Controlling Extracellular Matrix Degradation: Is The Promised Land In Sight?

or

Why I take a matrix metalloproteinase inhibitor every morning

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Many disease states, ranging in severity from periodontal disease to highly invasive metastatic tumors, are characterized by degradation of extracellular connective tissue matrix. The major components of ECM — the collagens, glycosaminoglycans and proteoglycans, elastin, laminin, etc. — are all subject to enzymatic degradation by multiple mechanisms, some quite specific and others very general. In the last two decades, we have identified pathologic increases in at least a dozen such enzymes during the course of inflammatory, degenerative and neoplastic processes, providing potential targets for development of pharmacologic agents. Both broad-based and specific inhibitors of many ECM-degrading enzymes are now approaching clinical utility. Although we have made great progress in alleviating the symptoms and signs of many inflammatory and degenerative diseases, preventing ECM destruction has been an elusive goal. In this brief review, I will outline the potential usage of inhibitors of matrix-degrading enzymes for human diseases, as well as the pitfalls that have slowed the pharmaceutical development of these agents and kept many of them on the drawing board.

Extracellular connective tissues generally consist of several classes of macromolecules that are associated with a number of additional small molecules and proteins. If the macromolecules are destroyed by physical disruption or enzymatic action, the tissues lose mechanical stability and impaired function results. For most connective tissues, the major macromolecular constituents are the collagens glycosaminoglycans, which occur either free or in association with protein (proteoglycan), elastin, laminin, fibronectin, etc.

The major class of enzymes capable of degrading these molecules is known as the matrix metalloproteinases. Clearly, excessive degradative action of one or more MMPs without accompanying repair will have an adverse effect on the integrity of the ECM. For example, the biomechanical properties of cartilage depend in large part on the incompressibility of water. Water is held in cartilage by heavily hydrated proteoglycans, which in turn are held in place by collagen fibrils. If the collagen fibrils are degraded, making the proteoglycans mobile, or if the proteoglycans themselves are directly attacked, loss of water leads to loss of the normal resistance to compression, which is the hallmark of healthy cartilage. Thus there is a strong rationale for inhibiting the action of enzymes that can degrade important structural macromolecules.

The Matrixins

There is a large class of zinc-dependent proteolytic enzymes known as the metzincins. Within this superfamily are four major subfamilies, one of which is the matrixins, or MMPs. The MMPs are the major degrading enzymes for many components of the ECM. This group [Table 1] includes the collagenases, gelatinases, stromelysins, membrane-type MMPs, as well as matrilysin and macrophage metalloelastase. A second relevant enzyme family, known as adamolysins (ADAMs and ADAMTSs, *vide infra*), includes members that possess sheddase or disintegrin activity.

MMPs have certain general properties. Most such enzymes have five areas of primary structure, namely, a) an initial signal peptide, b) a pro-domain that is cleaved off to convert an inactive zymogen to an active enzyme, c) a catalytic domain, d) the zinc-binding domain, and e) a hemopexin-like domain involved in substrate selectivity and binding. MMPs act extracellularly, are proteolytic, can be inhibited by chelators such as 1,10 o-phenanthroline, and can

ECM = extracellular connective tissue matrix

MMPs = metalloproteinases

Table 1. Current nomenclature for matrix metalloproteinases

MMP Number	Trivial name	MMP Number	Trivial name
1	collagenase-1, fibroblast collagenase	11	stromelysin-3
2	gelatinase A,72 kDa gelatinase	12	macrophage elastase
3	stromelysin 1	13	collagenase-3,rat osteoblast collagenase
4	not used	14	MT1-MMP
5	not used	15	MT2-MMP
6	not used	16	MT3-MMP
7	matrilysin	17	MT4-MMP
8	collagenase-2, "neutrophil" collagenase	18	collagenase-4
9	gelatinase B, 92kDa gelatinase	19	no trivial name
10	stromelysin-2	20	enamelysin

