Inflamm. res. 50 (2001) 183–186
1023-3830/01/040183-4 $ 1.50+0.20/0

Inflammation Research

Commentary

Selective matrix metalloproteinase (MMP) inhibition in rheumatoid arthritis – Targetting gelatinase A activation

C. Jackson, M. Nguyen, J. Arkell and P. Sambrook

Sutton Arthritis Research Laboratory, Royal North Shore Hospital, St. Leonards NSW 2065 Australia, Fax 612-99266269,
e-mail: cjackson@med.usyd.edu.au

Received 6 July 2000; returned for revision 12 October 2000; accepted by R. Day 23 November 2000

Abstract. The matrix metalloproteinases (MMP) are a large group of enzymes responsible for matrix degradation. They contribute to joint destruction in rheumatoid arthritis (RA) by directly degrading the cartilage and bone and indirectly promoting angiogenesis (formation of new blood vessels). Inhibition of MMPs is a primary therapeutic target in RA. However, the results of limited clinical trials performed to date are disappointing. Improvements in therapeutic benefit may be achieved by targetting specific MMPs. A subclass of the MMPs, the gelatinases, contribute directly to joint destruction as well as being vital during angiogenesis. Gelatinase A is released as a latent enzyme and must be activated to degrade the matrix. It has a unique mechanism of activation on the cell surface involving membrane-type MMP (MT-MMP). Recently, the serine protease, activated protein C (APC), has been shown to directly activate gelatinase A, without requiring MT-MMP. Inhibition of APC represents a selective approach to prevent gelatinase A activation and may prove to be of therapeutic benefit in RA.

Key words: Rheumatoid arthritis – Matrix metalloproteinase – Human endothelial cells – Gelatinases – Activated protein C

Matrix metalloproteinases (MMPs) and Arthritis

MMPs are a family of enzymes that play a central role in extracellular matrix turnover and remodelling based on their ability to hydrolyze major protein components of the ECM [1]. There are currently more than 20 MMPs, which differ in their substrate specificity but share a number of common structural and functional similarities. All MMPs have been assigned an MMP number and most also have a common name. A list of human MMPs involved in arthritis is shown in Table 1. They were originally classified into groups depending upon their substrate specificity. The collagenases degrade fibrillar collagen, stromelysins have relatively broad substrate specificity while gelatinases breakdown gelatin and basement membrane collagens. The most recently discovered MMP26 also degrades the basement membrane [2]. The membrane-type MMPs, unlike other MMPs, are cell-associated.

MMPs are secreted as inactive proenzymes that require activation by the removal of the propeptide, revealing the Zn-binding active site. The regulation of MMP activity occurs at various stages including gene activation and transcription, translation and secretion of latent enzyme, proenzyme activation and inactivation by endogeneous inhibitors, known as tissue inhibitors of MMPs (TIMPs). In normal physiology, MMP activity is associated with various processes such as connective tissue turnover, ovulation, trophoblast invasion, skeletal development, mammary gland involution and wound healing. Loss of control of MMP activity has been implicated in a number of diseases, including cancer, osteoarthritis (OA) and rheumatoid arthritis (RA) [3].

In RA, MMPs contribute to joint destruction in at least two ways. First, they can directly degrade the cartilage and bone. The major MMPs implicated in this process include stromelysin-1, collagenase-1, collagenase-3, gelatinase A and gelatinase B. Second, MMPs are important during angiogenesis (the formation of new blood vessels), which is a prominent feature of rheumatoid arthritis [4]. During angiogenesis, endothelial cells must degrade at least two distinct barriers, the microvascular basement membrane and the interstitium. The gelatinases are vital during these stages [5].

MMP Inhibitors in arthritis

The major contribution of MMPs towards ultimate destruction of the joint has led drug companies to develop agents that inhibit MMPs in arthritis. Possible strategies include

Correspondence to: C. Jackson

Table 1. Some human matrix metalloproteinases implicated in RA.

