Matrix Metalloproteinases & Implication in Periodontitis- A Short Review

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Abstract:
Matrix metalloproteinases (MMPs) are a group of enzymes which are responsible for the degradation of extracellular matrix during normal tissue turnover and also during inflammatory processes. The expression and activity of MMPs in adult tissues is normally quite low, but increases significantly in various pathological conditions that may lead into unwanted tissue destruction, such as inflammatory diseases, tumour growth and metastasis. The role of MMP-8 in periodontitis is the well-known example of the unwanted tissue destruction related to increased activity of MMPs. Degradation of the extracellular matrix may involve four distinct pathways. A body of evidence suggests that matrix components may be dissolved by extracellular matrix metalloproteinase (MMP)-dependent or plasmin (Pln)-dependent cleavage reactions and that larger fragment of matrix may be disposed by a phagocytic pathway by way of cleavage by lysosomal proteinases. Mineralized matrices appear to be degraded by a complex process mediated by osteoclasts which relies on degradation by lysosomal proteinases in a narrow pericellular compartment. Matrix metalloproteinases can specifically cleave and degrade collagens and connective tissue matrix at physiologic pH and temperature. The objective of this review article is to understand the complete mechanisms regulating the expression of MMPs and enzymatic activity is of great importance. Source for all the articles is electronic Pub-Med system, published in between 1997-2011 searched by using keywords like Matrix Metalloproteinases, Periodontitis, Extracellular Matrix, Collagen. Future trend should be directed towards the development of easy, reliable and fast diagnostic tools and the effective therapeutic strategies to reduce the levels of MMPs.

Keywords: Matrix Metalloproteinases, Periodontitis, Extracellular Matrix, Collagen.

Introduction:
The major forms of periodontal disease are thought to occur as bacterial "infections" in which certain microbes play a major part in the initiation and maintenance of the inflammatory process. However, the mechanisms by which organisms destroy connective tissue matrix, periodontal ligament fibers and alveolar bone are not completely understood. Active disease is characteristic of interaction of a number of factors which includes the susceptibility of the host, the presence of pathogenic organisms and the absence of beneficial species. Progression of the disease is manifested by formation of deep periodontal pockets which is mainly due to the destruction of extracellular matrix and alveolar bone loss. There are different pathways for metabolic degradation of extracellular matrix.

Matrix metalloproteinases (MMPs) are a family of Zn2+-dependent endopeptidases capable of cleaving extracellular matrix (ECM) and basement membrane (BM) molecules. During normal tissue remodelling and development, MMPs mediate important functions and their expression and activity is low. But during the destructive pathological conditions like periodontitis, cancer aberrant/increased MMP activity has been reported in several tissues. MMPs are a family of structurally related but genetically distinct enzymes that degrade extracellular matrix and basement membrane (BM) components.

Discovery of MMPs:
Gross and Lapier identified diffusible proteinases capable of degrading fibrillar collagen from involuting tadpole tail which leads to the discovery of MMP-1 in 1962. From there on, several families and subclasses of these MMPs have been characterized. (Table 1) MMP numbering is usually determined by the order of their discovery, MMP-1 being the first. However, MMP-4, -5 and -6 have been eliminated as a consequence of duplication. MMP nomenclature often includes a characteristic name, for example gelatinase, stromelysins etc. MMP-2 was the first purified from a malignant murine sarcoma cell line and also first identified type IV collagenase. MMP-7 has a high affinity for elastin and was isolated from a mixed tumor. MMP-8 was first cloned from the peripheral leukocytes of a patient with chronic granulocytic leukaemia from which messenger RNA (mRNA) was extracted. The MMP-9 was cloned from the HT1080 fibrosarcoma cell line. MMP-13 was first identified from the tumor cell surface and it increases invasiveness of cultured carcinoma cells. MMP-14 was first discovered from carcinoma of human breast and later cloned from interleukin-1 (IL-1) stimulated chondrocytes. MMP-14 was first identified from the tumor cell surface and it increases invasiveness of cultured carcinoma cells. MMP-18 was the first identifiable amphibian collagenase. MMP-26 was cloned from a cDNA library of human endometrial
Matrix metalloproteinases form a family of enzymes that degrade extracellular matrix, cytokine, phospholipids, virulence factors, bacterial endotoxin, cell-membrane associated proteins. In addition, these MMPs can modulate growth factor and cytokine activity. For the regulation, expression of MMPs from particular tissue is necessary. The basal expression of several MMPs (such as MMP-1,-3,-7,-8,-9,-10,-12 and -13) in cultured cells is low, and their transcription is induced by a variety of extracellular stimuli like stress, cytokines, growth factors, chemical agents etc. Tumor necrosis factor-α (TNF-α) is an inflammatory mediator that upregulates many MMPs. This TNF-α is proteolytically processed to a soluble homotrimer. TNF-α and Interleukin (IL)-1β does not stimulate MMP-2 but they stimulate MMP-9 production in keratinocytes of human gingival mucosa. The bacterial lipopolysaccharide (LPS) is a very potent inducer of both MMP-2 and -9. TGF-β is part of a super-family and requires activation to a mature form for intracellular signalling and receptor binding. TGF-β family has a dual role in both inhibitions as well as in activation of MMPs. TGF-β1 is the only member of the collagenase family that is inducible by a single stimulus and its expression is highly dependent on the cell type. Several cytokines, growth factors, and other mediators of inflammation regulate the expression of MMPs. The expression and activity of MMPs in non-immune cells is regulated by the availability of endogenous inhibitors. The release of extracellular matrix by MMPs can modulate growth factor and cytokine activity. Cleavage of the heparan sulphate proteoglycan by MMPs (such as MMP-1,-3,-7,-8,-9,-10,-12 and -13) in cultured cells is low, and their transcription is induced by a variety of extracellular stimuli like stress, cytokines, growth factors, chemical agents etc. Therefore the exact role of TGF-β family is still not known completely.

