J Periodont Res 2004; 39; 269–274
Printed in the UK.

JOURNAL OF PERIODONTAL RESEARCH
doi: 10.1111/j.1600-0765.2004.00740.x

Influence of the matrix metalloproteinase inhibitor batimastat (BB-94) on periodontal bone destruction in Sprague-Dawley rats

Magnús Jón Björnsson, Anne Havemose-Poulsen, Kaj Stoltze, Palle Holmstrup
Department of Periodontology, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, Denmark

Björnsson MJ, Havemose-Poulsen A, Stoltze K, Holmstrup P. Influence of the matrix metalloproteinase inhibitor Batimastat (BB-94) on periodontal bone destruction in Sprague-Dawley rats. J Periodont Res 2004; 39; 269–274.

Background: Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of degrading most macromolecules of the extracellular matrix. It has been assumed that an association exists between MMP activity and periodontal disease progression, but the precise role of MMPs in disease progression is still not fully clarified. Batimastat, or BB-94, is a synthetic broad-spectrum MMP inhibitor not previously examined in periodontal research. If there is an association between MMP activity and periodontal disease progression, then batimastat might be expected to reduce the progression of experimental periodontal disease in rats.

Objectives: The objective of the present study was to determine the effects of batimastat on periodontal status in healthy Sprague-Dawley (SPRD) rats as well as in rats with ligature-induced experimental periodontal disease.

Methods and Results: Periodontal bone destruction was used as a means of evaluating periodontal destruction by measuring periodontal bone loss on defleshed rat jaws and periodontal bone support on radiographs of the jaws. There was significantly more periodontal bone destruction in the groups treated with batimastat than in the placebo and control groups. This accounted for both ligated and non-ligated groups, irrespective of whether periodontal bone loss ($p < 0.05$) or periodontal bone support ($p < 0.05$) were measured.

Conclusion: In conclusion, the results of this study did not support the hypothesis that the MMP inhibitor batimastat could reduce the progression of experimental periodontal disease in rats. Instead, significantly increased bone destruction was found in rats treated with batimastat.

Professor Palle Holmstrup, DDS, PhD, DrOdont, Department of Periodontology, Faculty of Health Sciences, University of Copenhagen, Nørre Alle 20, 2200 København N, Denmark
Tel: +45 35326691
Fax: +45 35326699
e-mail: ph@odont.ku.dk

Key words: experimental periodontal disease; matrix metalloproteinase; matrix metalloproteinase inhibitor; rats

Accepted for publication March 9, 2004

Matrix metalloproteinases (MMPs) are a family of highly conserved Zn^{2+}-dependent proteolytic enzymes that collectively are capable of degrading most, if not all, macromolecules of the extracellular matrix. For detailed information on the MMP family, see recent books and reviews (1–3).

MMPs are implicatedin various pathological processes such as rheumatoid arthritis, cancer invasion and metastasis, atherosclerosis and periodontal disease (4–12) and it has been assumed that an association exists between MMP expression and periodontal disease

progression (13). Therefore, efforts have been made to inhibit the enzymes ever since the detection of the first collagenase (14). Inhibition of MMPs in periodontal disease has primarily focused on the use of tetracyclines, which mainly inhibit MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 with both chelation of active enzymes and by down regulation (3, 15). Non-antimicrobial and low dose tetracyclines have been shown to possess inhibitory effects on periodontal disease progression in both laboratory animals (16) and humans (17). Also, two non-tetracycline MMP inhibitors have recently shown beneficial effects on experimental periodontal disease in rats (18). Although there is some evidence on the role of MMPs in periodontal disease, their role in disease progression is still not fully clarified. A recent study showed that gingival fibroblasts from healthy controls dissolved type I collagen fibers twice as fast as fibroblasts from patients with juvenile, rapidly progressive, and slowly progressive periodontal disease (19). This result may indicate that there is no simple correlation between periodontal connective tissue breakdown and MMP expression by periodontal fibroblasts. Instead, the breakdown may rather be correlated to MMP activity as expressed by the ratio: activated MMPs/endogenous MMP inhibitors (TIMPs) (13, 19).

Inhibition of MMPs in an animal model of experimental periodontal disease is one of the methods that can be used to investigate the role of MMPs in periodontal disease (18, 20).