Class	Common name	MMP	Major Substrates
Collagenases	Collagenase 1	MMP1	Type I, II, III, VIII collagens
	Collagenase 2	MMP8	
	Collagenase 3	MMP13	
Gelatinases	Gelatinase A	MMP2	Types I, IV, V collagens, gelatin
	Gelatinase B	MMP9	
Stromelysins	Stromelysin 1	MMP3	Fibronectin, laminin, collagens IX, X, elastin
	Stromelysin 3	MMP11	
Membrane-type MMPs	MT1-MMP	MMP14	Activate latent gelatinase A
	MT2-MMP	MMP15	
	MT3-MMP	MMP16	
Others	?	MMP19	?

impeding the production of MMPs, blocking the active site of MMPs, increasing endogenous production of TIMPs and preventing the activation of MMPs. Most efforts to date have concentrated on blocking the active site of MMPs. Marimastat was the first orally available MMP inhibitor tested in clinical trials in cancer. It is classed as a broad spectrum inhibitor because it non-selectively inhibits all known MMPs, by binding to the active site. At least two agents have been tested in clinical trials for arthritis, Trocade (Ro 32-3555) (Roche) and BAY12-9566 (Bayer). Trocade, a potent inhibitor with relative specificity for the collagenases (1, 2 and 3), has been trialled in RA patients, whilst BAY12-9566, a relatively selective inhibitor of gelatinases and stromelysin-1 has undergone clinical trial in OA patients. The BAY12-9566 osteoarthritis trial was ceased early when a separate trial of small cell lung carcinoma showed the drug was performing worse than the placebo. Whether the negative findings were specific to the compound or to the small-cell lung cancer population is unknown. Interestingly, BAY 12-9566 differs from most other MMP inhibitors currently in clinical trial for cancer, by not producing musculoskeletal pain as the major side effect. The Trocade trial was stopped due to apparent low efficacy of the drug. Efficacy endpoints were typical of most RA trials and included the number of swollen and tender joints, physician and patient global assessment, ESR and CRP. Whether these outcome measures are appropriate for MMP inhibitors is unclear. Assessment of structural change using MRI would be of interest.

There are a number of possible reasons why MMP inhibitors appear to be unsuccessful to date. These trials have been stopped early and higher efficacy may have been obtained if they were taken to completion. The drugs are taken orally so bioavailability to the synovial joint may be impaired. Leung et al [6] have reported that anti-inflammatory therapy is required to assure entry of an MMP inhibitor into the inflamed joint space. Another factor to be considered is the stage of the disease being treated. Degradation of the bone and cartilage occurs later in the disease whilst angiogenesis appears early and continues to occur throughout the disease. MMP inhibitors may best be suited to treating the disease before cartilage and bone degradation occurs. A further considerable problem is the specificity of MMP inhibitors. Broad spectrum MMP inhibitors, such as Marimastat, block normal matrix turnover [7] and are more likely to cause side-effects and/or mask the beneficial effects of selective inhibitors. Second generation inhibitors, such as Trocade or BAY 12-9566, are more selec-tive in blocking a sub-class of MMPs. Nonetheless, the use of more specific inhibitors that target single MMPs which are involved in RA may be beneficial.

With these approaches in mind, the gelatinases seem ideal targets. Gelatinase A and B are well known for their ability to degrade the basement membrane collagens and partially-degraded collagen [5]. Gelatinase A is particularly important as it can activate collagenase and directly cleave type I collagen at a rate similar to that of interstitial collagenase [8]. As well as directly degrading the cartilage and bone, the gelatinases are vital during angiogenesis, which has been clearly demonstrated using in vitro and in vivo models. For example, Koivunen et al. [9] demonstrated that a synthetic peptide, CTTHWGFTLC, which specifically blocks gelatinases, inhibits endothelial migration in vitro and prevents tumour growth in mice, without any apparent toxicity effect. Itoh et al. [10] have reported a substantial reduction in angiogenic activity as well as in subsequent tumour progression in gelatinase A-deficient mice.

