doi:10.1111/j.1365-2591.2007.01223.x

The effect of chemical inhibition of matrix metalloproteinases on the size of experimentally induced apical periodontitis

L. Tjäderhane[1,2], T. Hotakainen[3], S. Kinnunen[3], M. Ahonen[3] & T. Salo[3,4]

[1]Institute of Dentistry, University of Helsinki, Helsinki, Finland; [2]Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital (HUCH), Helsinki, Finland; [3]Institute of Dentistry, University of Oulu, Oulu, Finland; and [4]Oulu University Hospital, Oulu, Finland

Abstract

L. Tjäderhane, T. Hotakainen, S. Kinnunen, M. Ahonen, T. Salo. The effect of chemical inhibition of matrix metalloproteinases on the size of experimentally induced apical periodontitis. *International Endodontic Journal*, **40**, 282–289, 2007.

Aim To determine the effect of matrix metalloproteinase (MMP) inhibition on periapical lesion formation in a rat model.

Methodology The pulp chambers of mandibular fist molars of adult SD rats were exposed to be infected by oral microbes. The experimental group was fed 20 mg kg^{-1} MMP-inhibitor chemically modified tetracycline-3 (CMT-3) daily in an oral gavage and the controls were fed the vehicle. After 2 and 4 weeks, the mandibles ($n = 10$ in both groups at both times) were radiographed, decalcified and subjected to histological analysis. Extension of necrosis in first molar distal root canals was measured from the histological sections, and periapical lesion sizes in the same roots were determined from radiographs and histological sections. Mann–Whitney *U*-test was used for the statistical analysis.

Results There was a statistically significant difference in the extension of necrosis in root canals between 2 and 4 weeks in the control group ($P < 0.05$), but not with MMP inhibition. Radiographically, MMP inhibition increased the periapical lesion size by 70% and 34% after 2 and 4 weeks respectively ($P < 0.05$ in after 2 weeks). In histological measurements, lesion size increased with MMP inhibition by 26% and 8% after 2 and 4 weeks respectively.

Conclusions MMP inhibition affects pulpal and periapical inflammation, increasing the rate of spreading of necrosis in root canals and the rate of periapical lesion formation.

Keywords: inflammation, *in vivo*, MMP inhibition, pulp, rodent, tooth.

Received 7 August 2006; accepted 9 October 2006

Introduction

Matrix metalloproteinases (MMPs) are a family of endopeptidases, which depend on zinc for their activity and the degradation of extracellular matrix proteins (Woessner & Nagase 2000). MMPs are regarded as one of the key factors in the uncontrolled tissue destruction observed in several inflammatory diseases, e.g. periodontitis (Ingman *et al.* 1996, Chen *et al.* 2000). Macrophages and polymorphonuclear leukocytes (PMN-cells) secrete several MMPs, including collagenases MMP-8 and -13 and gelatinases MMP-2 and -9. Also other inflammatory cells express several MMPs (Shin *et al.* 2002, Wahlgren *et al.* 2002).

Matrix metalloproteinase-13 is synthesized by pulp fibroblasts in abundance, but its level does not seem to be altered in moderately carious teeth (Sulkala *et al.* 2004). In advanced pulpitis, inflammatory cells may be responsible for most of the MMPs observed in the pulp tissue. MMP-1, -3 and -8 have been localized in the infiltrating neutrophils, macrophages, plasma cells and

Correspondence: Dr Leo Tjäderhane, Institute of Dentistry, University of Helsinki, PO Box 41, 00014 Helsinki, Finland (Tel.: +358-9-191-27261; fax: +358-9-191-27286; e-mail: leo.tjaderhane@helsinki.fi).

lymphocytes of acute pulpitis (Shin *et al.* 2002, Wahlgren *et al.* 2002), demonstrating the multicellular sources of MMPs in endodontic infections. However, Gusman *et al.* (2003) demonstrated increased MMP-9 levels in inflamed pulp, with no apparent changes in the levels of MMP-2 or -3, indicating the importance of MMP-9 in the pulp tissue breakdown. The samples collected from teeth during root canal treatment show abundant amounts of MMP-8 in inflamed and necrotic human pulp and periapical lesions, with subsequent decrease after a successful root canal cleaning, shaping and $Ca(OH)_2$ medication (Wahlgren *et al.* 2002). MMPs are also present in jaw cysts (Teronen *et al.* 1995a,b, Lin *et al.* 1997).

