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Matrix metalloproteinases (MMP-1, -8, -13) in chronic periapical lesions

Metaloproteinaze matriksa (MMP-1, -8, -13) kod hroničnih periapeksnih oboljenja

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Abstract

Background/Aim. Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of degrading almost all extracellular matrix and basement membrane components in many destructive pathological processes, such as chronic inflammation and bone-destructive lesions. The aim of this study was to determinate the correlation between concentration of collagenases (MMP-1, -8, -13) in chronic periapical lesions and their dimension calculated with software predilection through X-ray. **Metods.** Chronic periapical tissues were collected by periapical surgery from 60 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lesions, 10 cysts). Ten normal pulps used as controls were obtained by extirpation of the pulp of impacted third molars after their surgery. For rapid analysis of MMP-1, -8, -13 collagenase activities in the examined material Chemicon Collagenase Activity Assay Kit were used. From the X-ray trough software predilection (Image Tool3 Program) of the volume of chronic periapical tissue, correlation between concentration of MMPs in the periapical lesions and their dimension was confirmed. **Results.** Different concentrations of collagenases (MMP-1, -8 and -13) in chronic periapical process from different inflammation types showed different activity of MMPs. The obtained results showed the highest values of collagenases concentration (MMP-1, -8, -13) in chronic diffuse lesions (5.39 ng/ml). Low values of concentration of MMPs accompanied less serious lesions, whereas chronical periapical lesions of large dimension had high concentration of MMPs, which was proportional to progression of the lesion and destruction of bone tissue. **Conclusions.** This study confirmed the destructive role of collagenases (MMP-1, -8 and -13) in inflammation process, which directly depends on the concentration of MMPs in pathologically changed tissue.

Key words:
periapical diseases; matrix metalloproteinases; collagen.

Apstrakt

Uvod/Cilj. Matriks metaloproteinaze (MMPs) su proteolitički enzimi koji razgrađuju većinu komponenata ekstraćelijskog matriksa i bazalne membrane u mnogim destruktivnim patološkim procesima, kao što su hronično zapaljenje i destruktivne lezije kostiju. Cilj rada bio je određivanje korelacije između koncentracije kolagenaza (MMP-1, -8, -13) u hroničnim periapeksnim lezijama i njihovih dimenzija izračunatih na osnovu softverske predlekcije rendgenskog snimka. **Metode.** Hronično periapeksno tkivo sakupljeno je oralnohirurškim intervencijama na 60 zuba sa klinički i radiografski verifikovanim raznorodnim hroničnim periapeksnim lezijama (20 granuloma, 20 difuznih periapeksnih lezija, 10 cista). Deset normalnih pulpi korišćeno je kao kontrolna grupa koja je dobijena ekstirpacijom pulpe trećeg molara nakon hirurške intervencije. Za brzu analizu aktivnosti MMP-1, -8, -13 kolagenaza u ispitivanom materijalu korišćen je *Chemicon Collagenase Activity Assay Kit*. Snimanjem X-zracima, sa prethodno utvrđenim parametrima (*Image Tool3* program) zapremine hroničnog periapeksnog tkiva, potvrđena je korelacija između koncentracije MMPs u periapeksnim lezijama i njihovih dimenzija. **Rezultati.** Kolagenaze MMP-1, -8, -13 dobijene iz različitih tipova zapaljenja u hroničnim periapeksnim procesima pokazale su različitu aktivnost. Najviše kolagenaza MMP-1, -8, -13 ustanovljene su u hroničnim difuznim lezijama (5,39 ng/ml). Niske koncentracije MMPs bile su udružene sa malim lezijama, dok su hronične periapeksne lezije velikih dimenzija imale visoke koncentracije MMPs, proporcionalne sa proširenošću lezija i stepenom destrukcije koštanog tkiva. **Zaključak.** Kolagenaze MMP-1, -8 i -13 u zapaljenskom procesu imaju destruktivnu ulogu koja direktno zavisi od njihove koncentracije u patološki promenjenom tkivu.