Gelatinase A – a unique MMP

Human gelatinase A is regulated differently to other MMPs at both transcriptional and extracellular levels [5]. The promoter of gelatinase A gene lacks the TRE sequence as well as the known transactivator sequences, AP-1 and PEA-3 [11]. This may explain the lack of gelatinase A upregulation by agents such as phorbol myristate acetate, tumour necrosis factor-α or interleukin-1. In addition, the promoter region of gelatinase A has a unique noncanonical TATA box, which may be responsible for basal secretion of the enzyme [12]. Human endothelial cells, including those derived from RA synovium [13] constitutively secrete latent gelatinase A. The enzyme must be activated to allow for matrix degradation. Activation of gelatinase A is regulated differently to other MMPs (Fig. 1). Gelatinase A does not possess the propeptide sequences of other MMPs that are susceptible to proteolytic activation by other proteases such as plasmin or trypsin. Moreover, unlike most other MMPs where activation occurs in the extracellular milieu, activation of gelatinase A can occur on the cell membrane via another MMP, membrane-

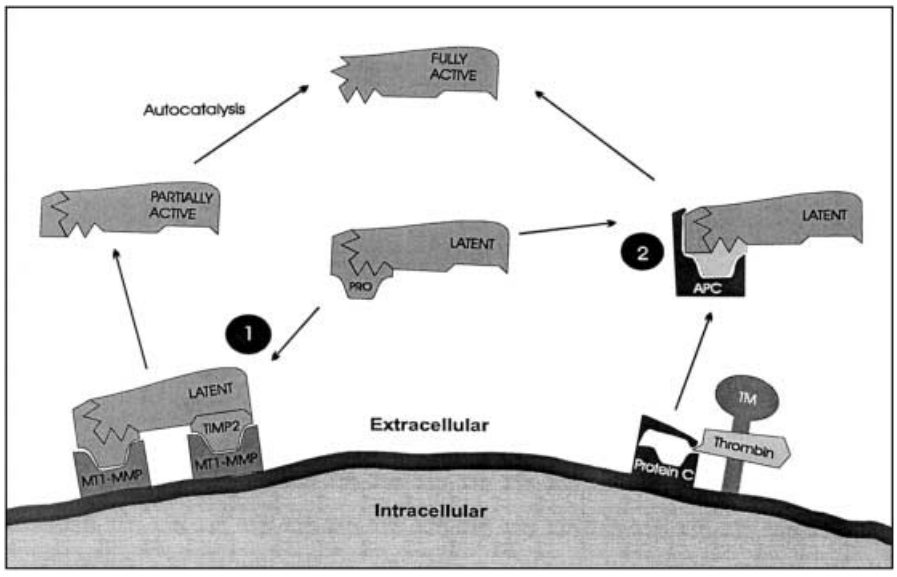

Fig. 1. Activation gelatinase A on the surface of endothelial cells. At least two separate mechanisms are known to activate gelatinase A on the endothelial surface. 1. MT1-MMP. TIMP-2 binds to the catalytic domain of active MT1-MMP. Latent gelatinase A interacts with TIMP-2 forming a trimolecular complex allowing a nearby free MT1-MMP to partially activate progelatinase A by initiating a cleavage in the propeptide domain of progelatinase A. The partially active gelatinase A is released and subsequently autocatalysed to the fully active form. 2. APC. Thrombin binds to thrombomodulin on the endothelial surface and activates protein C to form APC. APC directly and rapidly activates latent gelatinase A to the fully active form.

type MMP (MT1-MMP). Type I collagen, a common component of the extracellular matrix, activates gelatinase A by upregulating MT1-MMP [14]. Thrombin has recently been shown to be a rapid and efficient activator of gelatinase A in human endothelial cells. Thrombin operates via a novel pathway that is independent of MT1-MMP [15]. Preventing the activation of gelatinase A in arthritis may be achieved by inhibiting MT1-MMP, collagen or thrombin. However, all of these molecules have multifactorial actions and inhibition in RA is likely to cause side-effects.

Recently, we have shown that the serine protease, APC activates gelatinase A in human endothelial cells [16]. APC is generated by the interaction of thrombin and thrombomodulin activating protein C on the endothelial cell surface. Unlike some other serine proteases such as thrombin or plasmin, APC is a relatively selective enzyme, being responsible for physiological anticoagulation. The importance of APC as an anticoagulant is reflected by the findings that deficiences in this molecule result in familial disorders of thrombosis [17]. Activation of endothelial gelatinase A by APC is rapid and efficient [16]. It does not require MT1-MMP and occurs in the absence of cells, indicating that it acts directly. We have recently shown that thrombin-induced activation of gelatinase A is mediated through APC (J.Arkell, M.Nguyen and C. Jackson, unpublished observations). Since thrombin levels are dramatically increased in RA [18], it is feasible that APC plays an important role in activating gelatinase A in this disease.