The synthetic MMP inhibitor batimastat, or BB-94 ([(4-*N*-hydroxyamino)-2*R*-isobutyl-3*S*-(thienyl-thiomethyl)succinyl]-L-phenyl-alanine-*N*-methylamide), is a collagen peptide based hydroxamic acid that specifically mimics the site in the collagen substrate that is cleaved by the MMPs and works by a competitive, reversible inhibition (21, 22). Batimastat is a broad-spectrum MMP inhibitor with activity against, at least, MMP-1 (MMP-13 in rats) MMP-2, MMP-3, MMP-7, MMP-9 and MMP-14 (21, 22). It also binds to MMP-8 but the inhibitory potency is not known (23, 24). It is almost insoluble and consequently has a very poor bioavailability when administered orally. Thus batimastat has to be injected into body cavities (peritoneal and pleural cavities) resulting in sustained plasma concentrations (21). Batimastat has primarily been used in cancer research and has been shown to inhibit both tumour growth, invasion and metastatic spread and to prolong survival in rodent cancer models (25–29). If the hypothesis is true that an association exists between MMP activity and periodontal disease progression, the MMP inhibitor batimastat might be expected to reduce the progression of experimental periodontal disease in rats.

The purpose of the present study was to describe the effects of batimastat on periodontal status in Sprague-Dawley (SPRD) rats with ligature-induced experimental periodontal disease, as well as in periodontally healthy rats. This was accomplished by measuring periodontal bone loss and periodontal bone support in the rats after treatment with batimastat for 31 days.

Materials and methods

Animals

The animals used in the present study were 119 fully barrier-reared, outbred, male Mol:SPRD Han rats (M&B A/S, Ry, Denmark). The rats were bred under special conditions to prevent the development of periodontal disease before the start of the experiment (30). Furthermore, the animals were anaesthetized and examined for signs of periodontal disease prior to entering the study (30). When the animals reached the age of 8 weeks they were randomly moved to cages with two animals in each cage. A license to perform this study was obtained from the Danish National Experimental Animal Inspectorate.

Experimental design

The 119 rats were randomly divided into six groups (Table 1). The discrepancy in group sizes was due to failure in delivery of rats for the study and loss of animals during the study (see Results). Experimental periodontal disease was induced in the rats in groups 4–6 as follows. The rats were anaesthetized with a 2 ml/kg mixture of Hypnorm (Janssen Pharmaceutical, Beerse, Belgium) and Midazolam (Dumax-Alpharma AS, Oslo, Norway) and 4/0 silk ligatures (Perma-Hand® Seide, Ethicon GmbH, Norderstedt, Germany) were tied around the cervix of the second maxillary molar in the right and left side with a surgical knot on the palatal aspects of the teeth (31, 32). The animals in groups 4–6 were anaesthetized once a week and ligatures that were loose or missing were replaced by new ligatures (33, 34). Experimental periodontal disease was not induced in groups 1–3. Medication started simultaneously in all groups 3 days before placement of ligatures. The animals in groups 1 and 4 served as controls and received no medication. Groups 2 and 5 were placebo groups. The animals in both placebo groups received daily 12 ml/kg intraperitoneal (i.p.) injections of sterile phosphate-buffered saline (pH 7.4) containing 0.01% (v/v) Tween 80

Table 1. Experimental design and results from multiple comparisons of groups with different treatment[a]

			Periodontal bone loss			Periodontal bone support		
Group	Ligation	Treatment	*n*	Mean	$p < 0.05$	*n*	Mean	$p < 0.05$
1	No	Control	19	1.45	$1 \neq 2$	19	46.37	$1 = 2$
2	No	Placebo	19	1.58	$2 \neq 3$	19	46.34	$2 \neq 3$
3	No	Batimastat	20	1.75	$3 \neq 1$	20	43.91	$3 \neq 1$
4	Yes	Control	18	3.09	$4 = 5$	18	40.20	$4 = 5$
5	Yes	Placebo	17	3.31	$5 \neq 6$	16	38.61	$5 \neq 6$
6	Yes	Batimastat	18	3.63	$6 \neq 4$	18	32.88	$6 \neq 4$

[a]= means that there was not a significant difference between the two groups, whereas ≠ stands for a significant difference between groups.