Ključne reči:
zub, periapeksne bolesti; matriks metaloproteinaze; kolagen.

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Introduction

Matrix metalloproteinases (MMPs) are a family of host-derived enzymes responsible for degradation of most extracellular matrix (ECM) proteins during organogenesis, growth and normal tissue turnover, wound healing, tooth morphogenesis and tooth eruption [1]. Expression and activity of MMPs in adult tissues are normally quite low, but increase significantly in many destructive pathological processes, such as chronic inflammation and bone-destructive lesions [2].

This group of 23 human enzymes is classified into collagenases, gelatinases, stromelysins, membrane-type MMPs and other MMPs, mainly based on the substrate specificity and molecular structure [3].

Matrix metalloproteinases activity is controlled by changes in the delicate balance between the expression and synthesis of MMPs and their major endogenous inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) [4].

Based on structure and substrate specificity, MMPs are divided into five subgroups: collagenases, gelatinases/type IV collagenases, stromelysins (including matrilysin and metalloelastase), membrane-type MMPs, and others [5].

Collagenase-1 (MMP-1), collagenase-2 (MMP-8) and collagenase-3 (MMP-13) comprise a collagenase subfamily capable of initiating degradation of native fibrillar collagen types I, II, III, V and IX [6].

Collagenase-1 most effectively cleaves collagen type III. MMP-1 appears to be constitutively synthesized and secreted by fibroblasts and macrophages, and it is the most often associated collagenase with normal tissue remodeling. MMP-1 is currently shown to be produced by a variety of other cells such as osteoblasts and odontoclasts [7].

Collagenase-8 (MMP-8) is the most effective collagenase in initiating type I collagen degradation. Its main cellular source is polymorphonuclear leukocytes (PMNs), and the enzyme thus plays a key role in tissue destruction during inflammatory diseases [8].

Collagenase-13 (MMP-13) expression was originally documented in human breast cancer, and MMP-13 prefers type II collagen [9]. In normal physiology, MMP-13 is highly expressed in developing bone and cartilage [10]. Moreover, MMP-13 is expressed during many pathological conditions associated with excessive degradation of the extracellular matrix (ECM), such as osteoarthritic cartilage, oral mucosal epithelium during chronic inflammation and odontogenic keratocysts [11–13].

In normal conditions, the degradation and synthesis of ECM components is in balance, so that collagenases are expressed at very low levels, if at all, but their production and activation is rapidly induced whenever active tissue remodeling is required.

With respect to other literature findings that underline the role of collagenases (MMP-1, -8, -13) in chronic periapical process, the aims of this study were: quantitative measurements of tissue levels of collagenases (MMP-1, -8, -13) in chronic periapical process with enzyme method; determination of the dependence between collagenases (MMP-1, -8, -13) with the degree of tissue destruction of examination material (periapical tissue), as well as, character and differences between periapical lesions; and determination of the correlation between concentration of collagenases (MMP-1, -8, 13) in chronic periapical process and their dimension calculated with software predilection through X-ray.

Methods

This study included 50 patients, both male and female, investigated in the School for Dental Medicine (Clinic for Oral Surgery) in Skopje. Laboratory analyses were done in the School of Natural Sciences and Mathematics (Institute of biology) in Skopje.

On the basis of anamneses data, clinical intraoral and extraoral inspection and after detailed analysis of X-rays, diagnosis and indications for realizing oral surgery intervention was set up.

In each patient with detailed anamneses and extensive clinical investigation, the presence of subjective symptoms (pain, perusable sensibility) and objective symptoms (swelling, eventual exudation from the root canal and existing of fistula) were registered.

Using X-rays, condition of the periapical tissue was estimated in order to confirm bone resorption, the absence of lamina dura and existence of chronic periapical lesion. From X-ray to software predilection (Image Tool3 program) of the volume of chronic periapical tissue, correlation between concentration of MMPs in the periapical lesions and their dimension was confirmed.

Examination material was collected on the basis of clinical diagnosis after completely realized anamneses and clinical investigation with the analysis of radiological changes.