be inhibited by naturally occurring proteins known as TIMPs (tissue inhibitor of metalloproteinase). Once upon a time, MMPs were discovered by looking for enzymes that could degrade tissue constituents whose quantities were diminished by disease or during development. With the advent of molecular biology, MMPs and related enzymes are now routinely discovered and categorized by screening cDNA libraries for conserved amino acid sequences such as the cysteine switch (PRCGxPD) or the catalytic zinc-binding site (HExGHxxGxxHS/T) [1]. There are many variations on the basic MMP structural theme, not all enzymes meet all criteria, and chimera can be made that are functionally active despite deletion of certain elements.

The substrates for the MMPs also vary widely. In general, collagen in its triple-helical, undenatured form usually cannot be attacked by any protease other than a specific collagenase. Gelatin, i.e., denatured collagen, can be degraded by many proteases, but the gelatinases, MMP-2 and MMP-9, play a prominent role in much pathology. The list of MMP substrates (remembering that much of the data come from *in vitro* experimentation) also includes other non-fibrillar collagens, myelin basic protein, α1-antiproteinase, substance P, laminin, elastin, fibronectin, link protein, vitronectin, fibrillin, entactin, and heparan sulfate. Some MMPs even serve to catalytically activate other MMPs, often in conjunction with other MMP-related systems, e.g., the integrins. Such cascades of inter-related events clearly complicate pharmacologic intervention.

A new class of MMP-related enzymes has recently attracted much attention: the ADAMs or ADAMTSs [2]. ADAM stands for *a d*isintegrin *a*nd *m*etalloprotease; several similar enzymes have additional thromobospondin motifs and are now being called ADAMTS enzymes. In contrast to MMPs 1 to 13, these enzymes are membrane-bound and can cleave (shed) certain cell surface peptides such as the integrins, TNF-α, TGF-α, L-selectin, M-CSF, or Fas ligand (thereby earning the alternate designation as sheddases). They are classed with the MMPs based on structural similarities involving their catalytic domains and zinc-binding motifs.

The importance of this latter group in the current discussion is twofold. First, the major structural proteoglycan of cartilage, called aggrecan, is lost in end-stage arthritis, and it has long been believed that inhibition of aggrecan destruction would be therapeutically useful for chronic arthritic conditions. Although aggrecan can be cleaved by many proteases, including MMP-8, elastase and stromelysin, it was recognized several years ago that a unique cleavage signature that represented the action of an enzyme called aggrecanase could be identified. After several years of research, two forms of aggrecanase have finally been identified, and they both appear to belong to the ADAMTS family (ADAMTS-4 and ADAMTS-11) [3].

Secondly, there is a major debate in the MMP-inhibitor field (discussed below) regarding the advantages of broad spectrum vs. highly specific inhibitors. Part of that debate extends to inclusion of anti-TACE activity (TACE = TNF-α converting enzyme = ADAM 17). If TNF-α is a phlogistic cytokine and reduction of TNF-α levels is beneficial in a disorder such as rheumatoid arthritis, then wouldn't a dual enzyme inhibitor — one hitting both a connective tissue degrader as well as TACE — be doubly beneficial? Many inhibitors of conventional MMPs are also inhibitors of TACE [4].

The Rationale for Inhibition

The first vertebrate collagenase was discovered by Gross and Lapierre [5] observing the resorption of the metamorphosing tadpole. Within a few short years, excess collagenase had been identified in several human disease states, including dystrophic epidermolysis bullosa, periodontal disease, and rheumatoid arthritis. By 1969, several potential inhibitors had been characterized. During the ensuing 30 years, literally thousands of MMP-inhibitory compounds have been developed, and the potential market for such drugs is immense. Table 2 lists the major disease targets that have attracted the attention of the MMP-inhibitor industry and the enzymes primarily being targeted. Even in an industry where drug sales of $200 million per year is considered small change, the prospect of lifelong treatment