Several studies have shown that MMPs, especially excessive levels of MMP-8 and -9 originating from PMN leukocytes, are strongly associated with the progression of periodontitis (Sorsa *et al.* 2006). Moreover, MMP inhibition has been shown to reduce periodontal tissue destruction both experimentally and as an adjunctive treatment in patients. Markedly, lower MMP activity together with dramatically reduced marginal bone loss has been demonstrated with different MMP inhibitors in periodontitis induced with local lipopolysaccharide injections (Llavaneras *et al.* 1999, 2001, Ramamurthy *et al.* 2002a,b). In humans, low-dose doxycycline (LDD: MMP-inhibitor with no antibacterial efficacy) as an adjunct therapy to scaling and root planning or surgical therapy has repeatedly been shown to reduce MMP levels and attachment loss in periodontitis (Ashley 1999, Golub *et al.* 2001, Novak *et al.* 2002, Choi *et al.* 2004, Emingil *et al.* 2004a,b, Gapski *et al.* 2004, Lee *et al.* 2004, Gurkan *et al.* 2005).

The aim of this study was to examine the role of MMPs on periapical lesion formation. Based on the studies regarding the effect of MMP inhibition in periodontitis, a hypothesis was set that MMP inhibition would decrease the rate and size of periapical lesion formation.

Materials and methods

Animals and medications

Forty adult female Spraque–Dawley (SD) rats were weighted and randomly divided in two groups of 20 animals. From day 0 throughout the experiment, the first group (experimental group) received the MMP inhibitor chemically modified nonantimicrobial tetracycline-3 (CMT-3; Collagenex Pharmaceuticals, Inc., Newtown, PA, USA), suspended into NaCl, 5 days $week^{-1}$ 20 mg kg^{-1} body weight as oral gavage. With this dose, CMT-3 has previously been shown to inhibit caries progression in rat molars by MMP inhibition (Sulkala *et al.* 2001), and a respective CMT-1 dose has been shown to reduce periodontal bone loss in *Porphyromonas gingivalis*-infected rats (Ramamurthy *et al.* 1998). The second group received NaCl (the same consistency and pH as CMT-3 suspension) alone and served as a control group. The animals were weighted weekly to maintain the correct dosage.

Perapical lesion induction

On day one, animals were anaesthetized with i.p. injection of Ketamine HCl (10 mg kg^{-1}; Pfizer, New York, NY, USA) and Xylazine (5 mg kg^{-1}; Bimeda-MTC, Cambridge, ON, Canada), and pulpal exposure was performed on mandibular first molar crowns with dental burs (Komet ISO 806 104 (165524 014[858]; Komet Group, Besigheim, Germany) in dental handpiece. Exposed teeth were left open to ensure microbial infection of the pulp. Temgesic (0.04 mg; Schering-Plough, Pointe Claire, QC, Canada) intracutaneously was used to reduce pain for 2 days postoperatively.

Sample preparation

Two and four weeks after the pulp exposure, 10 animals from both groups were killed, the mandibles were dissected, defleshed and fixed in 4% paraformaldehyde for 48 h. The jaws were radiographed with Kodak Ultra Speed D intraoral film, using 60-kV and 0.12-s exposure (Lin *et al.* 2000), developed and scanned to computer using Canon slide Scanner (4000×4000 pix) (CanonScan FS4000US, Tokyo, Japan). Scanned images were analysed using Scion Image Beta 4.02 for Windows (Scion Corporation, Frederick, MD, USA), and periradicular lesions area (μm^2) from first molar distal root were measured.

After radiography, mandibles were demineralized in 10% ethylenediaminetetraacetic acid (EDTA) at room temperature for 2 weeks. Jaws including all molars were embedded in paraffin, and 6 μm thick sections were prepared. Sections were stained in Weigert Iron haematoxylin, Alcian Blue and Van Gieson (WAV staining) using a standard WAV staining protocol.

Sections in which both necrotic and vital tissue could be seen in the first molar distal root canals were selected for the measurements of the extension of necrosis in the root canals. The measurements were performed with Leica DM RB/E microscope equipped with Leica DFC 480 CCD camera (Leica, Heerbrugg,