Chronic periapical tissues were collected in periapical surgery from 60 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lessions, 10 cysts). Ten health pulps used as controls were obtained by extirpation of it from impacted third molars after their surgery. The examination material was frozen at –80 ºC as soon as possible and stored till analysis, but not longer than six months.

For quantitative analysis of MMP-1, -8, -13 collagenase activities in all of the examination material (chronic periapical tissues and health pulp tissue) Chemicon Collagenase Activity Assay Kit (ECM710) was used.

Chemicon Collagenase Activity Assay Kit was designed so as to achieve quick, convenient and sensitive evaluation of MMP-1, -8 and -13 collagenase activities in a 96-well microplate format. Biotinylated, native triple helical type I collagen was used as a substrate and was cleaved from the activated MMP-1, -8, -13 enzymes.

Each sample was macerated in phosphate-buffered saline – PBS (1.5 ml) and then homogenized in Eppendorf-Centrifuge, 10.000 g for 10 min. The supernatant was used for the analysis. In the homogenized mixture with Bradford micromethod using series of five standards of bovine-serum albumin and than measuring the absorbance on 450 nm with spectrophotometer, concentration of total proteins was de-

termined. With interpolation from the standard curve, concentration of the proteins in samples was measured.

A microplate reader (Anthos ht III) was used to measure the absorbance at 450 nm. Values of the absorbance of each standard were corrected according to protein concentration.

By adding 100 μl of stop solution to each well, the bright yellow convert to bright blue colored product and the enzyme reaction was stoped.

The MMPs concentration of each sample was normalized versus concentration of proteins in each sample. Standard curve was designed with software program Curve Expert 1.3. With interpolation of the values, MMP-1, -8, -13 collagenase concentrations were calculated.

Comparison of the values to determine the significant difference between the specimens of the examination material was performed using descriptive and analytical statistical methods from program Stat Soft Statistic 6.0.

Results

Different concentrations of collagenases MMP-1, -8 and -13 in chronic periapical lesions from different inflammation types showed different activities of MMPs. The obtained results showed the highest values of the concentration of collagenases (MMP-1, -8, -13) in chronic diffuse lesions (5.39 ng/ml) (Table 1).

Concentration of MMPs in all of the samples was < 0.09 ng/ml (Figure 1).

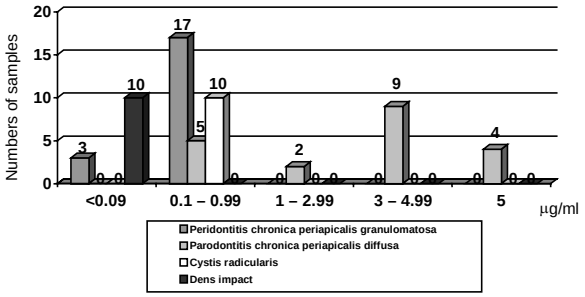

Fig. 1 – Concentration of matrix metalloproteinases in the samples of examinated material

Small values of concentrations of MMPs accompanied smaller lesions, whereas chronical periapical lesions of large dimension had higher concentration of MMPs, which was proportional to the progression of the lesion and destruction of bone tissue (Table 2).

There was a strong linear connection between the level of the lesions and concentration of MMPs in the patients with the diagnosis of *Parodontitis periapicalis chronica granulomatosa* ($R^2 = 0.868$, ANOVA F = 118.702), which means that 86% from the variable dates for the concentration of MMPs was due to variable data for the level of the lesion and the opposite (Figure 2).

