions

Subsequently, the cultures were incubated for a 5 days at 37°C in an 85% nitrogen (N_2), 10% carbon dioxide (CO_2) and a 5% hydrogen (H_2) atmosphere, achieved by use of the generating envelope systems in an anaerobiosis jar. Therefore, it was possible to make a visual assessment of the total number of viable bacteria unit-forming colonies.

Statistical analysis

The results, in unit-forming colonies per milliliter (ufc/ml), were transformed into $\log_{10}$ and submitted to descriptive analysis and the Wilcoxon statistical test. The calculation of the reduction in percentage before and after instrumentation and before instrumentation and after photodynamic therapy were submitted to Mann–Whitney statistical test.

Results

The results of the microbiological count (in ufc/ml) before instrumentation, after instrumentation and after photodynamic therapy are shown in Table 1. The statistical analysis was performed with the Bioestat 2.0 program, and the results, in ufc/ml, were transformed into $\log_{10}$ (Table 2). We used the Wilcoxon paired test for dependent samples to make a comparison before and after radicular canal instrumentation and before radicular instrumentation and photodynamic therapy (Tables 3 and 4). The reduction in percentage before and after radicular instrumentation, and the reduction in percentage before radicular instrumentation and photodynamic therapy, were submitted to Mann–Whitney statistical analysis (Tables 5 and 6).

Mathematical formula used to obtain the percentage of reduction:

$\alpha_i = ai - (di \text{ ou } dft)$

$$\begin{array}{ccccc} ai & & 100\% & & x = \alpha_i \text{ X } 100\% \\ \times & & & \Rightarrow & \hline \\ \alpha_i & & x & & ai \end{array}$$

Where α_1: numerical difference before and after the radicular instrumentation or before the radicular instrumentation and photodynamic therapy.

ai: before radicular instrumentation

di: after radicular instrumentation

dft: after photodynamic therapy

Table 1 Total viable bacteria count (in ufc/ml) before the chemical–mechanical instrumentation, after chemical–mechanical instrumentation and after photodynamic therapy

N=10	After photodynamic therapy	Before instrumentation	After instrumentation
1	12,640×10	5,220×10	0.132×10
2	932×10	65.32×10	6×10
3	0.132×10	0,106×10	0.013×10
4	53.2×10	4×10	0.013×10
5	40×10	1.32×10	0.264×10
6	1,200×10	0.613×10	0.013×10
7	1,320×10	0,132×10	0.026×10
8	0.132×10	0.013×10	0
9	2,2640×10	2,400×10	16×10
10	1.32×10	0.186×10	0.066×10

Table 2 Total viable bacteria count (log $_{10}$) before the chemical–mechanical instrumentation, after chemical–mechanical instrumentation and after photodynamic therapy

N=10	Before instrumentation	After instrumentation	After photodynamic therapy
1	4.102	3.718	0.054
2	2.969	1.815	0.845
3	−0.879	−0.975	0.006
4	1.726	0.602	0.006
5	1.602	0.121	0.102
6	3.079	−0.213	0.006
7	3.121	−0.879	0.011
8	−0.879	−1.886	0.000
9	4.355	3.380	1.230
10	0.121	−0.730	0.028

Discussion

The microbiota of deciduous teeth with necrotic pulp is composed mainly of *Streptococcus mutans* (85%) and black pigmented bacillus (30%) [4]. In the group comprising *Streptococcus*, the *mutans*, *intermedius* and *peptostreptococcus* genus stand out and *Prevotella intermedia* and *Fusobacterium nucleatum* are in smaller numbers [2, 3]. In relation to oxygen tension, there are aerobic bacteria, selected and mandatory anaerobic bacteria, and, in relation to the cell wall, gram-positive bacteria are remarkable (*Streptococcus* 85%) [2–4].