TIMP = tissue inhibitor of metalloproteinase

TACE = TNF-α converting enzyme

Table 2. Major disease targets for inhibitors of matrix metalloproteinases

Disease	Enzyme targets (MMP numbers)
Periodontal disease	MMP-8, MMP-13
Rheumatoid arthritis	MMP-8, MMP-13, MMP-3, aggrecanases
Osteoarthritis	MMP-8, MMP-13, aggrecanases
Cancer	MMP-2, MMP-9, MMP-7, MMP-13
Atherosclerosis/plaque rupture MMP-13	MMP-2, MMP-9, MMP-7, MMP-12,
Abdominal aortic aneurysm	MMP-2, MMP-7, MMP-9, MMP-12
Delayed hypersensitivity	MMP-3, MMP-9
Wound healing	MMP-1, MMP-3
Osteoporosis	MMP-1, MMP-13
Multiple sclerosis	

for the patient populations listed in Table 2 would certainly be expected to drive the development of many MMPIs.

There are, of course, natural inhibitors of MMPs. Many tissues contain proteins called TIMPs, of which four varieties have now been described; and in fact, TIMP inhibition is now often used as a criterion for categorizing a putative MMP as such. For a while, the pharmaceutical industry explored the use of recombinant TIMPs as therapeutic agents, but with the exception of possible gene transfer technology, the logistics of introducing a TIMP into the affected tissue are overwhelming (parenteral administration, purification, stability, cost, etc.). The proteins α1-antiproteinase and α2-macroglobulin are circulating natural inhibitors, but clearly, in disorders where ECM degradation ensues, these natural inhibitors are overwhelmed.

At one time or another, virtually every major pharmaceutical company has had an MMPI program. From 1987 to 1994 there were roughly 5 to 12 new MMPI patents per year; in 1995–1997 the number ranged from 23 to over 50 per year [6]. Is it not curious that it took 37 years after the discovery of the first MMP for the first product (low dose doxycycline, marketed as Periostat, approved in October 1998) to reach the market for the express purpose of inhibiting pathologically excessive collagenase. In fact, Periostat, the only such drug currently available, is not a low molecular weight agent specifically designed for the purpose of MMP inhibition, but a common pharmacologic agent found serendipitously to have a rather remarkable new property.

There are several obvious approaches to development of an MMPI (or inhibitor of any other enzyme, for that matter). First, one could hope to decrease expression of the enzyme at the mRNA level. It is therefore perhaps of interest that doxycycline has been shown to reduce expression of both iNOS [7] and MMP-8 [8]. Secondly, since most, if not all, MMPs are synthesized as inactive zymogens, one could try to suppress activation. *In vitro*, MMPs can be activated by many mechanisms; *in vivo*, the probable mechanism would be proteolytic, but the enzyme most likely to do the job has not been identified, so no specific inhibitor can be designed. The most common approach has been to directly inhibit the activity.

It is far beyond the scope of this discussion to delve into the numerous chemical approaches that have been attempted for development of MMPIs; many reviews are available [6,9–12]. The initial concepts were built around peptides that would mimic the collagen cleavage site, but oral bioavailability proved to be a formidable problem. Structure-based design has in some instances given way to mass screening. The pharmaceutical industry, using recombinant enzymes and peptide substrates, plus combinatorial chemistry to generate thousands of low molecular weight compounds, combined with X-ray crystallography and computer modeling to check the interaction of the MMPI with the active cleft of the enzyme, has had no trouble generating a large number of MMPIs with nanomolar (sometimes picomolar, sometimes micromolar) potency against selected targets. For example, AG3340, an MMPI in advanced stages of clinical evaluation, has picomolar effectiveness against gelatinases, stromelysin, and collagenase-3, but not collagenase 1 [13]. These problems of bioavailability have been substantially addressed by the medicinal chemists.

