Collagenase-3 (MMP-13) is expressed in periapical lesions: an immunohistochemical study

R. Leonardi[1], R. Caltabiano[2] & C. Loreto[2]

[1]Department of Medical and Surgical Science, II Dental Unit; and [2]Department of Anatomy and Pathology, University of Catania, Catania, Italy

Abstract

Leonardi R, Caltabiano R, Loreto C. Collagenase-3 (MMP-13) is expressed in periapical lesions: an immunohistochemical study. *International Endodontic Journal*, **38**, 297–301, 2005.

Aim To determine whether or not matrix metalloproteinase 13 (MMP-13) is present in periapical granulomas with and without epithelium.

Methodology Seventeen open periapical granulomas of pulpal origin (seven lesions without epithelium and 10 with proliferating epithelium) were fixed in formalin and then embedded in paraffin prior to being processed for immunohistochemical analysis. A monoclonal antibody against human MMP-13 was used to evaluate MMP-13 expression. Immunocomplexes were subsequently treated with the secondary antibody and then detected by means of streptavidin peroxidase. Immunoreactivity was visualized by development with 3,3′-diaminobenzidine.

Results An immunopositive cytoplasmatic reaction for MMP-13 was observed in all the specimens, although the immunostaining by anti-MMP-13 antibody was heterogeneous and its levels varied according to histopathological findings. In periapical lesions without epithelium MMP-13 immunolabelling was detected in a few fibroblast-like cells, and in some plasma cells within the granulomatous tissue. A clear upregulation of MMP-13 expression was detected in periapical lesions with epithelium, especially in small island and thin strands of epithelium.

Conclusions The expression pattern of MMP-13 demonstrates that it is involved in the conversion of a periapical granuloma with epithelium into a radicular cyst. This property is related to the ability of MMP-13 to influence not only the migration of epithelial cell but also the invasion of granulomatous tissue.

Keywords: granulation tissue, MMP-13, periapical lesions, rests of Malassez.

Received 13 September 2004; accepted 5 January 2005

Introduction

Periapical lesions are the most common osteolytic lesions in the maxilla and mandible (Bando *et al.* 1993, Hong *et al.* 2004) and include granulomas and radicular cysts. Both granulomas and radicular cysts are thought to represent two different stages of development of the same inflammatory process (Shear 1985). They are characterized by the infiltration of lymphocytes, plasma cells and macrophages into the granulomatous tissue (Gao *et al.* 1988, Lukic *et al.* 1990, Marton & Kiss 1993, Liapatas *et al.* 2003). A sequel to granuloma formation is the proliferation of the epithelial cell rests of Malassez associated with the inflammation, which may lead to the development of an inflammatory radicular cyst (Gao *et al.* 1988, Liapatas *et al.* 2003).

Although these lesions have been described histologically, very little is currently known about the precise mechanisms of growth inside the bone. Some cytokines have been described as being involved in the pathogenesis (Bando *et al.* 1993, Wang & Stashenko 1993, Fouad 1997, Lin *et al.* 2000, Huang *et al.* 2001), but matrix metalloproteinases 13 (MMPs), which have been detected recently, in periapical

Correspondence: Prof. Rosalia Leonardi, Istituto di II Clinica Odontoiatrica, Policlinico, Citta' Universitaria, Università di Catania, Via S. Sofia n.78, Catania, Italy (Tel.: +39 095 3782645; fax: +39 095 3782645; e-mail: rleonard@unict.it).

lesions, seem to play a major role in bone resorption (Teronen *et al.* 1995a,b, Lin *et al.* 1997, 2002, Kubota *et al.* 2000, 2001, 2002, Takata *et al.* 2000, Wahlgren *et al.* 2001, 2003).

Matrix metalloproteinases are a family of zinc-dependent endoperoxidases collectively capable of degrading all extracellular matrix components (Johansson *et al.* 2000, Khasigov *et al.* 2001). MMPs have been divided into four subfamilies: collagenases, gelatinases, stromelysins and membrane-type MMPs, according to their substrates and structures.

