Metalloproteases and tendinopathy

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Summary

Matrix metalloproteinases (MMP) are involved in the development of tendinopathy. These potent enzymes completely degrade all components of the connective tissue, modify the extracellular matrix (ECM), and mediate the development of painful tendinopathy. To control the local activity of activated proteinases, the same cells produce tissue inhibitors of metalloproteinases (TIMP). These latter bind to the enzyme and prevent degradation. The balance between the activities of MMPs and TIMPs regulates tendon remodeling, whereas an imbalance produces a collagen dis-regulation and disturbances in tendons. ADAMs (a disintegrin and metalloproteinase) are cell membrane-linked enzymes with proteolytic and cell signaling functions. ADAMTSs (ADAM with thrombospondin motifs) are secreted into the circulation and constitute a heterogenous family of proteases with both anabolic and catabolic functions. Further studies are needed to better define the mechanism of action, and whether these new strategies are safe and effective in larger models.

KEY WORDS: metalloproteases, tendinopathy, tendon healing.

Introduction

Tendinopathy is a spectrum of disorders varying from an asymptomatic condition of ultrasound (US) changes to severe pain, discomfort, and frank functional impairment. Biomechanical factors, functional alterations, aging, and metabolic disorders may predispose to it. Diagnosis is usually clinical but, at times, it needs to be confirmed by US and MRI. However, the definitive verdict comes from histology. This is a "failed healing response" to overuse or stress tendon injury, with haphazard proliferation of tenocytes, intracellular abnormalities in tenocytes, disruption of collagen fibers, and a subsequent increase in non-collagenous matrix[1,2]. These intratendinous changes may be part of normal ageing[3,4]; however, the exact pathophysiologic mechanism is unclear.
Many molecular changes occur within tendinopathic tendon, but much attention has to be paid to better understand the role of matrix metalloproteinases (MMP) in tendinopathy[5,6]. MMPs are a family of 24 zinc-dependent endopeptidases activated after proteolysis of an inactive pro-form. They degrade the extracellular matrix[7-12], but they are also involved in many normal and pathological processes: embryonal development, ovulation, wound healing, periodontitis, tumor invasion and metastasis, and soft tissue remodeling after injury[7,13-15]. In addition, MMPs are implicated in the degeneration process of the intervertebral disc[16,17] and of loosening after hip arthroplasty[18,19]. On the other hand, they are reversibly inhibited by tissue inhibitors of metalloproteinase (TIMPs), natural endogenous inhibitors of the MMPs[12,20,21]. The aim of this review is to describe the mechanism by which MMPs and TIMPs are involved in tendon remodelling, collagen dysregulation, and development of tendinopathy[22,23].

Enzymes of the extracellular matrix

The turnover of the extracellular matrix is a dynamic process in which synthesis and degradation are finely

balanced[24,25]. The matrix metalloproteinases (MMPs) are potent molecules able to completely digest and degrade the connective tissue and change the properties of the extracellular matrix (ECM)[26]. They take also part regulation to the inflammatory response mediated by chemokine and cytokine signaling and by neoepitopes expressed from the ECM[27]. There are 4 main groups of MMPs, organized based on the substrate preference of these enzymes. The most well known enzyme is the collagenase, synthetised by connective tissue fibroblasts[28]. Collagenases MMP-1, -8, and -13 cleave all subtypes of collagen, especially the triple helix fibrillar collagens types I, II, and III which confer mechanical strength to tissues[7-12]. Collagenase 4 (MMP-18) is often omitted from the list of human MMPs, but it has been found in human ligaments[29]. MMP-2 and MMP-14 play collagenolytic activities.

MMP-2 and -9 are gelatinases which degrade smaller collagen fragments released, and cleave denatured collagens and type IV collagen. Membrane-type MMPs (MT-MMPs) are cell membrane-linked proteases engaged in many activities. Stromelysins degrade proteoglycans, fibronectin, casein, collagen types III, IV, and V. Stromelysins (MMP-3 and -10) and matrilysin (MMP-7) are broad-spectrum proteinases engaged in the activation of other MMPs[30].