One conceptual problem that may have affected development of MMPIs has been findings from studies of knockout mice. Obviously, there is no greater inhibition possible than the knockout. In many instances, knockout mice do not appear to be any more or less susceptible to induced disease models than wild type controls, suggesting limited utility for a specific pharmacological inhibitor. But mice are not men, and there is a difference between knockout deletion of an enzyme starting in embryonic life versus pharmacological regulation of enzyme activity in an adult organism. Biologic systems are exceedingly complex and redundant, as are diseases such as rheumatoid arthritis, and it is naive to think that a simplistic approach based on a solitary target will by itself have a uniformly beneficial effect on the course of a complex disease.

The debate about specificity continues to plague the field. First, since there are at least a dozen potential enzyme targets, some companies have chosen to focus on one particular enzyme target which they believe to be at the heart of the disease target, e.g., the gelatinases (MMP-2 and/or -9) for cancer, or MMP-13 for arthritis. The chemists have infinite confidence in their ability to design highly selective inhibitors, much as they did for COX-1 and COX-2. Thus, the musculoskeletal side effects initially reported with the British Biotech drugs marimastat and batimistat, and attributed to concomitant inhibition of constitutive MMP-1 along with the intended effect on inducible MMP-8 and/or -13, led to second-generation agents that were designed to target primarily the latter and spare MMP-1. Secondly, since TACE is related to the MMPs, and some MMPIs were

MMPIs = MMP inhibitors

shown to be TACE inhibitors, some programs have focused on dual inhibition while others have tried to spare TACE. It remains unclear which approach will prove more useful.

Disease Targets

Arthritis was the first major target for MMPI development programs, but in the past 3 years cancer has emerged as the area in which many companies have elected to do their clinical trials, partly because the endpoints of the studies are perhaps easier to ascertain. Both rheumatoid arthritis and osteoarthritis, as well as atherosclerosis, osteoporosis, and abdominal aortic aneurysm are all disorders that would require long, expensive trials to establish efficacy. Periodontal disease, for reasons discussed in detail elsewhere [14], is a wonderful target disease in which the role of MMPs is clear, and in which demonstration of successful MMP inhibition along with clinical efficacy is relatively easy to prove. Thus it is perhaps no surprise that the only marketed agent with FDA-approved labeling as an MMPI is a sub-antimicrobial dose of doxycycline (20 mg b.i.d.) for adult periodontitis. One reason for this is the phenomenon referred to as *in vivo veritas*!

Many compounds are known with *in vitro* IC_{50} or Ki values in the nanomolar or even picomolar range, which are at best marginally effective in animal models; those that make it into the clinic are of course the exceptions. In contrast, certain tetracyclines, whose activity is comparatively unimpressive *in vitro* (inhibitory parameters that are low micromolar or high nanomolar), are nevertheless highly effective in animal models and in human disease. It is a great leap of faith from a 96-well microtiter plate to which you add an octapeptide substrate and purified recombinant enzyme to an organ or tissue where a blizzard of pathologic events is occurring simultaneously. In the MMPI field, there is no substitute for animal models and exploratory clinical trials.

Thus we come to a formidable problem: how do you prove that a drug designed to prevent connective tissue degradation actually does so in human disease? In animal models of rapidly progressive disease where large samples of tissue can be harvested ad lib, proving efficacy is relatively easy. In human rheumatoid arthritis or osteoarthritis for example, where tissues cannot be readily sampled, this is a very difficult proposition. The gold standard, i.e., radiologic progression, requires serial studies over time spans of 2 or more years combined with labor-intensive interpretations by trained observers. We know from three decades of experience that proving analgesic and/or anti-inflammatory effectiveness, which can be done over a short time frame, will have virtually no predictive value regarding progression of disease. An investigator faced with the goal of proving that a new drug can truly prevent joint damage faces the daunting prospect of a frighteningly expensive multi-year trial involving large numbers of patients and the monitoring of many markers of disease activity. I suggest that one of the major reasons for the lack of current availability of an MMP inhibitor is that although a potential drug may pass the *in vitro* and animal screens, the problems of proving efficacy in humans are so great that such agents are often shelved prematurely [15].

