Effect of Subgingival Irrigation with Different Substances in the Treatment of Periodontal Disease. A Histometric Study in Rats

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Abstract

The aim of this study was to evaluate the histometric effects of subgingival irrigation with different solutions as adjuvant for the treatment of periodontal disease in rats. Periodontal disease was induced by ligature in the first lower molars of 91 Wistar rats over the course of 28 days. After removal of the ligatures, the animals were subjected to scaling and root planing, followed by subgingival irrigation with different solutions (0.9% saline, 0.2% chlorhexidine, 0.1% and 0.5% sodium hypochlorite and 11% propolis extract). The animals were sacrificed 7 and 14 days after the treatment and tissue was processed for histometric analysis for evaluation of bone support and epithelial migration. The histometric analysis showed no statistically significant differences between the group treated with scaling and groups treated with subgingival irrigation ($p > 0.05$) regarding bone support and epithelial migration. Similarly, significant differences were not found among the different solutions used for subgingival irrigation. This study agrees with the position of the American Academy of Periodontology, which states that there is insufficient evidence to indicate the routine use of subgingival irrigation as adjuvant to periodontal treatment.

Key words: Periodontal diseases, therapeutic irrigation, chlorhexidine, sodium hypochlorite, propolis

Introduction

Periodontal disease (PD) is a biofilm-induced infection and the role of microorganisms in its causation and pathogenesis is well documented. The aim of periodontal therapy is to reduce the number of pathogenic microorganisms in contact with periodontal tissues; therefore, mechanical treatment including scaling and root planing (SRP) by the meticulous use of hand or power-driven scalers to remove biofilm, endotoxin, calculus and other plaque-retentive local factors is the basis of periodontal treatment (Drisko, 2001; Petersilka *et al.*, 2002). However, in some instances, the complex anatomy of the root and the contours of the periodontal pocket may hamper the treatment and avoid sufficient reduction of the bacterial load to make the tooth surface biologically acceptable (Schwach-Abdellaoui *et al.*, 2000). Furthermore, even after repeated treatments, some patients or sites fail to stagnate the PD (Lindhe and Nyman, 1984). For these cases of refractory subjects or non-responding sites, the adjunctive use of antibacterial agents, usually in the form of subgingival irrigants, has been proposed. In the treatment of PD, subgingival irrigation (SI) is used as a lavage to flush away the bacteria that are in contact with the periodontal tissues, in order to improve the outcome of SRP (Krishna *et al.*, 2011).

Among the different antibacterial agents, chlorhexidine digluconate is the most studied and used in periodontics because of its proven antimicrobial effects (Shiloah and Hovious, 1993), availability, low cost (Shahab *et al.*, 2011), safety, efficacy, substantivity and low toxicity (Krishna *et al.*, 2011).

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Studies have shown that subgingival irrigation with chlorhexidine is effective in reducing periodontal inflammation and in controlling subgingival plaque (Soh *et al.*, 1982) and is effective in periodontitis, improving all clinical parameters evaluated (Asari *et al.*, 1996). Sodium hypochlorite (NaOCl) presents properties of an ideal antimicrobial agent, including broad and fast antimicrobial activity, relative non-toxicity in low concentrations, and low cost (Slots, 2002). Some studies have also shown its use as a subgingival irrigant (Vieira *et al.*, 1982; Adcock *et al.* 1983; Kamagate *et al.*, 2005). Additionally, the use of natural products has also gained special attention in periodontics. *In vitro* studies have demonstrated the susceptibility of periodontal pathogenic microorganisms to propolis extract solutions, as well as no cytotoxicity toward gingival fibroblasts (Gebara *et al.*, 2002; Santos *et al.*, 2002; Sonmez *et al.*, 2005; Ozan *et al.*, 2007). A clinical study has also shown promising results after SI with propolis extract (Gebaraa *et al.*, 2003).

However, according to the Committee on Research, Science and Therapy of the American Academy of Periodontology, there is currently insufficient evidence to indicate that SI routinely should be used as a supplemental in-office procedure to augment the effects of SRP. Consequently, additional studies are needed to ascertain the full potential of SI as an adjunct to periodontal therapy (Greenstein, 2005).