(Sigma Chemical Co., St. Louis, MO, USA). The animals in the test groups, group 3 and 6, received daily i.p. injections of batimastat, 30 mg/kg (22). Batimastat was mixed in sterile phosphate-buffered saline, 2.5 mg/ml, containing 0.01% (v/v) Tween 80. This mixture was vortexed for 15–30 s, sonicated in an ultrasonic tub at 220–240 V, 50–60 Hz, for 10 min (Metason 120, Struers, Copenhagen, Denmark) and finally sonicated with a sonic probe (MSE ultrasonic disintegrator, 100 W) for 5 min.

Registration of periodontal bone destruction

Four weeks after the initial placement of ligatures all animals were killed. Periodontal bone destruction was used to evaluate periodontal tissue destruction by measurements of periodontal bone loss and periodontal bone support (35, 36). Briefly, periodontal bone loss was evaluated morphometrically by measuring the distance between the cemento-enamel junction and buccal alveolar bone crest at 15 sites in each upper jaw. The mean of the sums of the 15 measurements from the left and right side of the maxilla was used as a measure of periodontal bone loss in each animal. A higher value of periodontal bone loss indicates more periodontal bone destruction.

Periodontal bone support was measured on radiographs of the maxillary molars. This method measures the intrabony defects, not detected by the periodontal bone loss method. Briefly, the defleshed jaws were aligned in a way to achieve sufficient reproducibility, as described elsewhere (30). Radiographs were exposed for 20 s in a laboratory X-ray machine (Hewlett Packard Cabinet X-ray System, Faxitron Series) at 30 kV and 2.8–3.0 mA. The radiographs were scanned and digitized (Sprint Scan 35, model CS-3600, Polaroid Corporation, Cambridge, MA, USA) with a resolution of 2025 dots per inch. Measurements were performed on the mesial and distal aspect of the second molar in each side and the mean of the four registrations used to express periodontal bone support for each animal. The apex (A) of a distal root and the distal cusp tip (C) were located and the distance between A and C (AC) traced and measured in pixels. A line was traced from the deepest bony defect, intersecting AC at a right angle. Finally, the intersection of the two lines (B) was located and the distance from the apex to the intersection (AB) measured in pixels. Periodontal bone support was calculated according to the formula: periodontal bone support $= (AB/AC) \times 100\%$ (30, 35). Unlike periodontal bone loss, a higher value of periodontal bone support indicates less periodontal bone destruction. All measurements were performed blind with Digora for Windows 2.0 (Orion Corporation Soredex, Finland).

Statistical analysis

A 5% level of significance was used for all tests. Periodontal bone loss and periodontal bone support were subjected to ANOVA with the independent variables ligation (ligated and non-ligated) and medication (control, placebo and batimastat), as well as ligation–medication interaction. Duncan's multiple range test was then used to compare the effects of different treatment modalities (control, placebo, batimastat) among experimental periodontal disease and non-ligated groups. Data were transformed by weighting each squared residual with the reciprocal of the variance of the respective group if variance homogeneity could not be assumed. All statistics were performed with SAS System for Windows 8.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA).

Results

A total of eight rats were lost during the study and therefore not used for measurements of periodontal bone support and periodontal bone loss. Seven of these were lost during anaesthesia and one rat had to be killed due to illness. Finally, one animal could not be used for measurement of periodontal bone support due to an accident during preparation. Thus, a total of 111 out of 119 rats were available for measurement of periodontal bone loss and 110 for measurement of periodontal bone support (see Table 1). Two animals from group 6 got anaemic and lost weight, but recovered after suspension of medication for 1 and 3 days, respectively. One rat from group 1 and another from group 6 developed ascites. Data from the two sick rats and the rats with ascites did not influence the overall results.

Measurement of periodontal bone loss and periodontal bone support

Both periodontal bone loss and periodontal bone support data were transformed since homogeneity could not be assumed ($p < 0.0001$).