The subfamily of collagenases includes: the interstitial collagenase (MMP-1), the collagenase of neutrophils (MMP-8) and collagenase-3 (MMP-13). These enzymes disintegrate native fibrillar interstitial collagens by cleaving the single peptide bond in α-chains (Khasigov *et al.* 2003). Collagenases can also hydrolyse other substrates, but fibrillar collagens can be destroyed only by these enzymes (Khasigov *et al.* 2003).

MMP-13 is produced by fibroblasts, epithelial cells and malignant squamous epithelium, as well as plasma cells associated with bone destructive lesions (Johansson *et al.* 2000). Previously, Wahlgren *et al.* (2003) demonstrated the variable expression patterns of MMP-13 in odontogenic cysts and argued that it may play different roles in regulating focal proliferation, maturation and migration of the jaw cyst epithelium (Wahlgren *et al.* 2003).

On the basis of these observations, the present work was undertaken to identify MMP-13 expression in periapical granuloma with and without epithelium.

Materials and methods

Tissue specimens were obtained from the archives of the Department of Dentistry of Catania University, Italy. The formalin-fixed paraffin-embedded specimens included 17 periapical lesions of pulpal origin, obtained from periapical surgery or extraction. The 17 periapical granulomas were made up of seven lesions without epithelium and 10 with proliferating epithelium that had been diagnosed clinically, radiologically and histologically according to the WHO criteria. After removal, the tissues were fixed immediately in 10% neutral-buffered formalin at room temperature. They were embedded in paraffin wax, and 5-μm serial sections were collected on silane-coated glass slides. A well-characterized mouse monoclonal antibody against human MMP-13 (Ab-2, clone ID3, Neomarkers; Lab Vision, Fremont, CA, USA) was used to detect MMP-13. The antibody, diluted 1 : 100 in PBS, was applied directly to each section and the slides were incubated overnight (4 °C). Immunocomplexes were subsequently treated with the secondary antibody and then detected by means of streptavidin peroxidase, both were incubated for 30 min at room temperature (Vectastain ABC kit; Vector Laboratories, Burlingame, CA, USA). Immunoreactivity was visualized by development for 2 min with 0.1% 3,3′-diaminobenzidine and 0.02% hydrogen peroxide (DAB substrate kit; Vector Laboratories). Sections were counterstained with Mayer-haematoxylin, mounted with permount and examined by light microscopy.

Immunoreactivity was evaluated blindly by two observers, who independently assessed the immunostainings by means of light microscopy at 400× magnification high power field. Immunohistochemical reactions were scored using semiquantitative gradings (−, no staining; +, weak staining or strong stains in 11–25% of cells; ++, moderate staining or strong stains in 26–75% of cells; +++, strong staining of more than 76% of cells).

Amongst serial sections, two of every six were stained with haematoxylin and eosin (Bio-optica, Milan, Italy) for morphological studies. Negative controls consisted of sections in which primary antibodies were omitted and replaced with either nonimmune murine serum (X 0910; DAKO) or with 1% PBS-BSA. Positive controls consisted of breast carcinoma sections.

Results

Examination of haematoxylin-stained sections using light microscopy demonstrated a varied morphology of the periapical lesions. Seven specimens had a massive infiltration of inflammatory cells, identified on the basis of their morphological features as lymphocytes, plasma cells and macrophages. In 10 granulomas, rests of Malassez and epithelial strands or associated with inflammatory cells could also be observed. The epithelial cells were frequently arranged in layers to form small island, and strands and/or trabeculae of varying thickness. Many small, newly formed blood vessels, scattered within the lesions and concentrated in the regions with an intense inflammatory infiltrate, could also be seen in all specimens.