Because there are variations in the methodology of various studies, e.g., the sample size, depth of periodontal pockets, mechanisms of supra and subgingival biofilm control, type of irrigant solution used and frequency of irrigation, among others, it becomes difficult to make any valid conclusions about the potential effect of SI in the treatment of PD. Therefore, there is consensus among the authors about the need for additional studies to assess the benefits and limitations of SI as an adjunct to periodontal therapy. Furthermore, most of the available studies are based on clinical and microbiological parameters. Thus, there is a paucity of histometric data to report the effects of different solutions used for SI on periodontal tissues.

The purpose of this study was to evaluate the histometric effects of SI with different irrigation solutions (0.9% saline, 0.2% chlorhexidine, 0.1% and 0.5% sodium hypochlorite and 11% propolis extract) as adjuvant for the treatment of periodontal disease in rats. The null hypotheses tested are that neither SI nor the different solutions result in significant histological bone support improvement and linear extension epithelial migration in furcation regions.

Materials and methods

Ethical considerations

The Ethics and Animal Experimentation Committee of the Veterinary Medicine Course of the Maranhão State University approved this research, protocol # 039/2010.

Sample size and experimental design

Sample size was calculated using G*Power 3.1 software, considering ANOVA evaluation with an α-type error = 0.05. To detect possible statistically significant differences, seven rats per group were necessary.

This controlled, randomized, blinded study used a total of 91 rats (male, approximately 2 months old and weighing 200 g), distributed among the groups below. The hemi-mandibles in which each procedure was carried out were chosen by the flip of a coin.

- Control group (CO) – 7 animals: ligature in the first lower left molar for 28 days (positive control). The right side was used as negative control as no ligature was placed.
- Scaling group (SC) – 14 animals: ligature on the first lower right molar for 28 days. Ligature removal on the 28th day, followed by SRP.
- Saline group (SA) - 14 animals: ligature on the first lower left molar for 28 days. Ligature removal on the 28th day, followed by SRP and SI with saline at 0.9%.
- Chlorhexidine group (CHX) – 14 animals: same procedures as latter group, using 0.2% chlorhexidine digluconate as the solution.
- 0.1% NaOCl group (0.1%SH) - 14 animals: same procedures as the latter group, using 0.1% NaOCl as the solution.
- 0.5% NaOCl group (0.5%SH) - 14 animals: same procedures as the latter group, using 0.5% NaOCl as the solution.
- Propolis group (PRO) - 14 animals: same procedures as the latter group, using 11% propolis extract solution (Propomax®, Apis Flora, Brazil).

Periodontal disease induction

Periodontal disease was induced in the first lower molars by accumulation of bacterial biofilm using the ligature technique (Johnson, 1975). The ligature was set in position under general anesthesia, through intraperitoneal injection in association with 10% ketamine hydrochloride (Cetamin, Syntec, Brazil) and 2% xylazine hydrochloride (Xilazin, Syntec, Brazil).

A retraction cotton wire (ligature) was introduced in the interproximal space between the first and second lower molar using a Hollenback instrument Nr. 24 to adjust the ligature in the most cervical position possible, surrounding the first molar, with 2 knots on the buccal surface. The ligature remained in place for the next 28 days in all groups.

Treatment procedures

After removal of the ligature, the animals from the SC group were subjected to SRP with a sterile manual curette *Gracey Mini-Five 5/6* (Hu-Friedy, U.S.A.) previously sharpened as recommended by Garcia *et al.*, 2011.

If remaining biofilm or calculus was observed, scaling movements were repeated until a smooth root surface was reached. Animals from groups SA, CHX, 0.1%SH, 0.5%SH and PRO were subjected to SRP followed by SI with the respective solutions.

Subgingival irrigation was performed using a 30 gauge syringe tip (Ultradent, USA) in 6 different sites (vestibular, mesio-buccal, disto-buccal, lingual, mesio-lingual and disto-lingual), with 0.1 mL of the respective irrigating solution three times in each site.

In the CO group, the animals were sacrificed the day of ligature removal. In the other groups, the animals were sacrificed 7 and 14 days after the performance of PD treatment procedures (7 rats each time). The sacrifice was done with an overdose of anesthesia.