Both ligation and medication were significant as independent variables for periodontal bone loss ($p < 0.0001$), but no ligation–medication interaction ($p = 0.384$). The model was significant and explained 88% of the periodontal bone loss variation. Comparison within the non-ligated groups showed that there was significantly more periodontal bone loss in the placebo group than in the control group. The difference, however, was limited (Fig. 1). There was significantly more periodontal bone loss in the batimastat group than in both the placebo and control groups (Table 1 and Fig. 1). Comparison within the ligated groups showed that there was no significant difference between the control and placebo groups, but the batimastat group had significantly more periodontal bone loss than both the control and placebo groups.

Ligation ($p < 0.0001$) and medication ($p < 0.0001$), as well as ligation–medication interaction ($p = 0.046$), were significant as independent variables and interaction for periodontal bone support. The model was significant ($p < 0.0001$) and explained 72% of the periodontal bone support variation. Comparison within the non-ligated groups showed that the batimastat group had significantly less periodontal bone support than both the control and placebo groups, but there was no significant difference between the control and placebo groups. Within the ligated groups the batimastat group had significantly less

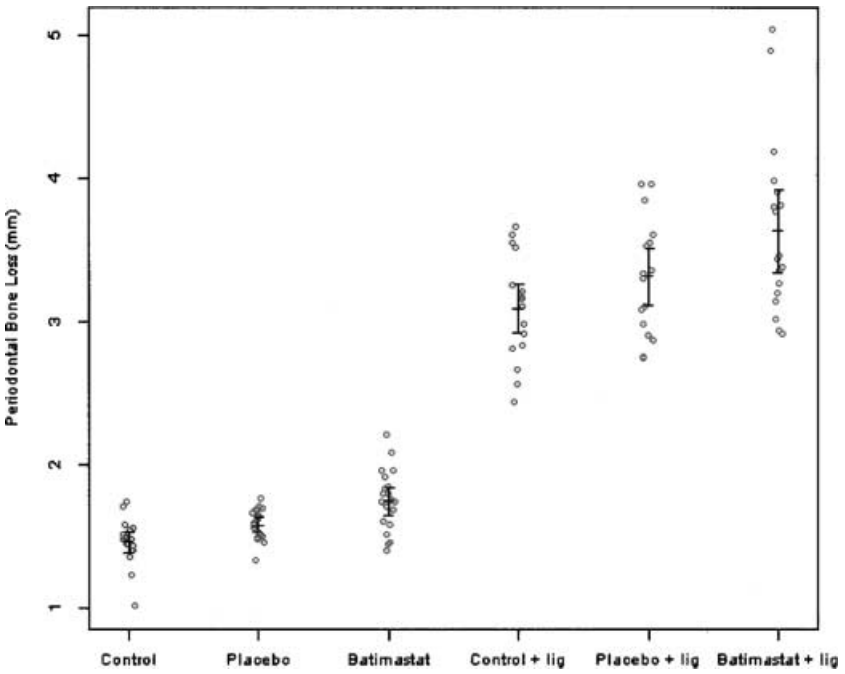

Fig. 1. Periodontal bone loss. Experimental periodontal disease was induced in the groups marked + lig. The vertical bars show the mean in each group and 95% confidence interval.