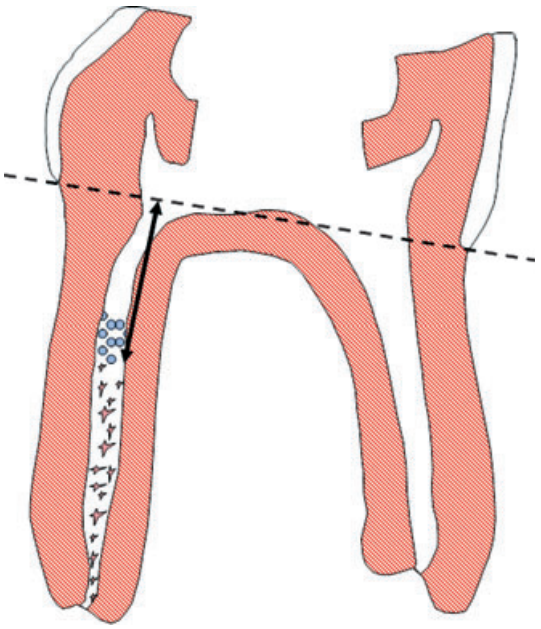

Figure 1 An illustration describing the technique to measure the extend of necrosis in root canals in the histological sections. A reference line was drawn between the first molar mesial and distal cemento–enamel junction points (dashed line), and a perpendicular line from the reference line was drawn to the borderline between accumulated inflammatory cells (blue dots) and vital tissue (star-shaped fibroblasts) in distal root canal. Estimation of the vitality of the root canal tissue was based on the observation of vital cells and blood vessels (see also Fig. 2a).

Switzerland), using Leica QWin software (Leica Micrsosystems Digital Imaging, Cambridge, UK) for image analysis. The reference line was drawn between the mesial and distal cementum–enamel junction (CEJ) (Fig. 1), and the distance (μm) between this line and the border between necrotic and vital tissue was measured. The limit between necrotic and vital tissue was determined by the extensive presence of inflammatory cells and the presence/absence of histologically vital cells in tissue (Figs 1 and 2a–c) in the root canals, using 25×, 50× and 100× magnification according to the need. Periradicular lesion areas (μm^2) were measured from histological section, using Nikon Optishot 2 (Zeiss 2.5/0.08 ocular, Carl Zeiss microImaging, Inc., Thornwood, NY, USA), Sony DXC-930P-3CCD Color Video Camera (Sony Corporation of America, San Jose, CA, USA) and MCID-M4 3.0 rev 1.1 software (Imaging Research Inc., St Catharines, ON, Canada). Only the sections in which the whole root canal including apical foramen could be seen were accepted for measurement to ensure that the section would represent the largest periapical lesion area ($n = 24$ in CMT-3 group, and $n = 18$ in the control group).

The statistical analyses were performed using SPSS version 12.0.1 (SPSS Inc., Chicago, IL, USA). Student *t*-test was used to compare the weight gains between the groups. Mann–Whitney *U*-test was used to determine the differences in lesion sizes between the groups. Nonparametric method was used, as the data did not meet the assumption of normal distribution.

Results

A summary of the data is provided in Table 1.

Body weight

In both groups, the body weight increased during the experimental period. The weight gains were 17.6% and 14.1% of the initial weight in the control and CMT-3 groups respectively. There were no statistically significant differences between the groups (Table 1).

Extension of root canal necrosis

Both control and CMT-3 groups had necrosis extending more apically after 4 weeks compared with

Table 1 Summary of the data (mean + SE) because of variation in samples available for the analysis in extension of necrosis in root canals, lesions measured from the radiographs and lesions measured from the histological sections, number of samples analysed are given in parenthesis

Group	Duration of experiment (weeks)	Initial weight (g)	Final weight (g)	Extension of necrosis in root canals (μm)	Radiographic lesion (μm^2)	Histological lesion (μm^2)
Control	2	202.9 ± 1.7	221.9 ± 3.4	461.2 ± 101.5 (19)	227 ± 37 (20)	616 ± 73 (8)
	4	202.5 ± 3.4	242.7 ± 5.0	1096.2 ± 205.8 (18)	309 ± 53 (20)	913 ± 246 (7)
CMT-3	2	205.4 ± 3.6	227.9 ± 4.0	535.7 ± 150.6 (20)	387 ± 33 (19)	831 ± 162 (11)
	4	197.1 ± 5.3	228.7 ± 6.9	981.8 ± 190.6 (19)	414 ± 48 (19)	988 ± 211 (8)