Once secreted into the extracellular space in the form of proenzymes (pro-MMP), some MMPs are stored within the cells (e.g. MMP-9 in neutrophil granules), others bind cellular membranes (e.g. MT1-MMP). These pro-MMPs are activated in the extracellular space by proteolytic cleavage[31-33]. The baseline production of MMPs is low, but their synthesis may be induced by cytokines (interleukin -1, -4, -6, and -10, and tumor necrosis factor-α), growth factors, extracellular MMP inducer (EMMPRIN) and cell-cell or cell-matrix interactions. These are stimulator mediated by signal intracellular pathways including the mitogen-activated protein kinase pathway[34-38].

Therefore, the structure and properties of the ECM vary based on the balance of tissue formation and breakdown. The remodeling process is followed by a repair response in which collagen fibers are involved[39]. Even though it is not properly defined how the new connective tissue is formed, it is know that new collagen is synthesized, some collagen fibers are destructed (turnover) of, part of fibers are weaved, and new collagen takes place in the existing structure of the tendon[40]. This regulation of all this is at levels of genetic transcription, pro-MMP activation, and inhibition of active enzymes.

The local activity of such activated proteinases is controlled by tissue inhibitors of metalloproteinases (TIMP). Released by the same cells which produce MMPs the inhibitors are bind to the enzyme, and prevent degradation[41]. The fine balance between MMPs and endogenous inhibitors is crucial to maintain the dynamic homeostasis and integrity of the extracellular matrix, and to control tendon remodeling. On the other hand, when there is some imbalance, collagen formation and tendon homeostasis undergo dis-regulation[22]. MMPs and TIMPs are also engaged in the development, morphogenesis, reproduction, tissue remodeling, apoptosis, and evolution of rheumatoid arthritis and osteoarthritis[42,7,13,10]. When referring to osteoarthritis and rheumatoid arthritis[20], it is somewhat oversimplified to consider MMPs as being solely tissue-degrading enzymes. Four main TIMPs reversibly inhibit all MMPs based on a 1:1 interaction with the zinc-binding site[36]. TIMP-1, -2, and -4 may are in various tissues, including the vascular system; TIMP-3 is sequestered within the ECM[43]. They also regulate angiogenesis and cellular proliferation[44]. Other factors such as the soluble α-1-antitrypsin and α-2-macroglobulin[45] and cell membrane-linked MMP inhibitors[43] may also work as additional endogenous inhibitors of MMPs. MMPs may also have anti-inflammatory action reasonably mediated by anti-inflammatory cytokines and chemokines[46,47].

ADAMs and ADAMTS

Two other groups of proteases are related to the MMPs. ADAMs (disintegrin and metalloproteinase) are cell membrane-linked enzymes involved in proteolysis and cellular signalling. ADAMTSs (ADAM with thrombospondin motifs) belong to a family of proteases with anabolic and catabolic activities, but their functions are only partially known. ADAMs bind to the cell membrane; ADAMTS are released into the peri-cellular space and in circulation. Thirty-three ADAMs have been identified, involved in the proteolysis of other membrane-bound proteins (e.g. growth factors in precursor state), cell signalling, and cell adhesion processes[48]. The well known types of ADAMTS are 19, distinguished into 4 groups. The aggrecanases (ADAMTS-1, -4, -5, -8, -9, -15, and -20) have proteoglycanolytic action, regulate angiogenesis and degradation of other proteins. ADAMTS-2, -3, and -14 belong to the second group, all with anabolic function and acting as procollagen N-propeptidases for collagen types I, II, and III. An ADAMTS-2 mutation has been discovered in Ehlers-Danlos syndrome type VII C (a condition with fragile skin, joint laxity, and hernias); ADAMTS-13, as cleavage factor of the von Willebrand factor, is supposed to be involved in thrombotic thrombocytopenic purpura. The function of the fourth group (ADAMTS -6, -7, -10, -12, -16, -17, -18, and -19) is still unclear[49]. TIMP-3 is the main TIMP which inhibits activity against some of the ADAMs and ADAMTS[48].