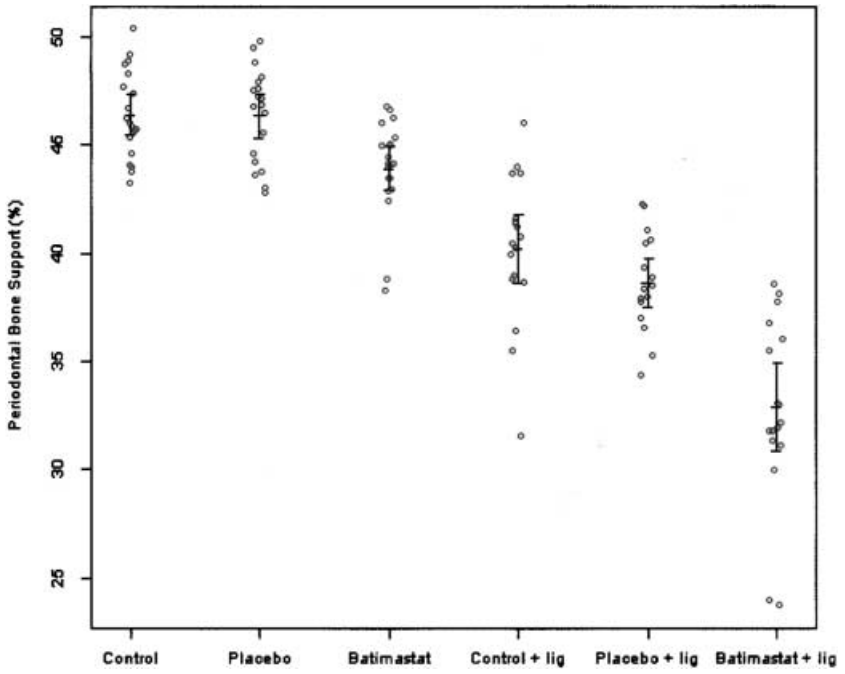

Fig. 2. Periodontal bone support. Experimental periodontal disease was induced in the groups marked + lig. The vertical bars show the mean in each group and 95% confidence interval.

periodontal bone support than both the control and placebo group, whereas there was no significant difference between the control and placebo groups (Table 1 and Fig. 2).

Discussion

This study provided the unexpected result that the rats which were treated with the synthetic MMP inhibitor batimastat had significantly more periodontal bone destruction than the control and placebo groups. These results were irrespective of whether experimental periodontal disease was induced in the rats with silk ligatures or not and whether periodontal bone loss or periodontal bone support was measured. Furthermore, the combination of batimastat and ligation further reduced periodontal bone support in the rats.

These results do not support the hypothesis that the MMP inhibitor batimastat could reduce progression of experimental periodontal disease in rats. Batimastat's effects on periodontal bone loss and periodontal bone support in the present study are neither in accordance with the results obtained with antimicrobial and non-antimicrobial tetracyclines (16) nor the effects of two non-tetracycline MMP inhibitors (18) on experimental periodontal disease in rats. The ability of tetracyclines to influence periodontal disease, irrespective of their microbiological effects, has to a great extent been attributed to their ability to inhibit certain MMPs (17, 37–39). Tetracyclines have both been used in sub-antimicrobial doses and chemically modified in order to prevent the formation of bacterial strains resistance to antibiotics. Their use has resulted in less periodontal breakdown and attachment loss in both laboratory animals and humans (17, 38, 40). However, tetracyclines' ability to inhibit experimental periodontal disease in rats and humans could also be attributed to properties other than their ability to inhibit MMPs, such as inhibition of interleukin-1β, tumour necrosis factor-α (TNF-α) and nitric oxide, as well as pro-anabolic effects on connective tissue and bone (for reviews see 15, 41). Therefore, it appears that the total effects of tetracyclines on periodontal disease are an assemblage of different mechanisms, which may be difficult to separate when the effects of non- or sub-antimicrobial tetracyclines on periodontal disease are valued. The broad-spectrum MMP inhibitor in this study is believed to have effect primarily on the matrix metalloproteinase pathway of tissue destruction and displays little detectable activity against other classes

of metalloproteinases (14). However, other hydroxamic-acid based MMP inhibitors (like batimastat) have been shown to be able to inhibit TNF-α converting enzyme, which belongs to the adamalysin family of metalloproteinases (42–45), and to inhibit TNF-α receptor shedding by a human colon adenocarcinoma cell line (46). It is currently not known if batimastat has this ability in rats and therefore unclear which role inhibition of TNF-α receptor shedding and TNF-α converting enzyme may have played in the present study.

It is possible that batimastat did not have specific local MMP inhibitory effects in the present study, as MMP expression and/or activity was not measured in gingiva or gingival crevicular fluid. However, analysis of data showed that it did have significant periodontal effects, measured as periodontal bone loss and periodontal bone support. The dosing used in the present study and the mode of administration are the same as in other studies where batimastat has shown its specific inhibitory effects (21, 22).

