

Diffusion of calcitonin through the wall of the root canal

Avaliação da difusão da calcitonina através da dentina radicular

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ABSTRACT: The aim of this study was to evaluate the *in vitro* diffusion of synthetic salmon calcitonin (CT), used as an intracanal medication, to the external root surface, with or without the presence of intact root cementum. Fifty-four human central incisors were used in the experiment, and were divided into two groups of 21 (test groups) and two groups of 6 teeth (control groups). After root canal preparation, 10 µl of calcitonin was inserted within the root canal chamber. The root was sealed and made externally impermeable. Specimens were then placed in tubes with saline solution buffered with phosphates and stored at 37°C. The diffusion of calcitonin was measured after 1, 4 and 7 days. To count calcitonin present at the external media (PBS), ELISA test (an antigen-antibody reaction) was used. Results showed that there was calcitonin diffusion through dentin in all of the test samples. The absence of cementum increased the diffusion of calcitonin ($p=0.05$). The highest counts of CT were obtained on day 7 for groups with or without cementum – showing a direct relation between time and diffusion of the medication.

DESCRIPTORS: Dental cementum; Tooth permeability; Root canal irrigants; Calcitonin.

RESUMO: O presente estudo determinou *in vitro* a difusão da calcitonina sintética de salmão, como medicação intracanal, até a superfície externa radicular, com e sem a presença de cimento radicular. Foram utilizados cinquenta e quatro incisivos centrais humanos, divididos em dois grupos com 21 dentes cada e dois grupos com 6 dentes cada (controle negativo). Após o preparo endodôntico, os dentes receberam 10 µl de calcitonina como medicação intracanal. Feitos o selamento e a impermeabilização externa da raiz, os espécimes foram acondicionados em tubos com solução salina tamponada com fosfatos e incubados em estufa a 37°C. Foram colhidas amostras da solução salina nos períodos de 1, 4 e 7 dias. Para a análise das soluções teste, utilizou-se teste ELISA. Os resultados mostram que existe a difusão da medicação testada até a superfície externa radicular em todas as amostras dos grupos teste. O fator ausência de cimento radicular permite a passagem de maiores quantidades de calcitonina, dados estatisticamente significantes ao nível de 5%. Os maiores valores de difusão foram encontrados no sétimo dia, tanto para o grupo com cimento como para o grupo sem cimento, mostrando uma relação direta entre tempo e difusão da medicação intracanal testada.

DESCRIÇÕES: Cimento dentário; Permeabilidade dentária; Irrigantes do canal radicular; Calcitonina.

INTRODUCTION

Inflammatory root resorption is one of the complications of traumatized mature teeth, especially in cases of luxation and avulsion. Current evidence suggests that this form of resorption is caused by an inflammation in the periodontal ligament, which is caused by bacteria present in the pulp chamber and dentinal tubules^{3,4}. It has been speculated that conventional endodontic treatment using a specific intracanal filling with the ability to diffuse and reach the active area of resorption could solve the problem¹³. Tooth ankylosis, another dental trauma complication, is associated with the absence of vital periodontal ligament and

represents the union of the root surface and the alveolar bone. In cases where limited damage has occurred, ankylosis reversal will occur. Otherwise, ankylosed teeth are gradually resorbed and replaced by bone². In dental trauma, calcium hydroxide has been used as a filling material with good effects on inflammatory resorption, but it also increases the risk of ankylosis in mature teeth with severe periodontal injury¹⁵. In the literature the use of a polypeptide hormone, calcitonin, has been proposed in an attempt to control the resorption process^{8,9,10,12}. It is well-established that calcitonin directly inhibits osteoclastic activity, both *in vivo*

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and *in vitro*^{5,6}. The motility of osteoclasts is suppressed by picomolar concentrations of calcitonin, also accompanied by the disappearance of a specialized membrane structure in resorption: the ruffle bordered, clear zone with an overall decrease in the size of the cells⁷. According to Pierce *et al.*¹⁴, (1989) osteoclasts and odontoclasts are similar structures with the only reported difference being the reduced size of the odontoclast. Based on that, the presence of calcitonin at the site of the active area of resorption can represent a reasonable multifactorial therapeutic intervention.

