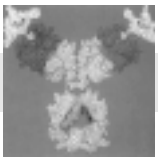

Potential Applications of Matrix Metalloproteinase Inhibitors in Geriatric Practice

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Key words: matrix metalloproteinases, extracellular matrix, degenerative processes, osteoarthritis, aortic abdominal aneurysm, macular degeneration

Abstract

Matrix metalloproteinases are a family of enzymes that degrade different components of extracellular matrix. They play an important role in normal physiologic processes of maintaining tissue integrity and remodeling, as in wound healing, processes of development, and regeneration. However, excessive expression of MMP has been observed in many disease states, including rheumatoid arthritis and osteoarthritis, vascular remodeling in atherosclerosis and aortic aneurysm formation, neoplastic processes, macular degeneration and many others.

IMAJ 2003;5:361–364

Traditionally, the role of extracellular matrix was considered to be structural support and an environment for cell-cell interaction. It has now become clear that ECM is a dynamic structure that plays a fundamental role in various physiologic functions, including specific control of remodeling in many tissues. Vascular and bone remodeling are examples of regulated systems that maintain matrix integrity by synthesis of ECM components followed by proteolytic degradation. The regulation of matrix synthesis and degradation is normally tightly controlled to maintain homeostasis. Matrix synthesis and degradation is regulated by a variety of cytokines [1]. If the balance between synthesis and degradation of extracellular connective tissue shifts towards degradation, it may result in various physiologic and pathologic events, including inflammatory, neoplastic and/or degenerative processes. Such a shift to degenerative process can play a key role in the development of diseases such as osteoarthritis, aortic aneurysm formation and macular degeneration – disorders commonly encountered in geriatric practice. In this paper we review the current literature on the role of MMPs in the pathogenesis of these three pathologic processes and the potential mechanisms for delaying and preventing them.

Biology of matrix metalloproteinases

MMPs are a family of Zn^{2+} and Ca^{2+} dependent enzymes promoting resorption of extracellular matrix in both physiologic and pathologic states. MMPs constitute the major class of enzymes capable of degrading components of ECM, and more than a dozen have been identified, cloned and sequenced.

The stimulation of a wide variety of cell types, including macrophages, synoviocytes, fibroblasts and chondrocytes leads to MMP gene expression and activation of MMP mRNA synthesis. In common with many genes, the inducible MMP genes (MMP1, 3, 9, and 13) contain activator protein 1 transcription factor-binding sites in their proximal promoters, which are required for induction by cytokines. The activation of MMP promoters involves MAPK signaling pathways that, in turn, are activated by interleukin-1 and tumor necrosis factor-alpha [2]. After pro-MMP molecules are assembled, they are secreted extracellularly in a latent inactive form as pro-enzymes. The molecules have to lose their "pro"-piece to acquire proteolytic activity and thereby activate the Zn-binding domain. The activity of MMP is kept under tight control at all three levels: transcriptional, activation of latent pro-enzymes, and functional. Blockade of the MAPK pathway leads to the inhibition of gene expression of MMPs, and has been shown in animal models to inhibit arthritis progression [2]. IL-1, TNFα and platelet-derived growth factor mediate the MAPK pathway and therefore stimulate synthesis of metalloproteinases. In turn, retinoids, gamma-interferon, IL-6 [3], and heparin [4] inhibit MMP synthesis. Corticosteroids non-discretionally inhibit the expression of pro-inflammatory genes and thereby suppress the synthesis of MMP. Plasmin has been demonstrated to be a potent activator of most MMPs by promoting cleavage of the pro-domain to convert an inactive zymogen to an active enzyme [3–5]. Proteolytic function of MMPs is regulated by a family of proteins called TIMPs (tissue inhibitors of metalloproteinases). Some of them bind to active forms of metalloproteinases, forming non-covalent complexes (TIMP-1), or stabilize the inactive form of the enzyme (TIMP-2), thereby inhibiting formation of active proteolytic enzymes [Table 1].

Many disease states are characterized by pathologically excessive degradation of extracellular connective tissue matrix. In addition to the MMP function as a driving force of chemical destruction of ECM, these enzymes were shown to be actively

MMP = matrix metalloproteinases
ECM = extracellular matrix

IL = interleukin
TNFα = tumor necrosis factor-alpha

Table 1. Regulation of matrix metalloproteinases

	Substrate	Stimulatory effect	Inhibitory effect
Transcription	MMPgenes	IL-1, TNFα, PDGF	TGFβ, IL-6, retinoids, heparin, corticosteroids
Activation	Pro-enzymes (zymogens)	Plasmin MT-MMP	TIMP
Functional proteolysis	Active enzymes	Positive feedback from active MMP and proteolysis products	TIMP Tetracyclins Synthetic inhibitors

