IMMUNOLOGICAL STUDY REGARDING THE ROLE OF $CA(OH)_2$ PASTE ON THE MMP8 EXPRESSION FOR TEETH WITH CHRONIC PERIAPICAL LESIONS

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ABSTRACT

Introduction MMP8 are secreted as inactive proproteins, stored in secondary granules within neutrophils and are activated by autolytic cleavage. The function of MMP8 is degradation of type I, II and III collagens. In this context, the concentration of MMP8 can be related to the intensity of inflammatory processes associated with chronic periapical lesions. **Objectives** The aim of our study was to measure changing of MMP8 within the periapical secretion of teeth affected by periapical lesions and treated with antibacterial medication, using immunological tests. **Methods** Study group included 22 patients with age 22-64 years. A number of 30 teeth with periapical lesions (periapical granuloma and diffuse periapical osteitis) were submitted to endodontic treatment and filled with $Ca(OH)_2$ paste. The periapical secretion was collected with paper points at baseline, after 14 days and after 28 days. The concentration of MMP8 was assessed using ELISA test Quantikinine (Human MMP-8 Immunoassay, R&D System, USA) based on quantitative sandwich enzyme immunoassay. **Results and discussions** At baseline the mean concentration of MMP8 was 31.3 ng/mL. The concentrations of MMP8 were closely related to the type of periapical lesion: 12.5 ng/mL for incipient periapical lesions, 18.1 ng/mL for small periapical granuloma and 91.6 ng/mL for extended periapical granuloma. The levels of MMP8 decreased gradually after 2 weeks and 4 weeks comparing with baseline. **Conclusion** Metaloproteinases (MMP8) could be used as biochemical markers of the periapical status of inflammatory processes in course of initial stage of endodontic therapy.

Key words: chronic periapical lesions, calcium hydroxide, metalloproteinases, MMP8

INTRODUCTION

The periapical reaction related to endodontic infection consists of a mixed inflammatory cell infiltrate, including large numbers of T cells, B cells, neutrophils, macrophages and plasma cells. The principal role of the proper endodontic treatment of the chronic periapical lesions is represented by the elimination of bacteria and endodotoxins associated with inflammatory reactions. Finally it reduces latent and active forms of enzymes related to the destruction processes. Matrix metalloproteinases (MMPs) are an important family of metal-dependent endopeptidases that represent the major class of enzymes responsible for degradation of

extracellular matrix (ECM) components. MMP8 are secreted as inactive proproteins, stored in secondary granules within neutrophils and are activated by autolytic cleavage. The function of MMP8 is degradation of type I, II and III collagens. MMP8 are secreted by PMN, monocites, macrofages, and fibroblasts. In this context, the concentration of MMP8 can be related to the intensity of inflammatory processes associated with chronic periapical lesions. In this context, clinical experience and radiographic exam must be completed with immunological tests that can confirm the initiation of healing processes.

Aim and objectives

The aim of our study was to measure, using immunological tests, the changing values of MMP8 within the periapical secretion of teeth affected by periapical lesions and treated with antibacterial medication based on $Ca(OH)_2$.

MATERIAL AND METHOD

The study group included 22 patients with age between 22-64 years. The patients were informed about study goals and gave written consent. A number of 30 teeth with periapical lesions (periapical granuloma and diffuse periapical osteitis) were submitted to classical endodontic treatment. The chronic periapical lesions were divided in three categories: incipient periapical lesions (8 teeth), periapical granuloma under 0,5cm (16 teeth), severe periapical lesions (6 teeth). The excluding criteria were as follows: teeth with acute periapical reactions, radicular cysts, severe coronal destructions, endoperiodontal lesions. The stages of endodontic treatment were as follows: mechanical and chemical disinfection (EDTA 17%, NaOCl3%), root canal medication ($Ca(OH)_2$ pastes) for 28 days, root canal filling using gutta-percha points and sealer (Endoflas, Sanlor) with lateral condensation technique. The stage of root canal medication consisted of two appointments with calcium hydroxide pastes, changed at intervals of 14 days. The periapical exudate was collected with paper points at baseline, after 14 days and after 28 days. The paper points were introduced in 50 μL sterile distilled water, in 1,5mL Eppendorf tubes, stored at -80^0C until immunochemical tests were performed. The concentration of MMP8 was assessed using ELISA test performed with kit Quantikinine (Human MMP-8 Immunoassay, R&D System, USA). This test is based on quantitative sandwich enzyme immunoassay. The evolution of periapical inflammatory reactions were also evaluated using clinical and radiographic examination, with radiographs taken at baseline and after 28 days.

RESULTS AND DISCUSSIONS

In figures 1-2 are presented examples of periapical lesions selected from study group.

The values of MMP8 at baseline, 14 days, 28 days are presented in Table I and Fig. 3. Related to the extension of chronic periapical lesions, minimal and maximum values of MMP8 were as follows: 0 ng/mL - 20 ng/mL (incipient), 5 ng/mL - 15 ng/mL (periapical granuloma with diameter under 0.5 cm), 5 ng/mL - 15ng/mL (periapical lesions with diameter over 0.5 cm). The mean values of MMP8 levels are presented in table 1:12.5 ng/mL for incipient periapical lesions, 18.1 ng/mL for small periapical granuloma and 91.6 ng/mL for extended periapical lesions.

The progressing decreasing of MMP8 levels is presented in Fig. 3. The mean value at baseline was 35ng/mL. After 2 weeks, 26 cases had a positive evolution associated with the absence of acute periapical reactions. For these cases values of MMP8 after 2 weeks ranged between 5 ng/mL-125 ng/mL. After 4 weeks, these 26 cases maintained their positive evolution and values of MMP8 after ranged between 0 ng/mL-15 ng/mL.