Even though the results of this study are not in accordance with the current hypothesis on the relationship between MMP activity and periodontal disease progression, they are in agreement with results from a preceding pilot study, conducted in the same way but with fewer animals and without placebo groups (results not shown). Some of the explanations for the results might be related to what is currently known about the role of MMPs in epidermal wound healing. Collagenase-1 expression by keratinocytes is induced in epidermal wound healing, where it is believed to provide migrating keratinocytes with a mechanism to maintain their course and directionality in the wound environment during re-epithelialization (47). Inhibition of MMPs with a hydroxamic acid inhibitor in a migration assay completely arrested keratinocyte migration and thus suggested that blockage of MMPs could have the potential of stopping wound healing (47). Furthermore, inhibition of MMPs in cutaneous wounds in pigs significantly decreased epithelial coverage (48). These studies indicate that the presence of MMPs is essential for cutaneous wound healing and that MMP inhibition may have detrimental effects on the healing process. Likewise, it has been suggested that periodontal disease might progress or persist because of the inability of periodontal cells to cope with a persistent wound healing situation (19). Still it has to be kept in mind that the mechanisms of bone remodeling are not identical to soft tissue remodeling, as bone is a mineralized tissue with a more complex matrix composition and hence degradation pathway (1).

Recently a mutant mouse deficient in MT1-MMP (MMP-14) was generated (49). This mouse showed growth impairment, delayed ossification of bones, impaired removal of calvarial cartilage, osteopenia, severe generalized arthritis and fibrosis of tendons. The progressive osteopenia was a result of increased bone resorption as well as diminished bone formation, with the largest number of osteoclasts found near bone/soft tissue interfaces (49). These results showed that MMP-14 is essential for the development and maintenance of the hard tissues of the skeleton. A recent study showed that ligature-induced periodontitis in rats increases the expression of MMP-2 and MMP-14 in periodontal cells (20). The authors suggest that these MMPs may play a role in the destructive as well as healing phases of a periodontal lesion (20). The rats in the present study were still in active growth like the MMP-14 deficient mice. The increased periodontal bone loss and decreased periodontal bone support in non-ligated animals in the present study could possibly be explained by increased bone resorption as well as diminished bone formation like that found in the mice. Furthermore, the difference between batimastat's and tetracyclines' effects on experimental periodontal disease in rats may perhaps be explained by the difference in their ability to inhibit MMP-14.

In conclusion, the results of this study did not support the hypothesis that the MMP inhibitor batimastat could reduce the progression of experimental periodontal disease in rats. Instead, a significantly increased bone destruction was found in rats treated with batimastat.

Acknowledgements

This work was supported by the FUT-Calcin Foundation.
Batimastat was kindly provided by British Biotech, Oxford, UK.

References

1. Birkedal-Hansen H, Moore WGI, Bodden MK *et al.* Matrix metalloproteianses: a review. *Crit Rev Oral Biol Med* 1993;**4:**197–250.
2. Parks WC, Mecham RP. *Matrix Metalloproteinases*. London: Academic Press, 1998.
3. Woessner JF, Nagase H. *Matrix Metalloproteinases and TIMPs*. Oxford: Oxford University Press, 2000.
4. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodont Res* 1993;**28:**500–510.
5. Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. *J Periodontol* 1993;**64:**474–484.
6. Haerian A, Adonogianaki E, Mooney J *et al.* Effects of treatment on gingival crevicular collagenase, stromelysin and tissue inhibitor of metalloproteinases and their ability to predict response to treatment. *J Clin Periodontol* 1996;**23:**83–91.
7. Larivee J, Sodek J, Ferrier JM. Collagenase and collagenase inhibitor activities in crevicular fluid of patients receiving treatment for localized juvenile periodontitis. *J Periodont Res* 1986;**21:**702–715.
8. Villela B, Cogen RB, Bartolucci AA *et al.* Collagenolytic activity in crevicular fluid from patients with chronic adult periodontitis, localized juvenile periodontitis and gingivitis, and from healthy control subjects. *J Periodont Res* 1987;**22:**381–389.
9. Ingman T, Sorsa T, Michaelis J *et al.* Immunohistochemical study of neutrophil- and fibroblast-type collagenases and stromelysin-1 in adult periodontitis. *Scand J Dent Res* 1994;**102:**342–349.
10. Sarment DP, Korostoff J, D'Angelo M *et al.* In situ localization and characterization of active proteases in chronically inflamed and healthy human gingival tissues. *J Periodontol* 1999;**70:**1303–1312.
11. Korostoff J, Wang JF, Sarment DP *et al.* Analysis of in situ protease activity in chronic adult periodontitis patients: Expression of activated MMP-2 and a 40 kDa serine protease. *J Periodontol* 2000;**71:**353–360.
12. Kubota T, Nomura T, Takahashi T *et al.* Expression of mRNA for matrix