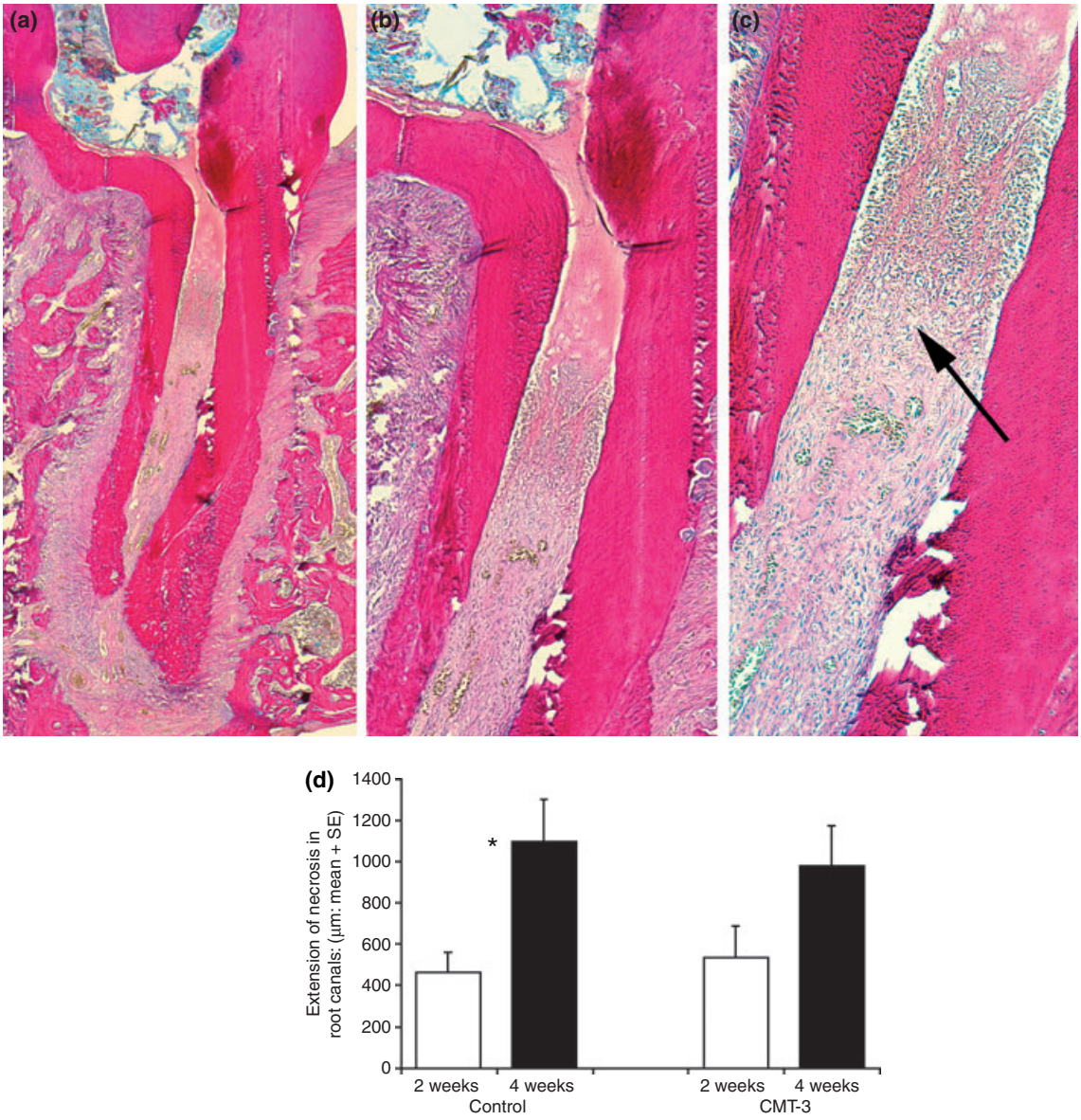

Figure 2 The extension of necrosis in root canals. (a–c) An example of first molar distal root canal 2 weeks after the exposure. The pulp chamber and coronal part of the root canal demonstrate complete necrotic destruction of tissue (a,b). Below necrotic tissue in root canal, immense and intensive accumulation of inflammatory cells can be observed (b,c); the tissue in this area appears necrotic. Immediately below accumulated inflammatory cells, first vital pulp tissue fibroblasts and blood vessels can be seen (c). The borderline between necrotic and vital tissue is indicated with the arrow. Magnifications: (a) 25×; (b) 50×; (c) 100×. (d) The extension of necrotic tissue in first molar distal root canals. In the control group, statistically significant difference was observed between 2- and 4-week measurements (*$P < 0.05$, Mann–Whitney *U*-test).

2 weeks (Table 1, Fig. 2a–c). There was a statistically significant difference between the extension of necrosis after 2 and 4 weeks in the control group ($P < 0.05$, Mann–Whitney *U*-test), but not in the CMT-3 group (Fig. 2d).