Interestingly, the ability of APC to stimulate gelatinase A activity appears to be independent of its anticoagulant activity. This is evidenced by the inability of its natural inhibitor, protein C inhibitor, to prevent gelatinase A activation (J. Arkell, M. Nguyen and C. Jackson, unpublished observations). These findings suggest that APC would make an ideal target for preventing gelatinase A activation. Inhibition of APC's ability to activate gelatinase A, without interfering with APC's anti-coagulation effect, makes an attractive therapeutic approach to RA.

Conclusion

Although early clinical trial results of MMP inhibitors in arthritis have not been encouraging, different approaches may lead to success. If the second generation of MMP inhibitors prove not to be successful, it is likely that a new third generation of specific MMP inhibitors will arise. These inhibitors would target single MMPs and alter their regulation at designated levels. Inhibition of gelatinase A activation induced by APC is one such target which may prove to be useful in RA.

References

[1] Birkedalhansen H. Role of matrix metalloproteinases in human periodontal diseases. J Periodontology 1993; 64: S474–S84.
[2] Park HI, Ni J, Gerkema FE, Liu D, Belozerov VE, Sang QXA. Identification and characterization of human endometase (Matrix Metalloproteinase-26) from endometrial tumor. J Biol Chem 2000; (In press).
[3] Cawston TE. Metalloproteinase inhibitors and the prevention of connective tissue breakdown. Pharmacology & Therapeutics 1996; 70: 163–82.
[4] Walsh DA. Angiogenesis and arthritis. Rheumatology 1999; 38: 103–12.
[5] Murphy G, Crabbe T. Gelatinase A and B. Methods in Enzymol 1995; 248: 470–84.
[6] Leung MK, Greewald RA, Ramamurthy NS, Moak SA, Koszulinski R, Dieudonne D et al. Tenidap and flurbiprofen enhance

uptake of matrix metalloproteinase inhibitor 4-dedimethylaminotetracycline in inflamed joints of adjuvant arthritic rats. J Rheumatol 1995; 22: 1726–31.
[7] Michaelides MR, Curtin ML. Recent advances in matrix metalloproteinase inhibitors research. Curr Pharm Des 1999; 5: 787–819.
[8] Aimes RT, Quigley JP. Matrix metalloproteinase-2 is an interstitial collagenase. J Biol Chem 1995; 270: 5872–6.
[9] Koivunen E, Arap W, Valtanen H, Rainisalo A, Kantor C, Gahmberg CG et al. Tumor targeting with a selective gelatinase inhibitor. Nature Biotech 1999; 17: 768–74.
[10] Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res 1998; 58: 1048–51.
[11] Frisch SM, Morasaki JH. Positive and negative transcriptional elements of the human type IV collagenase gene. Mol Biol Cell 1990; 10: 6524–32.
[12] Templeton NS, Stetler Stevenson WG. Identification of a basal promoter for the human Mr 72,000 type IV collagenase gene and emhanced expression in a highly metastatic cell line. Cancer Res 1991; 51: 6190–3.
[13] Jackson CJ, Arkell J, Nguyen M. Rheumatoid synovial endothelial cells secrete decreased levels of tissue inhibitor of MMP (TIMP1). Ann Rheum Dis 1998; 57: 158–61.
[14] Nguyen M, Arkell J, Jackson CJ. Three-dimensional collagen matrices induce delayed but sustained activation of gelatinase A in human endothelial cells via MT1-MMP. Inter J Biochem Cell Biol 2000; 32: 621–31.
[15] Nguyen M, Arkell J, Jackson CJ. Thrombin rapidly and efficiently activates gelatinase A in human microvascular endothelial cells via a mechanism independent of active MT1 matrix metalloproteinase. Lab Invest 1999; 79: 467–75.
[16] Nguyen M, Arkell J, Jackson CJ. Activated protein C directly activates human endothelial gelatinase A. J Biol Chem 2000; 275: 9095–8.
[17] Baker WF, Bick RL. Treatment of hereditary and acquired thrombophilic disorders. Semin Thromb Hemostasis 1999; 25: 387–405.
[18] Morris R, Winyard PG, Blake DR, Morris CJ. Thrombin in Inflammation and Healing – Relevance to Rheumatoid Arthritis. Ann Rheum Dis 1994; 53: 72–9.