The aim of this study was to evaluate calcitonin diffusion through root canal dentin to the external surface, when used as an intracanal filling.

MATERIAL AND METHODS

Fifty-four freshly extracted single rooted human teeth were selected. After extraction, teeth were placed in 10% sodium hypochlorite for 4 hours to remove the periodontal ligament, extensively washed with PBS (UBC, Vancouver, Canada) and observed with a stereomicroscope to detect cracks or any damage produced to the root area. Compromised teeth were discarded. Specimens selected were placed in individual vials containing 3 ml of PBS for 12 days prior to the experiment. Endodontic cavity accesses were made with round carbide burs (Dentsply-Maillefer, Ballaigues, Switzerland) n. 3 and 4, in high-speed motion. All teeth were cleaned and shaped to a minimum size of 40, 1 mm from the apex; the root canals were flared using the step-back technique followed by the use of Gates Glidden drills (Dentsply-Maillefer, Ballaigues, Switzerland). Irrigation during this phase was carried out with Endo-PTC cream (Fórmula e Ação, São Paulo, Brazil) and 10 ml of 0.5% of sodium hypochlorite (UBC, Vancouver, Canada), according to the Paiva, Antoniazzi¹¹ (1993) technique. For final irrigation, 10 ml of 17% EDTA (UBC, Vancouver, Canada) was used.

Specimens were then separated into two groups of 21 teeth each. In group 1, external root cementum was kept intact. In group 2, 0.5 mm of external root cementum was removed with diamond burs at low speed under PBS irrigation. Smear layer was removed with 17% EDTA for 5 minutes and specimens were washed with a final flush of PBS. Following that, a thin layer of Cavit (Cavit-W, Provisorische Verschluss ESPE GMBH, Seefeld/Oberlay, Germany) was placed at the apex (2 mm). Root canals were dried with paper

points and 10 µl of calcitonin was placed in the canals using a micropipette. A small cotton pellet and then a thin layer of Cavit cement were placed in the root chamber. External sealing was performed in G1 and G2 using 3 layers of cyanoacrylate (Crazyglue, Vancouver, Canada) at the apical area (over cavit cementum) and at the coronal area of the enamel-cementum junction.

The control groups (G3 and G4) consisted of six teeth each, selected randomly, and all external root surfaces were covered with cyanoacrylate. Each tooth was immediately placed in a specific vial containing 3 ml of PBS and incubated at 37°C.

Time intervals of 1, 4 and 7 days were determined to insert 50 µl aliquots of PBS in each specimen vial, and specimens were collected and analyzed to evaluate the rates of calcitonin release.

ELISA test was used to confirm the presence of calcitonin molecules in the model described above. The primary antibodies employed in the study were affinity purified rabbit antibodies against salmon calcitonin. A secondary antibody employed was alkaline phosphatase conjugated goat anti-rabbit IgG.

A 96-well ELISA plate was coated overnight at 4°C with individual aliquots of all groups (50 µl), leaving one lane as a blank. After washing twice with PBS, the nonsaturated bindings were blocked with 3% bovine serum albumine (BSA) in PBS for 2 hours at 37°C.

After two sequential washes with PBS, fifty µl of the buffer with 1% BSA/0.1% Tween 20/PBS containing rabbit antibody against salmon calcitonin (1:4,000 dilution) (Research & Diagnostic Antibodies, Berkley, CA, USA) was added to the wells and the plates were incubated at 37°C for one hour.

Another wash with PBS/0.1% Tween 20 (UBC, Vancouver, Canada) was performed and the plates were incubated for one hour at 37°C with 50 µl of alkaline phosphatase conjugated goat anti-rabbit-IgG antibody (ICN Biomedicals Inc., Costa Mesa, CA, USA), diluted in 1% BSA/0.1% Tween 20/PBS (1:4,000 dilution).

The plates were then washed four times with PBS/0.1% Tween 20 buffer, and developed with (0.5 mg/ml) of p-nitrophenyl phosphate (Sigma Chemicals Co., St Louis, MO, USA) in diethanolamine buffer (Quadra Logic Technologies Inc., USA). The optical densities were measured after 2 hours at room temperature.