Histological procedures and evaluation

After sacrifice, teeth were removed in block and placed for 48 hours in paraformaldehyde at 4% for fixation. The pieces were then placed in a solution of 7% EDTA for decalcification for 3 months, with a solution change every 2 days. The pieces were embedded in paraffin and submitted to serial sections of 5 µm in the mesio-distal direction, then stained with hematoxylin and eosin.

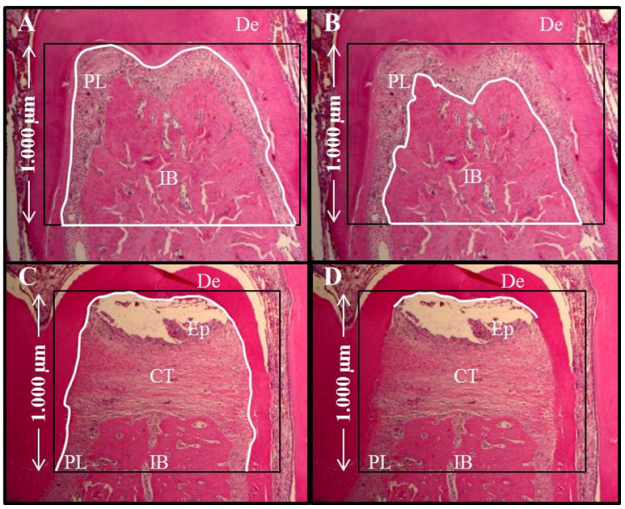

Figure 1. A) Photomicrography representing the area bounded in the region of furcation for histometric analysis. B) Area of the region of furcation filled by bone tissue whose value divided by the furcation bounded area corresponds to the percentage of the bone support. C) Linear extension of the delimited furcation for histometric analysis. D) Linear extension of furcation bare or coated by epithelial tissue, whose amount divided by the furcation delimited extension corresponds to the percentage of epithelial migration. De, dentin; IB, inter-radicular bone; Ep, epithelium; PL, periodontal ligament; CT, conjunctive tissue.

Images of four cuts of equidistant sections of each hemi-mandible were selected for histometric analysis. A blinded and calibrated examiner used Axio Vision Rel. 4.8 (Carl Zeiss, Germany) to determine the bone support (percentage of bone tissue in the area of 1,000 µm below the roof of the furcation in the inter-radicular region – *Figures 1A-1B*). Epithelial migration (linear extension of the root surface coated by epithelial tissue – *Figures 1C-1D*) was also measured by means of Image J 1.45s (National Institutes of Health, USA).

Statistical analysis

SPSS 18 (IBM, U.S.A) was used for all statistical procedures. A previous *t*-test was used to compare the data from the two sides of the control group in order to validate the induction technique of PD. Analysis of raw data demonstrated non-adherence to a Gaussian distribution (Kolmogorov-Smirnov) as well as non-homogeneity of the distribution (Levene). Therefore, a Kruskal-Wallis non-parametric procedure was performed followed by pair-wise comparison using the Mann-Whitney test with Bonferroni correction. Bone support and epithelial migration were the dependent variables, while solution used and time of sacrifice were the independent variables. The level of significance was set at 5%.

Results

Data referring to bone support and epithelial migration (%) are shown in *Figures 2-3*. Student's *t*-test between the CO groups showed significant statistical difference ($p < 0.05$), proving that the DP was effectively induced (data not shown).

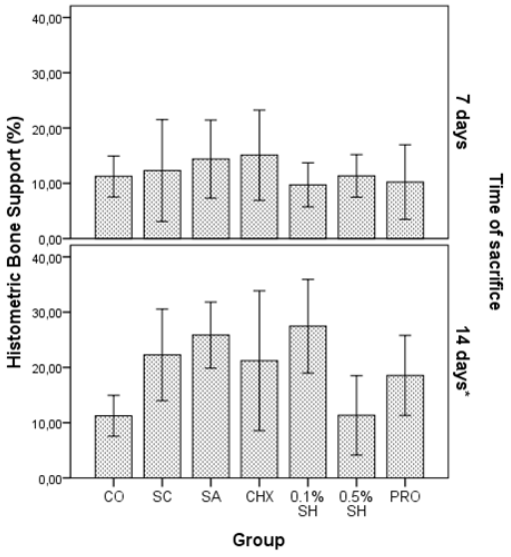

Figure 2. Mean and standard error of bone support for the different variables (%) – Statistical test Kruskal-Wallis. Bone support data referring to the type of treatment, irrigant solution used and time of sacrifice. *Significant differences between 7 and 14 days.