An immunopositive cytoplasmatic reaction for MMP-13 was observed in almost all the specimens, although the immunostaining by anti-MMP-13 antibody was heterogeneous and its levels varied according to the histopathological findings. Positive MMP-13 staining was found in strands of epithelial cells, as well as in

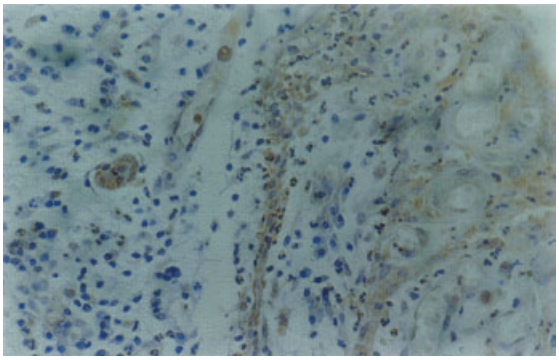

Figure 1 Immunohistochemical staining for MMP-13 in the periradicular tissue. Positive MMP-13 staining is observed: in epithelial cells, as well as in inflammatory infiltrate. Original magnification ×200.

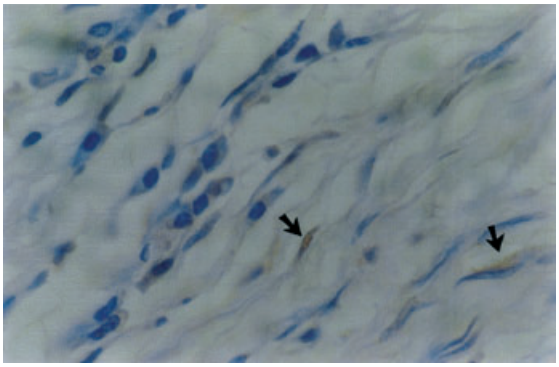

Figure 2 Immunohistochemical staining for MMP-13 in a periapical lesion without epithelium. Fibroblast-like cells (arrows) show a positivity to MMP-13. Original magnification ×400.

plasma cells, macrophage-like cells and fibroblasts (Fig. 1).

In periapical lesions without epithelium MMP-13 immunolabelling was detected in a few fibroblast-like cells (grade +), (Fig. 2), as well as in plasma cells located within the granulation tissue (grade ++).

However, a higher upregulation of MMP-13 was detected in periapical lesions with epithelium. In these small islands (Fig. 3a) and thin strands of epithelium (Fig. 3b), cells were strongly immunolabelled by MMP-13 antibody (grade +++), whilst epithelial cells forming thicker strands (Fig. 4) had a clear downregulation of MMP-13 expression. MMP-13 positivity was also detected in the plasma cells found in periapical lesions with epithelium. No staining was detected in the negative controls of periapical lesions.

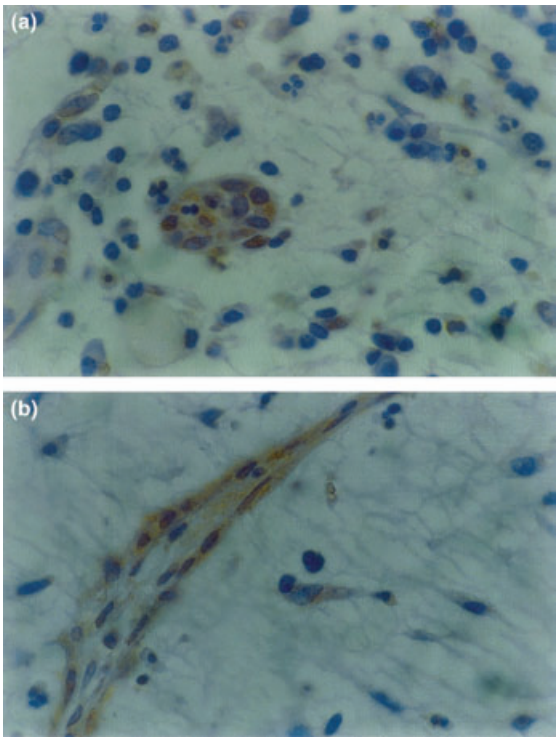

Figure 3 Immunohistochemical staining for MMP-13 in a periapical lesion with epithelium. MMP-13 is upregulated of MMP-13 in these small island (a) and thin strands of epithelium (b). Original magnification ×400.