ELISA determinations of calcitonin levels were made using corresponding levels of standard plot-

ted against the measured optical densities. Standard curves were generated for each ELISA plate analyzed. ELISA results found in optical density units were converted and expressed in percentage of calcitonin at the external media. These values were compared using ANOVA test and Tukey's test. To evaluate the possibility of unspecific binding affinity to any component except calcitonin, the PBS solution with all specimens were stored, before the experimental procedures, tested and showed negative values of optical density.

RESULTS

The diffusion characteristics of calcitonin through root canal dentin tubules to the external surface was observed by measuring the appearance of developed antigen-antibody complex at the suspended media in which the tooth was placed.

Table 1 shows mean percentages of calcitonin release in group 1 (with cementum), and group 2 (without cementum) in the external media.

In general, the release rates increased with time and were influenced by the presence or absence of cementum layer. When data for all specimens were evaluated together, the factor presence of cementum showed significant values, as demonstrated in Table 2.

Endodontic intervention is based on the enlargement and disinfection of the root canal, increasing dentin permeability in order to facilitate the filling material to diffuse through the external root surface, the site of the external progressive resorption¹³. Calcitonin has been indicated as an effective therapeutic strategy, since it directly inhibits osteoclast activity and suppresses inflammation^{6,8,10,12}.

This research was focused on the diffusion of calcitonin, when used as a filling material, through dentinal tubules, and in consequence reaching the surrounding periodontal ligament, which harbors the active resorbing cells.

Calcitonin release was noticed in both groups with and without cementum and significant statistical differences were observed. The presence of cementum is a barrier for calcitonin diffusion but it does not inhibit the diffusion. These results were in accordance to Wiebkin *et al.*¹⁶ (1996a), that previously showed the diffusion of radioactive calcitonin through root canal dentin. Calcitonin has been shown to have an affinity to root mineral structure. It seems that mineral structures from dentin and cementum have receptors for calcitonin

molecules. Those molecules bind one receptor to another by diffusion from the root canal to the external area, through a different concentration gradient¹⁷. The presence and absence of cementum are represented clinically in several avulsion therapeutic situations. In those cases in which treatment is performed just after the accident, the presence of an almost intact cementum layer is expected, and in other cases in which active resorption has already occurred this particular structure does not exist. In both cases, resorbing and inflammatory cells are present and calcitonin medicament at the site will provide an important barrier. Resorbing cells are the only cells that have receptors for calcitonin¹⁴. The hormone acts by inhibiting cell activity and suppressing inflammation^{10,12}. The clinical use of this therapy would be favorable in the initial phase of the avulsion-replantation treatment, since this period represents the highest cellular activity function. Osteoclast activity is directed to clean the area and other cells compete in order to remodel the original structures¹. Osteoclast inhibition would be favorable for the proliferation of periodontal and cementum cells facilitating periodontal tissue regeneration. However, further studies are necessary to characterize the effectiveness of this therapy to prevent or control external progressive root resorption.

It was also shown that time has a significant influence on the amount of calcitonin at the external surface. The highest percentage of calcitonin was observed on day 7 for both groups. These results suggest that the root canal system may work

TABLE 1 - Mean rate (standard deviation) release of calcitonin in groups 1 and 2.

Groups	Day 1	Day 4	Day 7
G1 (with cementum)	1.04 (1.66)	2.38 (3.02)	5.52 (3.54)
G2 (without cementum)	1.72 (1.81)	3.66 (3.73)	7.58 (2.79)

TABLE 2 - Mean rates of calcitonin diffusion in the presence or absence of cement.

	Mean	F calculated	F (p = 5%)
With cementum (n = 21)	2.92	5.79	4.08
Without cementum (n = 21)	4.32	1.06	3.08

ANOVA test $\alpha = 0.05$; n = number of teeth.

as a slow release system for this hormone. Wiebkin *et al.*¹⁷ (1996b) have suggested that root mineral non-specifically binds calcitonin. The number of binding sites is small, and, although apparently tight, the binding is reversible. Thus, despite the low concentration of calcitonin inserted into the root canal, the amount of calcitonin detected at the external root surface is likely to be representative of an intrinsically slow delivery system. Indeed, only low concentrations of the hormone are necessary to inhibit motility of osteoclasts⁷.