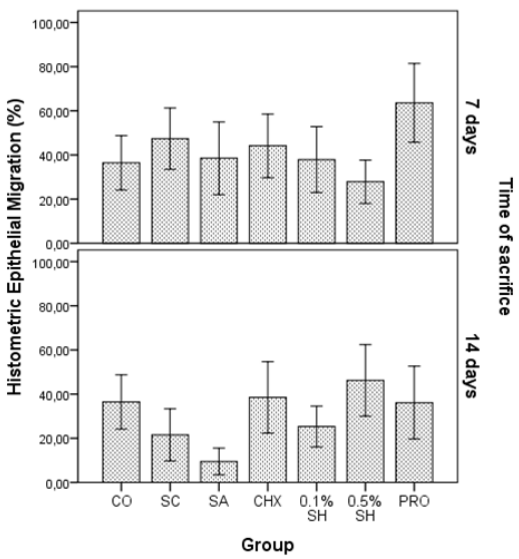

Figure 3. Mean and standard error of linear extension of epithelial migration for the different variables (%) – Statistical test Kruskal-Wallis. Epithelial migration data referring to the type of treatment, irrigant solution used and time of sacrifice.

The histometric analysis showed no statistically significant difference between the group treated with scaling alone and groups treated with subgingival irrigation ($p > 0.05$) with regard to bone support and epithelial migration. Similarly, solution type used did not significantly influence the histometric data. Groups sacrificed at 14 days after treatment showed mean bone support significantly greater than those sacrificed at 7 days ($p < 0.05$), whereas no significant difference was found for epithelial migration.

Discussion

The model of PD induction used in this study (Johnson, 1975) was effective, as determined by the control group. Before being exposed to SI, all teeth were subjected to SRP, since this procedure is established as the base of the majority of periodontal therapy protocols and significantly affects the composition of the subgingival microbiota (Drisko, 2001; Petersilka *et al.*, 2002). Therefore, analyses of SI and solution type were performed only after performing a "gold standard" baseline with SRP (Cobb, 2002).

Histometric analysis data confirmed the null hypothesis formulated, since neither SI with saline nor the different solutions displayed greater areas of bone support and lower linear extension of epithelial migration when compared to the SRP alone group. These results suggest that SI with different solutions provides no additional effects when used as an adjunct to basic mechanical therapy.

Animals sacrificed 14 days after treatment showed significantly higher bone support than those sacrificed at 7 days. This is expected because 14 days after effective treatment periodontal tissue should present less wounded histometric values.

The role of SI as adjunct in the treatment of PD remains controversial in the literature. Some studies have shown that SI provides no additional improvement to the therapeutic effects achieved by SRP alone (Wennstrom *et al.*, 1987a; Wennstrom *et al.*, 1987b; Krust *et al.*, 1991). On the other hand, some studies have shown synergistic effects (Soh *et al.*, 1982; Asari *et al.*, 1996; Gebara *et al.*, 2003), but in most cases the improvement is usually modest and temporary. After 12 months there was no significant difference between treatment methods regarding clinical parameters (Krück *et al.,* 2012).

Regarding the irrigation solutions, even with proven antimicrobial action, no significant differences could be found with respect to bone support and epithelial migration. It appears that the greatest shortcoming of irrigation therapy is the quick elimination of subgingivally placed drugs, which results in reduction of their substantivity (Greenstein, 2005). Thus, the routine use of SI is justified only because these drugs are inexpensive and relatively easy to use and have minimal risks (Quirynen *et al.*, 2002).

From the results obtained, this study agrees with the position of the American Academy of Periodontology, which states that there is insufficient evidence to indicate that SI routinely should be used as adjuvant to periodontal treatment to improve the effects of SRP.

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