Periapical lesion size in radiographs

Matrix metalloproteinase inhibition resulted in larger periapical lesions (Table 1, Fig. 3a), as measured from the radiographs (Fig. 3b). The increase in the lesion

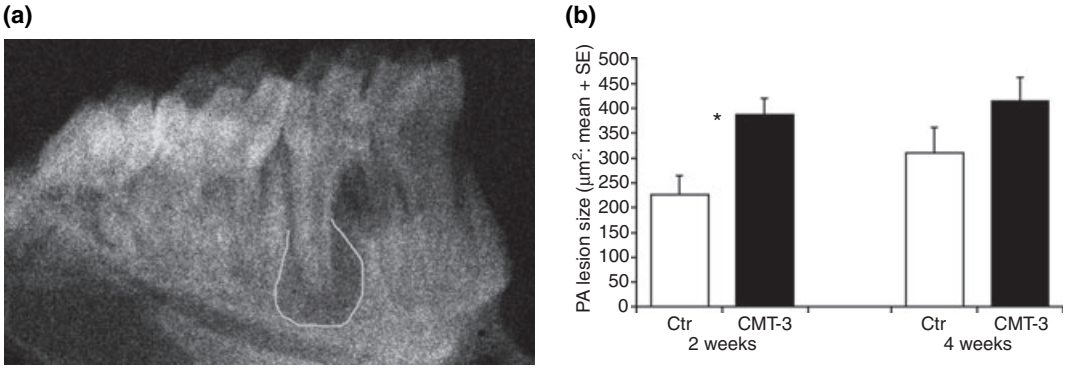

Figure 3 (a) A radiograph of a mandible of a rat after receiving CMT-3 for 2 weeks, with a large periapical lesion in first molar distal root (line). (b) With CMT-3, significantly larger lesions were observed in first molar distal root after 2 weeks (statistically significant difference to respective control; *$P < 0.05$, Mann–Whitney *U*-test). A smaller difference ($P > 0.05$) was observed after 4 weeks.

size in the CMT-3 group compared with controls was 70% and 34% after 2 and 4 weeks respectively. The difference was statistically significant ($P < 0.05$, Mann–Whitney *U*-test) after 2 weeks (Fig. 3b).

Periapical lesion size in histological sections

In the histological stainings (Table 1, Fig. 4a) the lesion areas were generally larger than in the radiological measurements. Basically, the histological staining

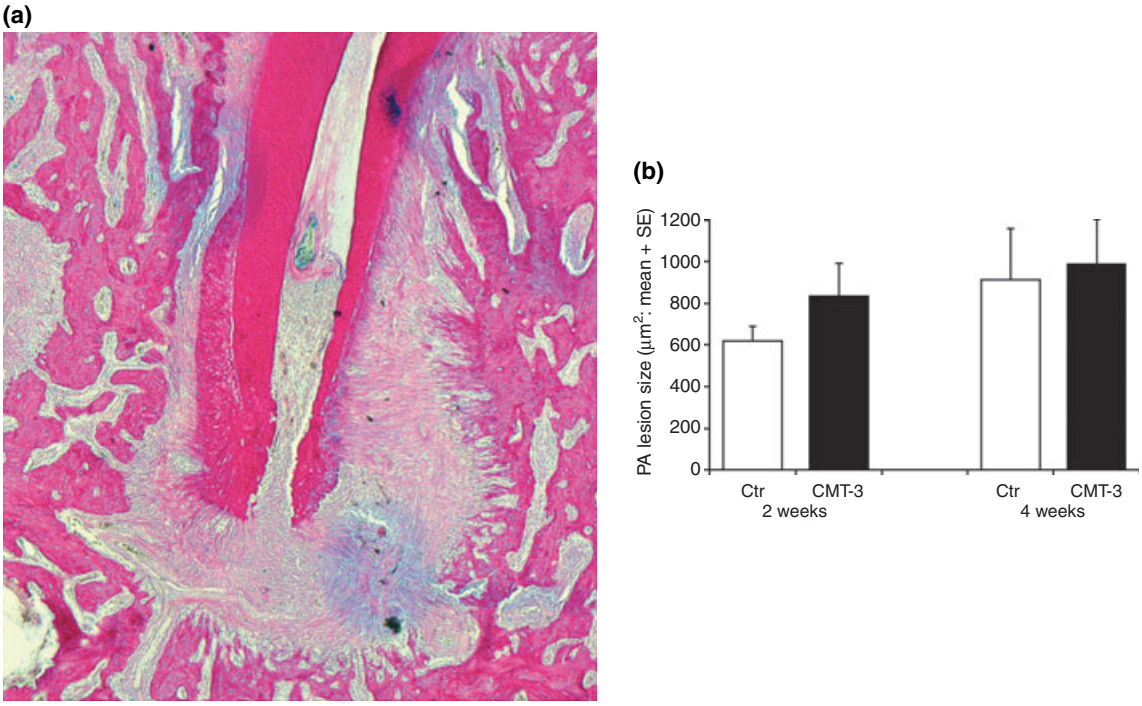

Figure 4 (a) A photomicrograph of representative histological section stained with Weigart Iron haematoxylin, Alcian Blue and Van Giesen (WAV). Periapical lesion 2 weeks after pulp exposure in first molar distal root, demonstrating marked bone resorption and clearly defined periapical lesion. (b) Periapical lesion sizes in histological measurements 2 and 4 weeks after pulp exposure, being in essence in line with radiographic findings.

confirmed the larger periapical lesions with MMP inhibition. In the CMT-3 group, the periapical lesions were 26% and 8% larger than in the control group after 2 and 4 weeks respectively (Fig. 4). However, because of the strict criteria for selection of samples to the analysis, the number of samples was markedly lower than with the radiographic analysis. Because of the small number of the samples analysed, statistically significant differences were not reached.