Table 1

Concentration of matrix metalloproteinases (ng/ml) in chronic periapical lesions and normal pulp tissue

Clinical diagnosis	n	min	max	median	$\bar{x}$ ±SD
Parodontitis periapicalis chronica granulomatosa	20	0.05	0.95	0.44	0.46 ± 0.29
Parodontitis periapicalis chronica diffusa	20	1.15	5.39	4.12	3.63 ± 1.46
Cystis radicularis	10	0.10	0.64	0.19	0.25 ± 0.16
Dens impacta	10	0.00	0.02	0.01	0.01 ± 0.009

Table 2

Concentration of metalloproteinases (MMPs) in examination material and radiografic values of chronic periapical lesions

MMP (ng/ml)	Radiografic values (mm^2)						Total
	< 0.299	0.3–0.499	0.5–0.699	0.7–0.899	0.9–1.999	> 2	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
< 0.09	1 (33.3)	2 (66.7)	/	/	/	/	3 (6)
0.1–0.99	8 (25)	12 (37.5)	1 (3.1)	3 (9.4)	7 (21.9)	1 (3.1)	32 (64)
1–2.99	/	/	1 (50)	/	/	1 (50)	2 (4)
3–4.99	/	/	/	5 (55.6)	2 (22.2)	2 (22.2)	9 (18)
> 5	/	/	2 (50)	/	/	2 (50)	4 (8)
Total	9 (18)	14 (28)	4 (8)	8 (16)	9 (18)	4 (8)	50 (100)

In the samples of the patients with chronic periapical granuloma, the values of collagenases (MMP-1, -8, -13) concentration in most cases (85%, 17 specimens) were 0.1–0.99 ng/ml. Collagenases concentration in the diffuse periapical lesions was 3–4.99 ng/ml (nine samples, 45%). Concentration of the MMPs in all 10 samples (100%) with clinical diagnosis *cystis radicularis* was 0.1–0.99 ng/ml. In the control group, the smallest concentrations of MMPs were registered.

There was a strong linear correlation between the level of the lesions and concentration of MMPs in the patients with the diagnosis of *parodontitis periapicalis chronica diffusa* ($R^2 = 0{,}922$, ANOVA F = 211.414), which means that 92% from the variable dates for the concentration of MMPs was due to variable data for the level of the lesion and *vice versa* (Figure 3).

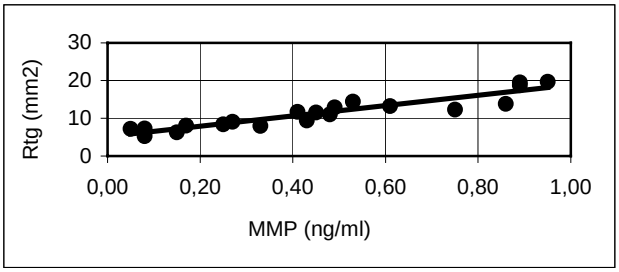

Fig. 2 –Correlation of the lesion and concentration of matrix metalloproteinases in *parodontitis chronica periapicalis granulomatosa*

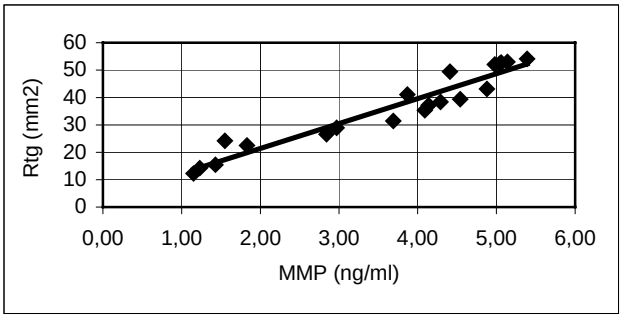

Fig. 3 – Correlation of lesion size and concentration of matrix metalloproteinases (MMPs) in *parodontitis chronica periapicalis diffusa*

The correlation line of dependence between the level of the lesion and concentration of matrix metalloproteinases in the patients with the diagnosis of *Cystis radicularis* had approximately equal values of the level of lesions, independently from the concentration of MMPs ($R^2 = 0.259$, ANOVA F = 4.144; Figure 4).