metalloproteinases and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. *Arch Oral Biol* 1996;**41:**253–262.
13. Havemose-Poulsen A, Holmstrup P. Factors affecting IL-1-mediated collagen metabolism by fibroblasts and the pathogenesis of periodontal disease: a review of the literature. *Crit Rev Oral Biol Med* 1997;**8:**217–236.
14. Brown PD. Synthetic inhibitors of Matrix metalloproteinases. In: Parks WC, Mecham RP, eds. *Matrix Metalloproteinases*. London: Academic Press, 1998: 243–261.
15. Golub LM, Lee HM, Ryan ME *et al*. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv Dent Res* 1998;**12:**12–26.
16. Chang KM, Ramamurthy NS, McNamara TF *et al*. Tetracyclines inhibit Porphyromonas gingivalis-induced alveolar bone loss in rats by a non-antimicrobial mechanism. *J Periodont Res* 1994;**29:**242–249.
17. Caton JG, Ciancio S, Blieden TM *et al*. Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. *J Periodontol* 2000;**71:**521–532.
18. Ramamurthy NS, Xu JW, Bird J. Inhibition of alveolar bone loss by matrix metalloproteinase inhibitors in experimental periodontal disease. *J Periodont Res* 2002;**37:**1–7.
19. Havemose-Poulsen A, Holmstrup P, Stoltze K *et al*. Dissolution of type I collagen fibrils by gingival fibroblasts isolated from patients of various periodontitis categories. *J Periodont Res* 1998;**33:**280–291.
20. Achong R, Nishimura I, Ramachandran H *et al*. Membrane type (MT) 1-matrix metalloproteinase (MMP) and MMP-2 expression in ligature-induced periodontitis in the rat. *J Periodontol* 2003;**74:** 494–500.
21. Rasmussen H, McCann PP. Matrix metalloproteinase inhibition as a novel anticancer strategy. A review with special focus on batimastat and marimastat. *Pharmacol Ther* 1997;**75:**69–75.
22. Brown PD. Matrix metalloproteinase inhibitors: a novel class of anticancer agents. *Adv Enzyme Regul* 1995;**35:** 293–301.
23. Grams F, Crimmin M, Hinnes L *et al*. Structure determination of human neutrophil collagenase complexed with a hydroxamate inhibitor. *Biochemistry* 1995;**34:**14012–14020.
24. Steinman DH, Curtin ML, Garland RB *et al*. The design, synthesis, and structure–activity relationships of a series of macrocyclic MMP inhibitors. *Bioorg Med Chem Lett* 1998;**8:**2087–2092.
25. Low JA, Johnson MD, Bone EA *et al*. The matrix metalloprotienase inhibitor batimastat (BB-94) retards human breast cancer solid tumor growth but not ascites formation in nude mice. *Clin Cancer Res* 1996;**2:**1207–1214.
26. Knox DJ, Bretton L, Lynch T *et al*. Synthetic matrix metalloproteinase inhibitor, BB-94, inhibits the invasion of neoplastic human prostate cells in a mouse model. *Prostate* 1998;**35:**248–254.
27. Watson SA, Morris TM, Robinson G *et al*. Inhibition of organ invasion by the matrix metalloproteinase inhibitor batimastat (BB-94) in two human colon carcinoma metastasis models. *Cancer Res* 1995;**55:**3629–3633.
28. Lein M, Jung K, Le DK *et al*. Synthetic inhibitor of matrix metalloproteinases (batimastat) reduces prostate cancer growth in an orthotopic rat model. *Prostate* 2000;**43:**77–82.
29. Eccles SA, Box GM, Court WJ *et al*. Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by the matrix metalloproteinase inhibitor batimastat (BB-94). *Cancer Res* 1996;**56:**2815–2822.
30. Björnsson MJ, Havemose-Poulsen A, Stoltze K, Holmstrup P. The influence of diet consistence, drinking water and bedding on periodontal disease in Sprague-Dawley rats. *J Periodont Res*; 2004;**39:**543–550.