Discussion

Matrix metalloproteinases have widely been accepted to act as tissue-destructive enzymes in inflammatory conditions, including marginal periodontitis. Surprisingly, this study demonstrates that in periapical inflammation, MMP inhibition increases the lesion growth rate, indicating that MMPs may have previously unknown anti-infective and/or anti-inflammatory properties.

In this study the rodent model, originally described in the classical study by Kakehashi *et al.* (1965), was used to find out the MMP inhibition effect in pathogenesis of apical periodontitis. This is the most widely used model to examine the pathogenesis of periapical lesions; for example, it has been used to examine the effects of immunosuppression on the development of periapical lesions in rats (Stashenko *et al.* 1995, Stashenko 2002). Instead of calculating the percentage of necrotic tissue in root canals of the total length of the canal, the extension of necrosis was measured using the CEJ as a reference point, because Balto *et al.* (2002) reported rapid and extensive apical root resorption occurrence in the rodent pulp exposure model. Apical root resorption was frequently, even if not consistently, observed also in this study (see Fig. 4a as an example).

The control group was able to limit the spreading of pulp necrosis better than the MMP-inhibited group, as there was a statistically significant difference between the 2 and 4 week time points in the control, but not in the CMT-3 group. In agreement, MMP inhibition also accelerated the growth of periapical lesion. To be visible in radiograph, a lesion has to cause 30–60% cortical bone loss (Razmus 1994, Wood *et al.* 1997). After 2 weeks, the lesions were radiographically significantly greater in size in the MMP inhibition group, but after 4 weeks the differences evened out, indicating markedly faster onset and progression of periapical lesion with MMP inhibition.

The findings provide functional confirmation to the previous descriptive data indicating that MMPs have a role in the pulpal (Shin *et al.* 2002, Wahlgren *et al.* 2002, Gusman *et al.* 2003, Tsai *et al.* 2005) and periapical (Teronen *et al.* 1995a,b, Lin *et al.* 1997, Wahlgren *et al.* 2002) inflammatory processes. Formation of periapical lesions is an inflammatory defensive reaction, aiming to prevent spreading of infection into bone. Therefore, the increased lesion size and accelerated lesion growth indicates that MMPs may possess anti-infective and/or anti-inflammatory properties in pulpal or periapical pathosis. There are two possible explanations for the findings. The accelerated lesion growth may be because of more rapidly advanced pulpal infection, as indicated by the absence of difference in the root canal necrosis after 2 and 4 weeks in CMT-3 group. Alternatively, as periapical lesion is a defensive barrier to avoid microbial invasion into the periapical bone (osteomyelitis), increase in lesion size may reflect the defensive compensation for the lack of MMP action. Previous studies have indicated anti-inflammatory properties for at least MMP-8 (collagenase-2) and MMP-9 (gelatinase B). While low-dose lung alveolar antigen exposure decreases mononuclear cell and lymphocyte accumulation in MMP-9 knock-out mice (Cataldo *et al.* 2002), higher antigen dosage induces increased inflammatory cell accumulation and proinflammatory cytokine and chemokine expression in MMP-9 knock-out mice in acute pulmonary inflammation model (McMillan *et al.* 2004). Comparable effect has been observed in MMP-8 knock-out mice after intratracheal LPS-induced inflammation (Owen *et al.* 2004) or albumin-induced asthmatic inflammation (Gueders *et al.* 2005), both demonstrating significant PMN accumulation. PMN accumulation may be caused by either increased PMN homing or, at least partially, decreased inflammatory cell apoptosis in MMP-8 knock-out mice.

Whatever is the mechanism behind the increased inflammatory response in pulpal and periapical tissues, the findings of this study are parallel with previous pulmonary tissue studies (McMillan *et al.* 2004, Owen *et al.* 2004, Gueders *et al.* 2005). To the best of our knowledge, the anti-inflammatory effect of MMPs has not previously been shown to occur in polymicrobial infection. Elimination of MMP anti-infective properties could accelerate the spreading of infection in the pulp chamber and the formation and growth of periapical lesion as a response to more rapidly advancing infection/inflammation in the root canals. As MMPs have traditionally been thought to be destructive enzymes in inflammatory diseases, these new potential modes of effect may open completely new insight into the

functional role of MMPs. Whether the anti-inflammatory/anti-infective effect is site or tissue specific or more general, and whether the effect is related to single MMP or several members of MMP-family, remains to be studied.