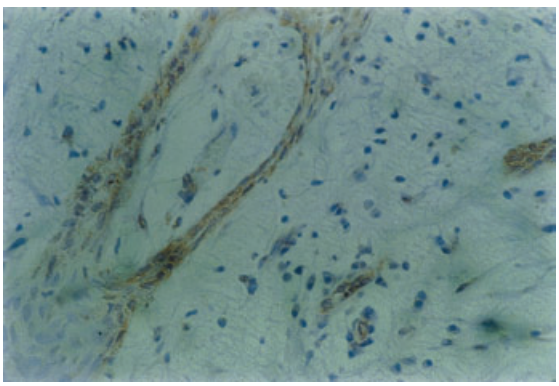

Figure 4 Immunohistochemical staining for MMP-13 in a periapical lesion with epithelium. Epithelial cells forming thicker strands show a clear downregulation of MMP-13 expression, contrary to epithelial cells forming thinner strands. Original magnification ×300.

Discussion

Matrix metalloproteinases collectively degrade extracellular matrix and basement membrane proteins. They

are often involved in chronic inflammation and bone-destructive lesions. Amongst MMPs, MMP-13 has an exceptionally wide substrate specificity compared with other MMPs. In addition to fibrillar type I, II and III collagens, MMP-13 degrades type IV, IX, X and XIV collagens, gelatine, tenacin-C, fibronectin and proteoglycan core proteins (Khasigov *et al.* 2001). MMP-13, originally discovered in breast cancer (Freije *et al.* 1994) is specifically expressed by tumour cells in squamous cell carcinoma of the head and neck and its expression correlates with their invasion capacity (Ala-aho *et al.* 2004). In fact, it is upregulated in the epithelial tumoural cells located at the invading front, which could explain the aggressive behaviour of some kinds of tumours (Alvarez Suarez *et al.* 2004). MMP-13 has also been described in human odontogenic tumours and its expression has often been related to the biological behaviour of the lesions (Wahlgren *et al.* 2003).

Molecular mechanisms of jaw cyst expansion probably involve interactions of some MMPs and the tissue inhibitors of MMPs (TIMPs). Therefore, there is an imbalance between MMP-1 and TIMP-1 production, that may lead to radicular cyst expansion (Lin *et al.* 2002).

An immunopositive reaction for MMP-13, with a varying degree of immunolabelling, that depends on the kind of lesion, was observed in the specimens. As far as plasma cells, macrophage-like cells and fibroblasts, immunolabelling is concerned, the present findings corroborate previous data on tissues other than periapical granulomas without and with epithelium. In fact, a previous study showed that plasma cells express MMP-13 focally in periapical granulomas, and it was therefore concluded that plasma cell MMP-13 has a particularly important role in benign and malignant bone-destructive lesions (Wahlgren *et al.* 2001). MMP-13 has also been associated with adult periodontitis. In these lesions both sulcular epithelial cells and macrophage-like cells can synthesize MMP-13 (Kiili *et al.* 2002). Furthermore, activated fibroblasts may also express MMP-13 (Ravanti *et al.* 1999).

The most original and striking finding of this investigation is the pattern of immunostaining shown by epithelial cells in periapical lesions with proliferating epithelium. In fact, MMP-13 was highly upregulated in the thinner epithelial strands of periapical lesions with epithelium, whilst just the scatter cells were immunolabelled by anti-MMP13 antibody in those areas where epithelium formed thicker strands or in periapical lesions without epithelium.

In man, epithelial rests of Malassez are not proliferative, but a proliferating epithelium is commonly found in inflammatory periapical lesions. The strong MMP-13 immunolabelling observed in the present samples appears to be associated with the migratory state of the epithelial cells.

In conclusion, MMP-13 expression may play a role in the pathogenesis of periapical lesions. Based on the present findings MMP-13 may provide the support for the conversion of a periapical granuloma with epithelium into a radicular cyst, due to the capability of the MMP to influence not only epithelial cell rest migration but also the invasion of the granulomatous tissue. Due to the possible important clinical significance of MMP-13 in the pathogenesis as well in the development of novel therapies (Ala-aho *et al.* 2004, Kirkwood *et al.* 2004, Teronen *et al.* 1997) further studies are required to clarify the roles of this MMP in periapical lesions.

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