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Effects of antimicrobial photodynamic therapy and surgical endodontic treatment on the bacterial load reduction and periapical lesion healing. Three years follow up

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Summary Besides the advances in endodontics, there are situations in which surgery is necessary to retain a tooth that otherwise would be extracted. This study analyzes the microbial reduction after conventional periapical surgery followed by antimicrobial photodynamic therapy (aPDT) in 3 years follows up. Twenty-eight teeth needing periapical surgery were enrolled in this study. Microbiological samples were taken after: (1) accessing the lesion, (2) surgical procedure, and (3) aPDT. The teeth received a full mucoperiosteal flap, osteotomy with a high-speed bur, manual curettage of lesion and of the root, root-end resection and retrograde cavities prepared using ultrasonic retro-tips. After the conventional procedure the cavities received an aqueous solution of methylene blue (60 μM, 3 min) and were irradiated with a diode laser λ = 660 nm (6 min, 15 J). After aPDT, a retrograde filling with mineral trioxide aggregate, flap repositioning, and sutures conventionally ended the procedure. In all the cases, a periapical X-ray exam was taken before and after the surgical procedure. The microbiological samples showed an overall significant reduction, surgical procedure achieved a mean reduction of about 3.5 log while aPDT achieved a 5 log reduction. After 36 months of follow up the patients had, in average a reduction of 78% of the periapical lesion area. It appears that surgical endodontic

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treatment associated with antimicrobial photodynamic therapy highly improves the microbial reduction compared to the traditional technique and this could directly affect the treatment prognosis and periapical lesion healing.

Introduction

Besides the advances in endodontic treatment [1], there are situations in which surgery are necessary to retain a tooth that otherwise would be extracted [2].

Endodontic surgical treatment prevents or minimize the invasion of bacteria and their by-products from the root canal into periradicular tissues [3] and indications for periradicular surgery is the removal of non reachable bacteria with conventional endodontic treatment, due to complex root canal anatomy, procedural accidents, irretrievable materials in the root canal, symptomatic cases, horizontal apical fracture, biopsy and corrective surgery [4,5].

Antimicrobial photodynamic therapy (aPDT) has been studied [6,7] as a promising approach to eradicate oral pathogenic bacteria that cause endodontic diseases [8], periodontitis [9], periimplantitis [10] and caries [11—13].

The combination of endodontic treatment and aPDT has been shown as an effective approach in reducing bacterial load in *in vitro* and *in vivo* models [14—19]. However, in the literature there is no information about the effect of aPDT associated with conventional endodontic surgery regarded to microbial reduction and even more if the noxious components produced on the periapical tissue could slow down the healing process.

This study evaluates the long-term effectiveness of aPDT and conventional endodontic surgery in reducing the bacterial load and its effects on bone healing in cases of large periapical lesion.

Materials and methods

Twenty-eight teeth were enrolled in this study from 22 patients with periapical lesions who had been previously treated with endodontic treatment. All the patients were in good health, checked by individual anamnesis interview, and between the ages of 17 and 52 years. All the teeth (incisives, canines and premolars) presented signs and symptoms of periapical periodontitis and apical bone lesion detected by radiography. The protocol was reviewed and approved by the Institutional Review Board of the São Paulo University.

A full mucoperiosteal flap, osteotomy, manual curettage (periapical lesion and external root surface), root-end resection, and retrograde cavities preparation using ultrasonic retro-tip were performed as a conventional treatment (Fig. 1).

After the conventional procedure, the cavities received an aqueous solution of methylene blue (60 μM — 3 min of pre-irradiation time) and a diode red laser ($\lambda = 660$ nm, $P = 40$ mW for 3 min, $E = 7.2$J). The cavity was then dried and the tip of the laser was changed to allow access to the retrograde cavity. The irradiation inside the retrograde cavity was performed with an optical fiber ($\Phi = 200$-mm, MMOptics, São Paulo, Brazil) [20].

Subsequently to aPDT, a retrograde filling with mineral trioxide aggregate (MTA Angelus — Londrina, Paraná, Brazil), flap re-positioning, and sutures ended the procedure.

Microbiological samples, using sterile swabs, were taken, after osteotomy and accessing the bone lesion, after surgical procedure and after aPDT.

The samples were removed from the anaerobic transport medium (VMGA III), placed in BHI broth, and vortexed for 30 s. Serial dilution and colony-forming units (CFU) enumeration were performed [21] after incubation, on BHI agar plates, inside a microaerophilic chamber with 5% oxygen, 15% carbon dioxide, and 80% nitrogen for 72 h at 37 °C. At each stage of the treatment (initial, after endodontic surgery, and after aPDT), CFUs were counted. Survival fractions were calculated from each patient taking into account its initial bacterial load [14].

The method of culture was selected to assess the microbial load usually found in infected root canals [22]. However, no attempt was made to identify the specific microbial flora during the process.

A periapical radiograph, with an individual positioning support, was taken before and after the treatment and after 36 month to evaluate the decrease of the periapical lesion area using Image J (National Institute of Health, USA) software.

Statistical analysis

Statistical comparisons between means were performed with a paired *t* test using Microsoft Excel; 2-tailed *p* values are reported.

Results

The microbiological samples confirmed the presence of infection in all teeth. The initial infectious burden varied widely between patients. This variation was probably caused by differences of the individual root anatomy, duration of the infections and dimension of lesion (Fig. 2). After conventional therapy, the mean infectious burden was reduced to approximately 3.5 log. The mean infectious reduction after subsequent aPDT was around 1.5 log. The overall mean log reduction after the combined therapies was 5 logs and this was significantly greater than that achieved by endodontic surgery alone ($p < .0005$). None of the lesion treated had 100% microbial reduction after surgical treatment, whereas in 8 lesions were not possible to detect bacteria after combinations of endodontic treatment and aPDT.

Radiographic follow-up showed a decrease of the lesion area for all teeth. The decrease of the lesion area did also vary widely between individual teeth, probably for the same reasons the contamination vary and also due the individual