The experimental model used in this project was effective to determine calcitonin concentrations in aqueous solution. The use of high affinity purified antibodies and the blocking of nonsaturated sites in the plate provided accurate specific calcitonin molecules bindings, eliminating the possibility of nonspecific bindings, which would

alter the results. Prior to the experimental phase, stored PBS solution was tested, showing negative values of optical density. The same results were obtained testing the control groups. Specific antigen-antibody ELISA tests demonstrated in this study showed to be an efficient model for the study of diffusion and medicament release through root canal dentin.

CONCLUSIONS

This paper showed the ability of calcitonin to diffuse through root canal system to the external surface. The presence of root cementum plays a role on the process, reducing rates of diffusion. Root canal works as a slow release system for calcitonin when used as filling material, since the highest percentages were obtained on day 7 for both tested groups.

REFERENCES

1. Andreasen JO, Andreasen FM. Text book and color atlas of traumatic injuries to the teeth. 3rd ed. Copenhagen: Munksgaard; 1994.
2. Andreasen JO. Analysis of pathogenesis and topography of replacement of root resorption (ankylosis) after replantation of mature permanent incisors in monkeys. *Sweed Dent J* 1980;4:231-40.
3. Andreasen JO. Exarticulations in traumatic injuries of the teeth: text book. 2nd ed. Copenhagen: Munksgaard; 1981.
4. Andreasen JO. The effect of pulp extirpation or root canal treatment on periodontal healing after replantation of permanent incisors in monkey. *J Endod* 1981;7:245-52.
5. Azria M. The calcitonins, physiology and pharmacology. Switzerland: Karger; 1989.
6. Chambers TJ, Magnus CJ. Calcitonin alters behavior of isolated osteoclasts. *J Pathol* 1982;136:27-40.
7. Chambers TJ, Moore A. The sensitivity of isolated osteoclasts to morphological transformation by calcitonin. *Clin Endocrinol Metabol* 1983;57:819-24.
8. Lage-Marques JL, Conti R, Antoniazzi JH. Conduta clínica frente ao traumatismo dental. *Rev Assoc Paul Cir Dent* 1994;4:1529-33.
9. Lage-Marques JL, Silva JRS, Prokopowisch I. Traumatismo dental: avulsión, nuevos conceptos. *FOLA Oral* 1995;1:68-72.
10. Manotas JD, Solano A, Osório M, Guerrero F. Efectividad de la calcitonina para detener la reabsorción externa posterior a un reimplante. *Rev Fed Odontol Colombia* 1992;45:19-31.
11. Paiva JG, Antoniazzi JH. Endodontia: bases para a prática clínica. 2^a ed. São Paulo: Artes Médicas; 1993.
12. Pierce A, Berg JO, Lindskog S. Calcitonin as an alternative therapy in the treatment for root resorption. *J Endod* 1988;14:259-64.
13. Pierce A, Lindskog S. The effect of an antibiotic/corticosteroid paste on inflammatory root resorption *in vivo*. *Oral Surg Oral Med Oral Pathol* 1987;64:216-20.
14. Pierce AM. Experimental basis for the management of dental resorption. *Endod Dent Traumatol* 1989;5:255-65.
15. Vanderas AP. Effects of intracanal medicaments on inflammatory resorption or occurrence of ankylosis in mature traumatized teeth: a review. *Endod Dent Traumatol* 1993;9:175-84.
16. Wiebkin OW, Cardaci SC, Heithersay GS, Pierce AM. Therapeutic delivery of calcitonin to inhibit external inflammatory root resorption. I. Diffusion kinetics of calcitonin through the dental root. *Endod Dent Traumatol* 1996a;12:265-71.
17. Wiebkin OW, Cardaci SC, Heithersay GS, Pierce AM. Therapeutic delivery of calcitonin to inhibit external inflammatory root resorption. II. Influence of calcitonin binding to root mineral. *Endod Dent Traumatol* 1996b;12:272-6.

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