Other MMPs inhibitors

Tetracycline antibiotics are pharmacological inhibitors of MMPs. They usually bind to the zinc site of the MMP enzymes, and block their activity. Doxycycline is probably the most potent MMP inhibitor, by inhibiting MMPs -1, -2, -7, -8, -9, -12, and -13[50,51]. They also inhibit MMP gene expression level, and reduce the activation via the inflammatory cascade and through reactive

oxygen species. Chemically modified tetracyclines may prevent unwanted effects on the endogenous microbial flora. For instance, low-dose doxycycline, 20 mg twice a day instead of the standard dose of 100 mg twice a day, still retains its MMP-inhibitory efficacy[52,53], but may reduce effects on the microbial flora of the vagina or the gut[54]. Synthetic MMP inhibitors are a large heterogeneous group of compounds, modified to produce increased inhibitory potency and increased specificity against particular MMPs[55]. Bisphosphonates, inhibitors of osteoclastic bone resorption, also have potent MMP-inhibitory properties, probably through cation-chelation of zinc[56,36].

Metalloproteasis in tendinopathy

MMPs degrade the extracellular matrix and may predispose to painful tendinopathy and tendon rupture[57,6]. Associations between variants of the MMP-3 gene and painful Achilles tendinopathy have been found[58]. Although enzyme inhibitors have not been extensively evaluated in the treatment of tendinopathy, 2 trials have showed that patients with patellar and Achilles tendinopathy may be well responsive to peritendinous injections of aprotinin, a general protease inhibitor[59,60]. In a randomized trial of patients with Achilles tendinopathy, combined aprotinin and eccentric exercises did not significantly improve outcomes when compared to placebo, but results were satisfactory in both groups[61]. Aprotinin is no longer available in most countries to use as an injectable MMP inhibitor (in small doses), even though it had been used in Europe for tendinopathy since the 1970s[60]. The major indication for aprotinin was to reduce bleeding in major surgery (esp. cardiac) using large intravenous doses. Recent data have recently shown that the risk profile outweighs the benefit for this indication[61]. Aprotinin also has been shown to predispose to anaphylactic reactions in some cases[62,63] without any superiority to other injectables products such as PRP (platelet rich plasma) or glucose prolotherapy. Tranexamic acid, which inhibits MMPs indirectly, through plasmin inhibition[64], could theoretically be used in tendinopathy, but laboratory and clinical evidences would be required.

The neurotransmitter substance P (SP) is a pain mediating neurotransmitter[65,66] which may regulate the gene expression of MMPs and TIMPs in fibroblasts[67], and would be responsible for altered regulation profile of MMPs and TIMPs. When SP is administered exogenously, it seems to enhance proliferation of fibroblasts and tendon healing[68,69]. Achilles tendinopathy could be a model tendinopathy and, probably it will be a model for treatment studies, but the molecular profiles of other tendinopathies, such as supraspinatus tendinopathy, should be taken into account[70].

There is evidence of rotator cuff disease associated with altered matrix composition. Specifically, total collagen concentration is reduced whereas the proportion of type III collagen is significantly increased[71]. Changes in collagen composition have been also detected in macroscopically normal tendons before rupture. This suggests that an altered pattern of collagen synthesis and turnover, probably related to the aging process, may precede the occurrence of chronic tendinopathy and, over time, tendon rupture. It has been shown that human cuff tendons may produce MMPs and TIMPs when placed in an organ culture[22]. In torn rotator cuff tendons, both mRNA and protein levels of MMP-13 are increased. Rotator cuff tendons contain type I collagen[72,73]. MMP-13, MMP-1 and MMP-8 degrade type I collagen. In both tearing and remodeling of rotator cuff tendons[74], MMP-13 cleaves gelatin much more efficiently than MMP-1 and MMP-8[9,10,12,74]. MMP-13 induce excessive degradation of the extracellular matrix, and is play a main role in pathological conditions such as osteoarthritis, rheumatoid arthritis, cutaneous and intestinal ulcers, and periodontal inflammation[11,75,12]. However, degradation is important for healing and remodeling of connective tissues[13]. The increased MMP-13 levels may be expression of active tissue remodeling or of a healing response after injury; it is unknown whether these levels are secondary to the effects of rotator cuff tearing itself or responsible of the pathogenesis of rotator cuff tearing[70]. The fact that MMP-3 mRNA (stromelysin) levels are decreased in torn rotator cuffs suggests that MMP-3 may be the result of failed matrix remodeling and inadequate homeostasis of tendons[70,71,76].