PDGF = platelet-derived growth factor

Table 2. Major disease targets for inhibitors of matrix metalloproteinases

	Trivial name	Disease
MMP1	Collagenase-1, fibroblast collagenase	Wound healing, osteoporosis
MMP2	Gelatinase A,72 kDa gelatinase	Cancer, atherosclerosis
MMP3	Stromelysin 1	Delayed hypersensitivity, wound healing, rheumatoid arthritis
MMP3	Matrilysin	AAA, cancer
MMP8	Collagenase-2, neutrophil collagenase	Periodontal disease, rheumatoid arthritis, osteoarthritis
MMP9	Gelatinase B92kDa gelatinase	Atherosclerosis, delayed hypersensitivity, cancer, osteoarthritis, abdominal aortic aneurysm
MMP12	Macrophage elastase	Abdominal aortic aneurysm, atherosclerosis
MMP13	Collagenase-3, rat osteoblast collagenase	Periodontal disease, osteoarthritis, rheumatoid arthritis, wound healing, atherosclerosis

involved in the processes of cell-cell interaction and angiogenesis [6,7], playing an important role in different types of tissue injury. MMPs have been identified in the course of inflammatory, degenerative and neoplastic processes [Table 2].

MMP and cardiovascular disease

ECM remodeling associated with smooth cell migration and proliferation was demonstrated in arterial response to intimal injury in atherosclerosis. Zempo et al. in 1996 [6] demonstrated the role of MMPs in smooth muscle cell proliferation, migration and intimal thickening, using synthetic MMP inhibitor BB94 (Bamtistat) *in vitro*. Similar results were shown on the formation of a neointima after balloon injury to the rat carotid artery [6]. Use of Batimstat in another animal model significantly reduced late lumen loss after balloon angioplasty by inhibition of constrictive arterial remodeling, whereas neointima formation was not inhibited by MMP inhibition [8]. MMPs are considered to play a central role in the pathogenesis of abdominal aortic aneurysms, by participating in the same mechanism that is involved in the injury of atherosclerosis. Extracellular matrix protein degradation in the abdominal aorta by metalloproteinases coupled with smooth muscle cell destruction, proliferation, and migration regulated by MMP, leads to a loss of architectural integrity and AAA formation. McMillian and Pearce [9] demonstrated elevated MMP9 plasma level in patients with AAA as compared to patients with symptomatic aorto-iliac occlusive disease and to healthy controls. Moreover, patients with multiple aneurysms had significantly higher levels of plasma MMP9 than did patients with isolated AAA. MMP9 levels were measured in 48 hour supernatants of organ culture tissue explants from the same group of patients and showed increased levels in patients with aneurysm, suggesting that the injured aortic intima could be the source of MMP9 [9]. Saito et al. [4] demonstrated immunoreactivity to MMP9 of macrophages and leukocytes in the adventitia and media and suggested that leukocyte-derived MMP9 is associated with aortic wall degeneration and aneurysm formation. They also hypothesized that activation of MMP9 may be caused by increased tissue-type plasminogen activator levels in the walls of AAA.

Doxycyline was shown to cause a slight reduction of activated gelatinase (MMP2) and significant reduction of MMP9 when given preoperatively to patients with abdominal aneurysms [10]. In addition, Kenagy and colleagues [11] showed that heparin inhibits proliferation of arterial smooth muscle cells *in vivo* and *in vitro,* as well as regulating the production of MMP9 and MMP3 activity. This finding underscores an additional role of heparin in the management of abdominal aneurysm.

MMP and osteoarthritis

Aging of cartilage is characterized by degradation of the collagen fibrils that hold hydrated proteoglycans in place, facilitating mobility of proteoglycans and release of water molecules. This in turn leads to decreased cartilage resistance to compression, creating conditions for cartilage and subchondral bone destruction. MMPs play an important role in matrix degradation in osteoarthritis, and their expression in this disease has been extensively investigated. In osteoarthritis, MMP expression is restricted to active cartilage lesions. Experiments with transgenic mice showed that over-expression of the constitutively active form of the human collagenase 3 gene (MMP13) in hyaline cartilage resulted in erosions closely resembling that seen in osteoarthritis [2]. Masuhara et al. [12] demonstrated high serum levels of MMP3 and MMP9 in a group of patients with rapidly destructive hip osteoarthritis and higher rates of MMP expression by synovial cells in rapidly progressive destructive osteoarthritis as compared to the ordinary form of the disease. The special class of metalloproteinase-related enzymes (aggrecanases, TACE) consists of membrane-bound enzymes with structural similarity to MMP. They possess unique qualities in addition to proteolytic activity of conventional MMPs, being able to cleave some cell surface peptides such as integrins, TNFα, L-selectin, macrophage colony-stimulating factor and Fas ligand, thereby contributing to local cytokine regulation [13]. This makes inhibition of aggrecanases a very attractive dual