MMPs can themselves modulate growth factor and cytokine activity. Cleavage of the heparan sulphate proteoglycan by MMP-3 and -13 results in the release of basic fibroblast growth factor. proTNF-α can be processed into the biologically active form by MMP-3 and -13. TGF-β is released from the decorin after it has been cleaved by MMP-2, -3 and -7. TGF-β and Interleukin (IL)-1β does not stimulate MMP-2 but they stimulate MMP-9 production in keratinocytes of human gingival mucosa. Therefore the exact role of TGF-β family is still not known completely.

### Transcriptional Regulation of MMPs:

MMP expression is regulated by proteins of extracellular matrix, cytokine, phospholipids, virulence factors, bacterial endotoxin, cell-membrane associated proteins. In addition, these MMPs can modulate growth factor and cytokine activity. For the regulation, expression of MMPs from particular tissue is necessary. The basal expression of several MMPs (such as MMP-1,-3,-7,-8,-9,-10,-12 and -13) in cultured cells is low, and their transcription is induced by a variety of extracellular stimuli like stress, cytokines, growth factors, chemical agents etc. Tumor necrosis factor-α (TNF-α) is an inflammatory mediator that upregulates many MMPs. This TNF-α is proteolytically processed to a soluble homotrimer. TNF-α and Interleukin (IL)-1β does not stimulate MMP-2 but they stimulate MMP-9 production in keratinocytes of human gingival mucosa. The bacterial lipopolysaccharide (LPS) is a very potent inducer of both MMP-2 and -9. TGF-β is part of a super-family and requires activation to a mature form for intracellular signalling and receptor binding. TGF-β family has a dual role in both inhibitions as well as in activation of MMPs. TGF-β1 is the only member of the collagenase family that is inducible by a single stimulus and its expression is highly dependent on the cell type. Several cytokines, growth factors, and other mediators of inflammation regulate the expression of MMPs. The expression and activity of MMPs in non-immune cells is regulated by the availability of endogenous inhibitors. The release of extracellular matrix by MMPs can modulate growth factor and cytokine activity. Cleavage of the heparan sulphate proteoglycan by MMP-3 and -13 results in the release of basic fibroblast growth factor. proTNF-α can be processed into the biologically active form by MMP-3 and -13. TGF-β is released from the decorin after it has been cleaved by MMP-2, -3 and -7. TGF-β and Interleukin (IL)-1β does not stimulate MMP-2 but they stimulate MMP-9 production in keratinocytes of human gingival mucosa. Therefore the exact role of TGF-β family is still not known completely.