Resistant bacteria persist after endodontic treatment in deciduous and permanent teeth [1, 3, 4, 14, 17, 18]. Therefore, chemical-mechanical endodontic treatment using irrigation solutions does not completely eradicate bacterial strains [1, 16]. The elimination of bacteria and their subproducts is a critical factor for effective endodontic treatment [17, 18]. In deciduous teeth, radicular canal anatomy, presence of accessory foramina in the furcation area, ectopic root reabsorption and lower tolerance towards long treatment make it difficult for chemical–mechanical instrumentation to be used; therefore, studies of new alternatives for microbial reduction in deciduous teeth with necrotic pulp are of the utmost importance.

Currently, photodynamic therapy is regarded as an additional resource to achieve microbial reduction. This technique consists of the use of a dye with photosensitizing properties, irradiation with low-intensity laser, and the production of free radicals and singlet oxygen, toxic products that alter the local cell environment, which may result in reduced growth or their death [2, 3, 12, 14]. The term "lethal photosensitization by laser" refers to the process of radiation emissions using a low-power laser device.

In this study, a low-power diode laser was used, with a 660 nm to 680 nm wavelength, 4 J/cm^2 light fluency, and 100 mW power output, which activates the sensitizing agent, producing a lethal effect on specific cells and, in this case, viable bacteria after chemical–mechanical instrumentation of radicular canals of deciduous teeth with necrotic pulp.

Ortho-toluidine, 75 μg/ml, and methylene blue dyes are used as sensitizing agents in modern photodynamic therapy. Ortho-toluidine blue dye, 75 μg/ml, used in association with low-intensity diode laser with a 665 nm wavelength and light fluency of 30 J/cm^2, resulted in an 83.2% reduction of *Enterococcus faecalis* bacteria in the radicular canals of permanent teeth [14]. However, the same laser used with a light fluency of 222 J/cm^2 associated with 25 μg/ml methylene blue dye was able to eliminate 97% of bacteria inside the radicular canals of permanent teeth [14]. An 84.9% reduction of *Streptococcus mitis* and an 98.9% reduction in *Streptococcus sanguis* were achieved in the

Table 3 Arithmetic average, standard deviation and result of Wilcoxon test before and after radicular canal instrumentation

Before instrumentation	After instrumentation
1.93 (1.93)[a]	0.49 (1.89)[b]

Different letters indicate statistically significant differences (*P*= 0.0051)

Table 4 Arithmetic mean, standard deviation and result of Wilcoxon test before radicular canal instrumentation and after photodynamic therapy

Before instrumentation	After photodynamic therapy
1.93 (1.93)[a]	0.22 (0.43)[b]

Different letters indicate statistically significant differences (*P*= 0,0218)

Table 5 Percentage of reduction before and after radicular instrumentation and before radicular instrumentation and photodynamic therapy

N=10	Before and after instrumentation	Before instrumentation and photodynamic therapy
1	58.70	99.99
2	92.99	99.35
3	19.69	90.15
4	92.48	99.97
5	96.70	99.34
6	99.94	99.99
7	99.99	99.99
8	90.15	100.00
9	89.39	99.92
10	85.90	95.00

Table 6 Arithmetic average, standard deviations and results of Mann–Whitney test of the percentage of reduction before and after radicular instrumentation and before and after radicular instrumentation and photodynamic therapy

Before and after instrumentation	Before instrumentation and photodynamic therapy
82.59 (25.05)[a]	98.37 (3.27)[b]

Different letters indicates statistically significant differences (P= 0.0126)

fissures of permanent teeth with arsenic–gallium–aluminum (AsGaAl) irradiation (low-intensity laser), having a power density of 6 J/cm^2, and 75 μg/ml of ortho-toluidine dye [12]. The use of toluidine blue dye, 200 μl/l, with a low-intensity diode laser having a 663 nm wavelength and 4.8 J/cm^2 power density resulted in a reduction greater than 99% of *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Prevotella intermedia* and *Streptococcus intermedius* in the radicular canals of permanent teeth [2]. According to the literature, microbial reduction depends on the dye used and its concentration, the laser's parameters, and the bacteria studied. Variations in the bacterial cell walls (gram$^+$or gram $^-$) influenced the dye's absorption. Chemical structure, molecular weight, and bacteria exposure time to the photosensitizer before irradiation are important factors in photodynamic therapy, and they explain the high microbial reduction 83,2% to 97% [14] in permanent radicular canals when dye, concentration, and laser energy were modified.