Statistical test Wilcoxon was used to determine the existence of statistical differences between MMP8 levels at baseline, 14 days, 28 days (Tables II - IV).

The decreasing of MMP8 after 2 weeks of root canal medication with calcium hydroxide is statistically significant (Tables II. a,b).

The decreasing of MMP8 between day 14 and day 28 is not statistically significant comparing with the changing of MMP8 values between baseline and day 14 (Tables III. a,b).

Fig. 1. Incipient periapical lesion

Fig. 2. Extended periapical granuloma (Ø > 0,5 cm)

Incipient periapical lesion	Small periapical lesion	Extended periapical lesions
12,5 ng/mL	18,1 ng/mL	91,6 ng/mL

Table I. MMP8 levels related to the type of periapical lesion

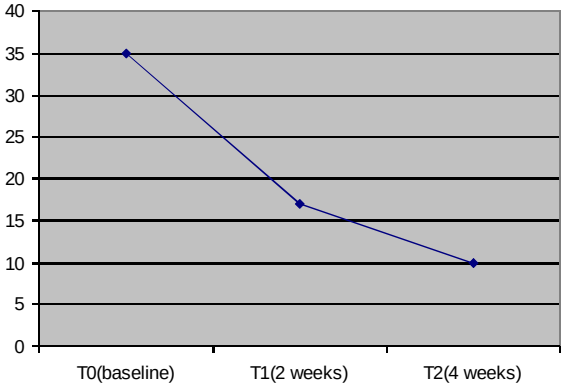

Fig. 3. The mean values o MMP8 at baseline (T0), 2 weeks (T1), 4 weeks (T2)

Ranks

		N	Mean Rank	Sum of Ranks
M2 - M1	Negative Ranks	18[a]	9,50	171,00
	Positive Ranks	0[b]	,00	,00
	Ties	8[c]		
	Total	26		

a. M2 < M1
b. M2 > M1
c. M2 = M1

Test Statistics[b]

	M2 - M1
Z	-3,800[a]
Asymp. Sig. (2-tailed)	,000

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test

Table II. a-b Test Wilcoxon for M1 (baseline) and M2 (2 weeks)

Ranks

		N	Mean Rank	Sum of Ranks
M2 - M3	Negative Ranks	8[a]	6,00	48,00
	Positive Ranks	6[b]	9,50	57,00
	Ties	12[c]		
	Total	26		

a. M2 < M3
b. M2 > M3
c. M2 = M3

Test Statistics[b]

	M2 - M3
Z	-,289[a]
Asymp. Sig. (2-tailed)	,772

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

Table III. a-b Test Wilcoxon for M2 (2 weeks) and M3 (4 weeks)

Ranks

		N	Mean Rank	Sum of Ranks
M1 - M3	Negative Ranks	4[a]	6,50	26,00
	Positive Ranks	16[b]	11,50	184,00
	Ties	6[c]		
	Total	26		

a. M1 < M3
b. M1 > M3
c. M1 = M3

Test Statistics[b]

	M1 - M3
Z	-3,027[a]
Asymp. Sig. (2-tailed)	,002

a. Based on negative ranks.
b. Wilcoxon Signed Ranks Test

Tabel IV. a-b Test Wilcoxon for M1 (baseline) and M3 (4 weeks)

The decreasing of MMP8 between baseline and day 28 is statistically significant comparing, following antibacterial and antiinflammatory effects of hydroxil ions released from calcium hydroxide paste (Tables IV. a,b)

The reduction of MMP-8 after 2 weeks and 4 weeks can be correlated with the absence of acute periapical reactions, blocking of destruction processes of organic matrix and osteoclastic processes at the level of mineral tissues.

Our results sustain literature data related to relation between changing of MMP-8 level accordingly to severity and therapeutical stage of chronic periapical lesions. Andronovska B.&col. (2008) correlated the levels of collagenases (including MMP8) with severity of chronic periapical lesions, similar result with our study [1]. Ma Z.&col. (2011) demonstrated the role of PMN on the secretion of metalloproteinases (including MMP8) after the interaction with E. faecalis [4]. Francisco WG&col. (2010) prove that teeth with apical periodontitis submitted to root canal treatment using calcium hydroxide presented a lower inflammatory cell infiltrate with a moderately organized connective tissue, a lower prevalence of bacteria, and a lower number of MMP-positive cells, similar to healthy teeth submitted to treatment [2]. Gendron&col. (1999) observe the inhibition of metalloproteinases secretion after repeated cleanings of root canals using chlorhexidine [3]. Wahlgren&col. (2002) show that the absence of MMP8 decreasing after two appointments of intracanalar medication with calcium hydroxide pastes can be considered failure of endodontic treatment [8]. Metzger (2000), Takahashi (1998) and Tjaderhane (2001) recommend the introduction in dental practice of chair-side tests for the assessment of periapical lesions activity (5, 6, 7).

CONCLUSIONS

- The decrease of MMP8 concentration in periapical exudates following intracanal medication with calcium hydroxide proves the its role in the initiation of healing periapical processess;
- The changing of MMP8 concentration, in

course of initial stages of endodontic treatment, can indicate the success or potential failure of clasical endodontic therapy of the extensive chronical periapical lesions;

– Metalloproteinases MMP8 could be used as biochemical markers to assess the activity of periapical inflammatory processess and the efficiency of the endodontic therapy.

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