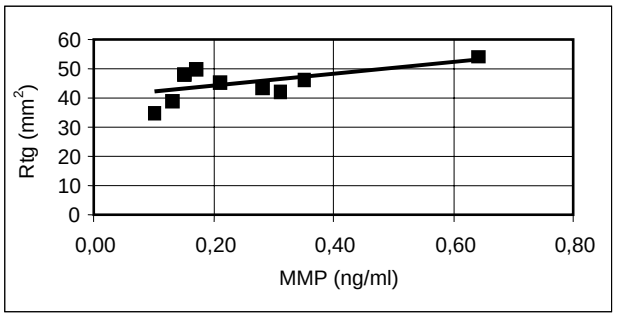

Fig. 4 – Correlation of the lesion size and concentration of matrix metalloproteinases in *cystis radicularis*

Discussion

Matrix metalloproteinases form a family of structurally related, but geneticly different endopeptidases which expression and activity in normal tissue are very low instead of significant increasing during pathological conditions that leads to unfavorable tissue destruction [14].

Degradation and synthesis of ECM components in normal, health tissues are in constant balance, so to maintain this concision, collagenases are expressed at very low levels and the enzyme activity is exactly controlled [15].

Our results confirmed the presence of small concentration of collagenases (MMP-1, -8, -13) in pulp tissues of impacted third molars. The measured concentration of MMPs in health pulp tissues of impacted third molar varied from the minimal value of 0.00 ng/ml to the maximal of 0.02 ng/ml.

Vu and Werb [16] showed the participation of MMPs in tissue remodeling and many other cell functions. According to these authors, MMPs adapt cellular behavior, for example, by inducing cell migration in normal growth and tissue remodeling, such as wound healing and angiogenesis.

The results from quantitative enzyme method in our study demonstrated that the concentrations of collagenases (MMP-1, -8, -13) in chronical periapical lesions were significantly higher than those of the control group ($p < 0.05$). Our results also confirmed the high significant difference ($p < 0.05$) among chronic periapical processes with different clinical diagnosis, which showed different activity of collagenases (MMP-1, -8, -13).

Results in the study of Tjäderhane et al. [17] have demonstrated that MMPs have a role in periapical lesion formation, because MMP inhibition significantly increase the lesion level. Accordingly, MMPs may be involved in defensive reactions against microbes in dental pulp or periapical area. These authors confirmed that the increase of the lesion level might be due to more rapidly advanced pulp infection.

Statistical analysis made with Pearson Chi-Square test showed that there was a high statistical significance between concentration of MMPs and radiographic values of chronic periapical lesions ($\chi^2 = 49.496$; df = 20; $p = 0.000$, means $p < 0.001$).

In the study of Leonardi et al. [18] expression pattern of MMP-13 demonstrated that it was involved in the conversion of periapical granuloma with epithelium into radicular cyst. According to these authors, this property was related to the ability of MMP-13 to influence not only the migration of epithelial cell but also the invasion of granulomatous tissue.

Wahlgren et al. [19] revealed with immunohistochemical analysis and *in situ* hybridization that plasma cells expressed MMP-8 and MMP-13 focally in periapical granulomas, odontogenic cysts and malignant plasmacytomas. These authors confirmed that MMP-8 and MMP-13 from plasma cells could participate in bone organic matrix destruction at sites of chronic inflammation and neoplastic growth. They also demonstrate that MMP-13 was more frequently expressed than MMP-8 in plasma cells of strongly recurring keratocysts and malignant plasmacytomas.

According to Jnakowska-Antczak et al. [20], metalloproteinases can be one of important factors decided about kinetics of periapical bone destruction and may act on periapical bone regeneration after apitectomy and radiectomy.

Results of the study of Wahlgren et al. [2] indicate that MMP analysis from periapical exudates could be used to indicate and monitor inflammatory activity and the success of treatment in teeth with periapical lesions.

Conclusion

According to the obtained results we can conclude that MMP-1, -8, -13 actively participate in tissue destruction and granulation tissue formation in chronic periapical lesions. This study opens a new opportunity for chronic periapical lesions diagnostics and monitoring of inflamed tissue condition, based on destructive role of collagenases (MMP-1, -8, 13) in inflammation process, which is directly dependent on thair concentration in pathologicly changed tissue.

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