The chemical–mechanical instrumentation with a 2.5% sodium hypochlorite irrigation solution reduced 90% of *Proteus mirabilis* and *Pseudomonas aeruginosa* found in the radicular canals of permanent teeth. The use of chemical and mechanical instrumentation, together with 2.5% sodium hypochlorite solution and photodynamic therapy (laser diode with a wavelength of 660 nm, 9.6 J/cm^2 density power, polyethylenimine and chlorine 10 μl/ photosensitizing agents) resulted in a 98% reduction of this microbiota [16], substantiated in this study wherein a total additional reduction of 15.78% was observed after the use of photodynamic therapy in the viable bacteria in deciduous teeth with necrotic pulp. Chemical-mechanical instrumentation followed by photodynamic therapy is an important clinical resource for microbial reduction, since the canal instrumentation promotes smear layer and radicular dentin debris removal, leading to dentin tubules opening and increasing the dye permeability in the radicular dentin.

Chemical–mechanical instrumentation using 0.5% sodium hypochlorite and 17% ethylene diamine tetra-acetic acid (EDTA) reduces the number of viable bacteria of in the radicular canals of permanent teeth by 93.25%. Photodynamic therapy using a diode laser with a 670 nm wavelength and 1.8 J power in conjunction with azulene dye reduces 99.2% of viable bacteria in the radicular canals of permanent teeth. The helium–neon (He–Ne) laser used with a 5 μmol/l to 200 μmol/l concentration of toluidine resulted in a 99.9% reduction of *Streptococcus intermedius* in the radicular canals of deciduous teeth with necrotic pulp [3].

The 82.59% reduction in total viable bacteria found in the radicular canals of deciduous teeth with necrotic pulp observed in this study was associated with the mechanical action of files on the radicular dentin and with the oxygen released by the chemical reaction between the urea peroxide present in Endo PTC and 0.5% sodium hypochlorite, thereby eliminating a large part of the obligatory anaerobic microbiota and substantiated by the reports in literature [2, 3, 12, 14, 17]. The 98.37% reduction in the microbiota of radicular canals with necrotic pulp was associated with the action of photodynamic therapy using a low-intensity diode laser with 660 nm wavelength and 4 J/cm^2 power density per 40 s, in conjunction with 0.005% toluidine blue. Toluidine blue dye is a phenothiazine that absorbs, through chlorine, the laser with 660 nm wavelength. The microbial reduction from the photodynamic therapy is the result of oxy-reduction by singlet oxygen and free radicals in the bacterial cytoplasm membrane, causing the outflow of its contents and microbial death [2, 3, 12, 14, 16].

Its is important to mention that pediatric dentistry research that relates photodynamic therapy and endodontic treatment in deciduous teeth is rarely seen [3]. The literature highlights permanent dentition as the focus of this kind of study [2, 12, 14, 16]. Anatomical, histological and chemical differences are found between permanent and deciduous teeth, such as a less thick enamel and dentin in deciduous teeth, higher mineralization in permanent dentition, physiological root reabsorption in deciduous teeth, etc. However, this research showed a microbial reduction similar to the results related in the literature for the permanent ones. The difference between these two dentitions were observed mainly because of the method employed. Quantification of total viable bacteria was used, instead of the quantification of species and genus separately.

Photodynamic therapy may be an additional resource for microbial reduction in deciduous teeth with necrotic pulp, since, in this study, there was an additional 15.78% reduction in the total number of bacteria. Therefore, in pediatric dentistry, the treatment of children and the anatomical and physiological characteristics of these teeth may limit the extent of chemical–mechanical instrumentation. The combined use of photosensitizing dye and a low-intensity laser appears to be a feasible, low-cost and non-traumatic alternative to complement classic endodontic therapy to be used in today's treatment of deciduous teeth.

Conclusion

Photodynamic therapy may be used as an adjunct in the endodontic treatment of deciduous teeth, resulting in a significant reduction in the total number of viable bacteria in deciduous teeth with necrotic pulp.

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