Conclusions

Matrix metalloproteinase inhibition increased the rate of pulpal necrosis and lesion formation in experimentally induced apical periodontitis. Together with previous studies with rat pulmonary tissue model, this finding indicates that MMPs may actually have either anti-inflammatory or anti-infective properties.

Acknowledgements

The study was financially supported by European Society of Endodontology (ESE) grant to Leo Tjäderhane. The financial support from the Academy of Finland (grant no. 104337 and no. 111724) and the Finnish Dental Society Apollonia are also acknowledged. We thank Ms Päivi Moilanen and Ms Sirpa Tausta for their help treating animals, and Ms Maritta Harjapää, Ms Eeva-Maija Kiljander and Ms Sanna Juntunen for their help in histology, and Dr Jukka Rosberg for help in radiography.

References

Ashley RA (1999) Clinical trials of a matrix metalloproteinase inhibitor in human periodontal disease. SDD Clinical Research Team. *Annals of the New York Academy of Science* **30**, 335–46.

Balto K, White R, Mueller R, Stashenko P (2002) A mouse model of inflammatory root resorption induced by pulpal infection. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **93**, 461–8.

Cataldo DD, Tournoy KG, Vermaelen K et al. (2002) Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen induced airway inflammation. *American Journal of Pathology* **161**, 491–8.

Chen CS, Cox SW, Eley BM, Mäntyla P, Rönka H, Sorsa T (2000) Matrix metalloproteinase-8 levels and elastase activities in gingival crevicular fluid from chronic adult periodontitis patients. *Journal of Clinical Periodontology* **27**, 366–9.

Choi DH, Moon IS, Choi BK et al. (2004) Effects of subantimicrobial dose doxycycline therapy on crevicular fluid MMP-8, and gingival tissue MMP-9, TIMP-1 and IL-6 levels in chronic periodontitis. *Journal of Periodontal Research* **39**, 20–6.

Emingil G, Atilla G, Sorsa T, Luoto H, Kirilmaz L, Baylas H (2004a) The effect of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid matrix metalloproteinase-8 levels in chronic periodontitis. *Journal of Periodontology* **75**, 106–15.

Emingil G, Atilla G, Sorsa T, Savolainen P, Baylas H (2004b) Effectiveness of adjunctive low-dose doxycycline therapy on clinical parameters and gingival crevicular fluid laminin-5 gamma2 chain levels in chronic periodontitis. *Journal of Periodontology* **75**, 1387–96.

Gapski R, Barr JL, Sarment DP, Layher MG, Socransky SS, Giannobile WV (2004) Effect of systemic matrix metalloproteinase inhibition on periodontal wound repair: a proof of concept trial. *Journal of Periodontology* **75**, 441–52.

Golub LM, McNamara TF, Ryan ME et al. (2001) Adjunctive treatment with subantimicrobial doses of doxycycline: effects on gingival fluid collagenase activity and attachment loss in adult periodontitis. *Journal of Clinical Periodontology* **28**, 146–56.

Gueders MM, Balbin M, Rocks N et al. (2005) Matrix metalloproteinase-8 deficiency promotes granulocytic allergen-induced airway inflammation. *Journal of Immunology* **175**, 2589–97.

Gurkan A, Cinarcik S, Huseyinov A (2005) Adjunctive subantimicrobial dose doxycycline: effect on clinical parameters and gingival crevicular fluid transforming growth factor-beta levels in severe, generalized chronic periodontitis. *Journal of Clinical Periodontology* **32**, 244–53.

Gusman H, Santana RB, Zehnder M (2003) Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed dental pulps. *European Journal of Oral Science* **110**, 353–7.

Ingman T, Tervahartiala T, Ding Y et al. (1996) Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *Journal of Clinical Periodontology* **23**, 1127–32.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effect of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surgery, Oral Medicine, Oral Pathololgy* **20**, 340–9.

Lee HM, Ciancio SG, Tuter G, Ryan ME, Komaroff E, Golub LM (2004) Subantimicrobial dose doxycycline efficacy as a matrix metalloproteinase inhibitor in chronic periodontitis patients is enhanced when combined with a non-steroidal anti-inflammatory drug. *Journal of Periodontology* **75**, 453–63.

Lin SK, Chiang CP, Hong CY et al. (1997) Immunolocalization of interstitial collagenase (MMP-1) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in radicular cysts. *Journal of Oral Pathology & Medicine* **26**, 458–63.