No significant difference between MMP-1 mRNA levels of normal and torn tendons have been found[70], but Yoshihara et al.[77] and Zhen et al.[78] demonstrated elevated levels of MMP-1, MMP-3, and glycosaminoglycans in the synovial fluid in patients with massive rotator cuff tears. The MMP-2 activation during the healing process after supraspinatus tendon tearing may induce extracellular matrix degradation in both the tendon edge and reparative tissue[70,79].

Metalloproteasis in tendon healing

Data from unloaded healing rat flexor tendons have suggested that MMP-9 and MMP-13 mediate tissue degradation during the early phase of healing, whereas MMP-2, -3, and -14 mediate both tissue degradation and later remodeling[80]. As observed in an experimental study, systemic treatment with the MMP inhibitor doxycycline weakens rat Achilles tendons during healing, highlighting the key role of MMPs in tendon healing[81]. Specific MMPs may be deleterious for tendon healing whereas MMP inhibitors could enhance it. For instance, MMP-7 levels inversely correlate with tendon strength in humans[82], whereas MMP-13 expression, strongly up-regulated in rotator cuff rupture[70], could be responsible of tendon degradation and stress-deprivation[83]. In rabbits undergoing anterior cruciate ligament reconstruction, intra-articular injections of endogenous MMP inhibitor α-2-macroglobulin improve strength and histological features of tendon to bone healing[84]. In tendon rupture, transient reduction of the tendon strength may occur immediately after

surgical repair[85,86]. At that stage, MMPs may induce degradation close to the sutures, making the tendon tissue around the suture weak[87]. Even if MMPs inhibition could improve tendon suture-holding capacity, doxycycline-coated sutures could be used to promote early tendon suture-holding capacity in a rat model[88].
TIMP-2, TIMP-3, and TIMP-4 mRNA levels are decreased in suffering tendons[7]. High levels of TIMP-3 are associated with cells undergoing apoptosis both *in vitro* and in *vivo*[43,89-92]. Because there is an increased number of apoptotic cells tendon tears compared to controls, decreased TIMP-3 mRNA levels suggest that TIMP-3 may not play a role in apoptosis in tendon tearing[70]. On the other hand, the expression of TIMP-1 controls and inhibits the excessive degradation of the matrix by MMP-2[79].
Local administration of a-2- macroglobulin, an endogenous MMP inhibitor, at the greater tuberosity footprint induces histological changes at the healing enthesis after rotator cuff repair[93], with a statistically significant reduction in local collagen degradation 2 and 4 weeks after the operation. The reduction of MMP activity was associated with increased formation of fibrocartilage 2 weeks after the operation, and improved collagen organization after 4 weeks. The local administration of an MMP inhibitor in the peri-operative period may favor the tendon-bone healing[65].
Membrane type 1 matrix metalloproteinase (MT1-MMP, also called MMP-14) is a membrane-bound matrix metalloproteinase involved in the embryologic development of musculoskeletal tissues[94]. Gulotta et al., in a recent study on rats, have hypothesized that this gene involved in the formation of tendon-to-bone insertion sites during embryogenesis, could induce regeneration[95]. They found significantly improved outcome in tendon-to-bone healing after application of adenoviral MT1-MMP transduced MSCs compared to application of MSCs alone. In rotator cuff surgery, the over-expression of MT1-MMP leads to improved biomechanical strength over the tendon-bone interface after 4 weeks from the index surgery. The exact role of MT1-MMP in this process is unknown, but it in involved in cell surface activation of MMP-2, and proteolytic activity[96]. Tendon to bone healing would be induced by 2 mechanisms. Membrane type 1 matrix metalloproteinase may digest unwanted scar tissue and restore an environment similar to the native insertion site. The second mechanism would be based on COX-2 inhibition[97]. MT1-MMP probably up-regulates COX-2, with beneficial effects on tendon healing, by inducing bone and cartilage formation in the fibrovascular scar tissue.
Doxycycline-mediated inhibition of interstitial collagenase (MMP-13) favorably influences early healing after tendon repair: collagen organization, biomechanical and histologic parameters are significantly improved[98]. The exact mechanism by which tetracycline antibiotics inhibit MMP 13 remains to be defined[98].

Conclusions

Biologic modulation of endogenous MMP activity to basal levels may reduce pathologic tissue degradation and favorably influence healing after tendon disease[70,77-79]. Further studies are needed to better define the mechanism of action, and whether these new strategies are safe and effective in larger models.

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