### Implication of MMPs in Periodontal diseases:

Matrix metalloproteinases form a family of enzymes that mediate multiple functions related to periodontal inflammation both in the tissue destruction and immune responses. The expression and activity of MMPs in non-
inflamed periodontium is low but is drastically enhanced in the inflammatory conditions. MMP-2 & MMP-9 levels are increased during inflammatory conditions like periodontal diseases. Consequence of degradation of the extracellular matrix and basement membrane leads to the development of periodontitis. Degradation of ECM is mainly due to the proteinases which are produced either from periodontopathic bacteria or from host itself. In periodontal diseases, matrix metalloproteinases play key roles in the degradation of the extracellular matrix, basement membrane. Many periodontopathic microorganisms like porphyromonasgingivalis are capable of producing bacterial collagenases. Collagenases produced from bacteria or host can be differentiated by many ways. One way to differentiate is by the way they cause collagenolysis. Host collagenase cleaves the collagen at a single site whereas bacterial collagenase attacks at multiple sites. Thus host collagenases leads to the breakdown of collagen in characteristic 3/4 and 1/4 fragments, whereas bacterial collagenase results in more than 200 peptide fragments. The reasons for mammalian collagenase for attacking at only one specific site are relatively low hydroxyproline content and reduced helical stability at that site and presence of the collagenase-susceptible glycine-leucin and glycine-isoleucine peptide bonds at that site. For collagen breakdown to occur it is necessary to first remove their associated proteoglycans and fibronectin. Matrix metalloproteinase-3 is effective in degrading proteoglycans and fibronectin. Some matrix metalloproteinases produced from inflammatory cells such as neutrophils and macrophages are destructive in nature. Intracellular regulation by cytokines, arachidonic acid metabolites and growth factors leads to the excess expression of fibroblast-type matrix metalloproteinases. This is responsible for the increased concentration of collagenase in the periodontal pocket and thus solved the controversy concerning the origin of the excess collagenase in the periodontal pocket. Whereas, Epithelial cells produced MMPs which facilitate the apical migration and lateral extension of the junctional epithelium and thus the subsequent loss of connective tissue attachment. TNF-α has the ability to induce periodontal pocket epithelial cells to produce matrix metalloproteinase-13.

Discussion:

Full-mouth profile of periodontitis patients showed statistically significant relationship between pocket depth and active MMP-8. Gingival epithelium and fibroblasts in periodontal ligament in rat periodontitis model was positively related with MMP-2 and MMP-3 respectively. In 2011 Leppilahti et al. done an interesting study, they evaluated whether oral rinse MMP-8 levels and TIMP-1 level can differentiate the subjects with different periodontal condition; and they concluded that oral rinse MMP-8 together with TIMP-1 analysis may have potential in periodontal diagnosis. Positive correlation was found between MMP-8 concentration in shallow crevices and attachment loss whereas in 2008 higher levels of MMP-8 was seen in saliva and MMP-9 in Gingival crevicular fluid in patients with the periodontitis compared to the gingivitis patients. On the other hand Gingivitis patients showed higher levels of MMP-2. Thus they concluded that MMPs can be used as a diagnostic and prognostic marker for the detection of Periodontitis and gingivitis. Another study detected MMP-1,8,13 activity level which was accounted for 0-1%, 94-96%, 3-4% respectively, of the total collagenase activity in the gingival crevicular fluid of adult periodontitis patients by Western blot analyses. Study done by Lee et al. showed that in progressive lesions total collagenase activity was 50-60% higher and MMP-8 level was 18-fold higher in progressing periodontitis compared to stable periodontitis. Tenget al. published a very interesting study, they monitor MMP-9 activity in gingival crevical fluid for a maximum of 10 months. They analyses the MMP-9 activity from the patients rinse and did not sample individual sites for gingival crevice fluid. They used the subject itself as the unit of investigation. Results shows the 2-fold increase of mean activity of MMP-9 in samples from patients with recurrent attachment loss, and these levels were decreased significantly following adjunctive metronidazole therapy.

All these findings suggest that MMP level is clearly elevated in the inflammatory processes and suggests a promising role for MMP-8 and MMP-9 with regards to periodontitis. However, Mancini and co-worker proposed that MMP levels in gingival crevicle fluid can be used as a novel screening test for active periodontal destruction.

Diagnostic test for MMPs:

Increasing positive correlation between MMPs and periodontitis lead Mantyla et al to develop monoclonal antibodies for MMP-8 which can be utilized in a chair-side test for MMP-8. This leads to the development of a chair-side dip-stick test for MMP-8. This chair side test is a sensitive, specific and rapid. This test is useful in detecting MMP-8 levels in GCF and peri-implant sulcular fluid (PISF). This test measures the GCF MMP-8 level in 5 minutes and can be performed by a dentist rapidly and without any specific equipment. It differentiates healthy and gingivitis sites from periodontitis sites. Also reduction in MMP-8 levels can be observed after successful periodontal treatment. This test is also useful tool for monitoring of peri-implantitis.
Conclusion:
From this review it can be concluded that MMPs are the important family of endopeptidases capable of degrading ECM and basement membrane leading to the periodontal diseases. These MMPs are mainly responsible for the degradation of collagen fibers. High levels of activity are found mainly in gingival crevicular fluid in inflammatory conditions like periodontitis, cancer etc. Therefore detection and reduction of these levels are important for inhibiting the progressive lesions. By keeping all this in mind the therapeutic interventions should focus on reducing the levels of activity MMPs.

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