31. Samejima Y, Ebisu S, Okada H. Effect of infection with Eikenella corrodens on the progression of ligature-induced periodontitis in rats. *J Periodont Res* 1990;**25:**308–315.
32. Sanavi F, Listgarten MA, Boyd F *et al*. The colonization and establishment of invading bacteria in periodontium of ligature-treated immunosuppressed rats. *J Periodontol* 1985;**56:**273–280.
33. Fischer RG, Klinge B. Clinical and histological evaluation of ligature-induced periodontitis in the domestic ferret. *J Clin Periodontol* 1994;**21:**230–239.
34. Lindhe J, Ericsson I. Effect of ligature placement and dental plaque on periodontal breakdown in the dog. *J Periodontol* 1978;**49:**343–350.
35. Klausen B, Evans RT, Sfintescu C. Two complementary methods of assessing periodontal bone level in rats. *Scand J Dent Res* 1989;**97:**494–499.
36. Chang KM, Ramamurthy NS, McNamara TF *et al*. Tetracyclines inhibit Porphyromonas gingivalis-induced alveolar bone loss in rats by a non-antimicrobial mechanism. *J Periodont Res* 1994;**29:**242–249.
37. Golub LM, Lee HM, Lehrer G *et al*. Minocycline reduces gingival collagenolytic activity during diabetes. Preliminary observations and proposed new mechanism of action. *J Periodont Res* 1983;**18:**516–526.
38. Golub LM, Sorsa T, Lee HM *et al*. Doxycycline inhibits neutrophil (PMN) -type matrix metalloproteinases in human adult periodontitis gingiva. *J Clin Periodontol* 1995;**22:**100–109.
39. Golub LM, Lee HM, Greenwald RA *et al*. A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 1997;**46:**310–319.
40. Golub LM, Evans RT, McNamara TF *et al*. A non-antimicrobial tetracycline inhibits gingival matrix metalloproteinases and bone loss in Porphyromonas gingivalis-induced periodontitis in rats. *Ann New York Acad Sci* 1994;**732:**96–111.
41. Seymour RA, Heasman PA. Tetracyclines in the management of periodontal diseases. A review. *J Clin Periodontol* 1995;**22:**22–35.
42. Parvathy S, Karran EH, Turner AJ *et al*. The secretases that cleave angiotensin converting enzyme and the amyloid precursor protein are distinct from tumour necrosis factor-alpha convertase. *FEBS Lett* 1998;**431:**63–65.
43. Black RA, Rauch CT, Kozlosky CJ *et al*. A metalloproteinase disintegrin that releases tumor-necrosis factor-α from cells. *Nature* 1997;**385:**729–733.
44. DiMartino M, Wolff C, High W *et al*. Anti-arthritic activity of hydroxamic acid-based pseudopeptide inhibitors of matrix metalloproteinases and TNF alpha processing. *Inflamm Res* 1997;**46:**211–215.
45. Gearing AJH, Beckett P, Christodoulou M *et al*. Processing of tumor necrosis factor-α precursor by metalloproteinases. *Nature* 1994;**370:**555–557.
46. Lombard MA, Wallace TL, Kubicek MF *et al*. Synthetic matrix metalloproteinase inhibitors and tissue inhibitor of metalloproteinase (TIMP)-2, but not TIMP-1, inhibit shedding of tumor necrosis factor-α receptors in a human colon adenocarcinaoma (Colo 205) cell line. *Cancer Res* 1998;**58:**4001–4007.
47. Pilcher BK, Dumin JA, Sudbeck BD *et al*. The activity of collagenase-1 is required for keratinocyte migration on a type 1 collagen matrix. *J Cell Biol* 1997;**137:** 1445–1457.
48. Agren MS. Matrix metalloproteinases (MMPs) are required for re-epithelialization of cutaneous wounds. *Arch Dermatol Res* 1999;**291:**583–590.
49. Holmbeck K, Bianco P, Caterina J *et al*. MT1-MMP-deficient mice develop dwarfism, ostopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* 1999;**99:** 81–92.