Lin SK, Hong CY, Chang HH et al. (2000) Immunolocalization of macrophages and transforming growth factor-beta 1 in induced rat periapical lesions. *Journal of Endodontics* **26**, 335–40.

Llavaneras A, Golub LM, Rifkin BR et al. (1999) CMT-8/clodronate combination therapy synergistically inhibits alveolar bone loss in LPS-induced periodontitis. *Annals of the New York Academy of Science* **878**, 671–4.

Llavaneras A, Ramamurthy NS, Heikkilä P et al. (2001) A combination of a chemically modified doxycycline and a bisphosphonate synergistically inhibits endotoxin-induced periodontal breakdown in rats. *Journal of Periodontology* **72**, 1069–77.

McMillan SJ, Kearley J, Campbell JD et al. (2004) Matrix metalloproteinase-9 deficiency result in enhanced allergen-induced airway inflammation. *Journal of Immunology* **172**, 2586–94.

Novak MJ, Johns LP, Miller RC, Bradshaw MH (2002) Adjunctive benefits of subantimicrobial dose doxycycline in the management of severe, generalized, chronic periodontitis. *Journal of Periodontology* **73**, 762–9.

Owen CA, Hu Z, Lopez-Otin C, Shapiro SD (2004) Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *Journal of Immunology* **172**, 7791–803.

Ramamurthy NS, Schroeder KL, McNamara TF et al. (1998) Root-surface caries in rats and humans: inhibition by a non-antimicrobial property of tetracyclines. *Advances in Dental Research* **12**, 43–50.

Ramamurthy NS, Rifkin BR, Greenwald RA et al. (2002a) Inhibition of matrix metalloproteinase-mediated periodontal bone loss in rats: a comparison of 6 chemically modified tetracyclines. *Journal of Periodontology* **73**, 726–34.

Ramamurthy NS, Xu JW, Bird J et al. (2002b) Inhibition of alveolar bone loss by matrix metalloproteinase inhibitors in experimental periodontal diseases. *Journal of Periodontal Research* **37**, 1–7.

Razmus TF (1994) Caries, periodontal disease, and periapical changes. *Dental Clinics of North America* **38**, 13–31.

Shin SJ, Lee JI, Baek SH, Lim SS (2002) Tissue levels of matrix metalloproteinases in pulps and periapical lesions. *Journal of Endodontics* **28**, 313–5.

Sorsa T, Tjäderhane L, Konttinen YT et al. (2006) P. Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. *Annals of Medicine* **38**, 306–21.

Stashenko P (2002) Interrelationship of dental pulp and apical periodontitis. In: Hargreaves KM, Goodies HE, eds. *Seltzer and Bender's Dental Pulp*. Hanover Park, IL: Quintessence Publishing Co., Inc., pp. 389–409.

Stashenko P, Wang CY, Riley E, Wu Y, Ostroff G, Niederman R (1995) Reduction of infection-stimulated periapical bone resorption by the biological response modifier PGG glucan. *Journal of Dental Research* **74**, 323–30.

Sulkala M, Wahlgren J, Larmas M et al. (2001) The effect of MMP-inhibitors on human salivary MMP activity and caries progression in rats. *Journal of Dental Research* **80**, 1545–9.

Sulkala M, Pääkkönen V, Larmas M, Salo T, Tjäderhane L (2004) Matrix metalloproteinase-13 (MMP-13, collagenase-3) is highly expressed in human tooth pulp. *Connective Tissue Research* **45**, 231–7.

Teronen O, Salo T, Konttinen YT et al. (1995a) Identification and characterization of gelatinases/type IV collagenases in jaw cysts. *Journal of Oral Pathology & Medicine* **24**, 78–84.

Teronen O, Salo T, Laitinen J et al. (1995b) Characterization of interstitial collagenases in jaw cyst wall. *European Journal of Oral Sciences* **103**, 141–7.

Tsai CH, Chen YJ, Huang FM, Su YF, Chang YC (2005) The upregulation of matrix metalloproteinase-9 in inflamed human dental pulps. *Journal of Endodontics* **31**, 860–2.

Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjäderhane L (2002) Matrix metalloproteinase-8 (MMP-8) in pulpal and periapical inflammation and periapical root-canal exudates. *International Endodontic Journal* **35**, 897–904.

Woessner JF, Nagase H (2000) *Matrix Metalloproteinases and TIMPs*. Oxford, UK: Oxford University Press.

Wood NK, Goaz PW, Jacobs MC (1997) Periapical radiolucencies. In: Wood NK, Goaz PW, eds. *Differential Diagnosis of Oral and Maxillofacial Lesions*, 5th edn. St Louis, MO: Mosby, pp. 252–78.