AAA = abdominal aortic aneurysm

therapeutic target for osteoarthritis. Suppression of aggrecanases would maintain structural integrity of cartilage by decreasing aggrecan proteolysis and at the same time suppress autocrine cytokine-driven MMP production by chondrocytes, thereby turning off localized cartilage destruction. However, the pharmaceutical industry effort to date has mostly been directed towards MMP inhibition. The rationale is based on the theory of imbalance between excessive MMP levels, coupled with inadequate TIMP expression. This disequilibrium was shown by Martel-Pelletier et al. [14] to facilitate the joint destructive process in osteoarthritic cartilage extracts. Multiple synthetic compounds have been synthesized and used in animal models of osteoarthritis and human clinical trials. RO32-35555 (Cipemastate) is a selective inhibitor of collagenases MMP1, 8, and 13. It was shown to effectively inhibit cartilage degradation, joint space narrowing, and osteophyte formation in the mouse model of osteoarthritis [15]. Another MMP inhibitor, Ro-130830, was shown to be effective in preventing cartilage damage in a rabbit meniscectomy osteoarthritis model [14]. Unfortunately, while initial trends in the development of MMP inhibitors showed promise in animal models and early clinical trails, most had major setbacks due to significant side effects or lack or efficacy. The therapeutic benefits of using MMP inhibitors to treat osteoarthritis remain to be seen. Future osteoarthritis therapies may include cocktails of MMP-directed therapy that targets gene expression, enzyme regulation and/or enzyme activity.

MMP and macular degeneration

Age-related macular degeneration is characterized by accumulation of abnormal deposits between the retinal pigment epithelial basement membrane and the inner collagenous layer of Bruch's membrane, called drusen. These deposits are formed in the extracellular matrix of the retina, partly as a result of failure to dispose of ECM molecules and partly due to an inflammatory immune reaction. Leu [16] gave an excellent review of multiple investigators on the role of MMP and MMP inhibitors in the aging eye. Expression of TIMP-3 immunoreactivity in drusen of the aged eye along with the expression of MMP2 and 9 was repeatedly demonstrated by many investigators. Levels of TIMP expression in age-related macular degeneration were higher than in controls. The theory of imbalance in MMP activity and tissue inhibitors of metalloproteinases leading to degenerative process in the eye was supported by the discovery of TIMP-3 gene mutation in Sorsby's fundus dystrophy – a genetic degenerative disease of macula, resembling age-related macular degeneration. Reduced MMP inhibitory activity and increased secretion and activation of gelatinase A and B were observed in TIMP-3 mutant retinal cell lines with a similar mutation [17]. Although mutations in TIMP-3 have not been detected in age-related macular degeneration, data are available supporting the theory of functional dysequilibrium. Leu and co-workers [16] analyzed the expression from the three major classes of MMPs and TIMPs, and their distribution in retinal pigmental epithelial cells, choroid, drusen particles, and Bruch's membrane in donor eyes with and without history of age-related macular degeneration. They demonstrated that age-related macular degeneration samples had increased expression of MMP. Interestingly, the MMP immunoreactivity was detected only on the surface of drusen, and high TIMP-3 immunoreactivity was noted in the center of the particles. They speculated that drusen is formed as cold spots for proteolysis due to high concentrations of TIMP-3, and their formation in turn contributes to progression of age-related macular degeneration. However, there is another theory of drusen formation, which is based on the MMP involvement in the process of neovascularization that may play a role in later stages of age-related macular degeneration. In their experiments in an animal model of choroidal neovascularization, Kvanta et al. [18] showed enhanced expression of MMP2 mRNA by macrophages with wide distribution in choroids, subretinal space and inner retina and correlation with new vessel growth. The recently published work of Hangai et al. [19] addressed the role of MMP in cellular interaction and regulation of retinal neovascularization, hypothesizing the possible mechanism via exposure of critical regulatory ECM epitopes as a result of proteolysis that leads to initiation of angiogenesis. They demonstrated the co-localization of these cryptic epitopes and MMP9 in early stages; later MMP2 expression was co-localized with angiogenic vessels. It is very tempting to speculate that synthetic MMP inhibitors could be applied to treat acute macular degeneration or other degenerative processes of the eye.

Summary

We have attempted to describe matrix degradation and repair dysregulation leading to MMP/MMPI, which could play a key role in various degenerative processes and is a common attribute of osteoarthritis, abdominal aortic aneurysms and macular degeneration formation, in spite of different tissue targets. Therefore, finding the means of controlling this disequilibrium and targeting MMPs at different levels of the regulatory mechanisms could provide a step towards solving the problem of the degenerative process that lies at the core of multiple illnesses in geriatric practice.

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