To address this problem, it is first necessary to clarify what endpoint is being sought. There is little reason a priori to believe that an MMPI, for example, would be anti-inflammatory in the conventional sense, i.e., relieve pain, swelling and stiffness. Surely any patient with rheumatoid or osteoarthritis being treated with an MMPI would also be receiving maximal anti-inflammatory and/or disease-modifying anti-rheumatic drug therapy, so it seems senseless to me to assess an anti-arthritic MMPI the way one would assess a non-steroidal anti-inflammatory drug. If MMP-9 is involved in cancer metastasis and/or local invasion, and the MMPI at hand is not cytotoxic, then the endpoint is tumor growth, number of metastases, or some correlate thereof, e.g., survival.

One answer to this dilemma might be the use of surrogate diseases and surrogate markers. As I have argued elsewhere [14], adult periodontitis is a good surrogate disease for rheumatoid arthritis. The pathophysiology is highly analogous, but ease of tissue sampling, shorter length of study, and rapidity of progression make this condition much easier to assess. Few other surrogate diseases come to mind, but surrogate markers for many diseases are now of great interest. Since collagen and proteoglycan, for example, have distinct MMPI-mediated cleavage products for which assays are now in development, such molecular moieties can be used as surrogate markers [16].

It has long been a basic tenet of the MMP field that triple-helical collagen can only be degraded by a collagenase. If excessive collagen breakdown occurs, elevated levels of breakdown products should become apparent in serum and/or urine, and specificity is enhanced if the breakdown products contain moieties known to be collagen-specific. It follows that if a collagenase inhibitor is administered therapeutically in a situation in which excessive collagenase activity transpires, there should then be a decrease in the amount of collagen-unique end product(s) of digestion that can be detected. Since there are assays now available for at least five different collagen cross-link degradation products, these appear to be the appropriate surrogates for demonstration of efficacy *in vivo* for human disease [16]. I believe that this is where the primary outcome for MMP inhibitors lies.

There is in fact only one study in the entire literature in which a collagenase inhibitor was administered orally to a group of patients suffering from enhanced connective tissue degradation due to increased collagenase levels. The results showed that both the pathologically elevated enzyme levels were normalized and that excess collagen breakdown was also reduced. This was a study of sub-antimicrobial dose doxycycline given to a small cohort of subjects with periodontal disease [17]. Gingival collagenases (MMP-8 and -13) and ICTP (a collagen degradation marker) in gingival crevicular fluid were both normalized by the MMP

inhibitory treatment. Interestingly, it appears that MMP-13 was the major target in these tissues.

As of September 1999, this author knows of Phase II/III MMPI clinical trials underway for cancer (Agouron, British Biotech, Bayer), rheumatoid arthritis (Bayer, Roche), age-related macular degeneration (Agouron), and osteoarthritis (Roche). A non-industry trial with doxycycline is underway for osteoarthritis (Dr. K. Brandt, Indiana) and others are being planned for plaque rupture, osteoporosis, adult respiratory distress syndrome, and abdominal aortic aneurysm. In many instances, routine clinical data are being supplemented by the "banking" of serum, urine, and tissue samples in the hope that surrogate markers will become available to support the clinical data.

In the meantime, since I happen personally to be afflicted with periodontal disease, and since sub-antimicrobial dose doxycycline has no apparent toxicity (including alteration of normal flora), I am contently taking this drug, knowing that I may well be minimizing the chances of plaque rupture, aneurysm expansion, neoplastic invasion, and perhaps other MMP-mediated phenomena yet to be attributed to this ubiquitous and multi-faceted class of enzymes.

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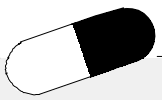
Capsule



Timing vaccinations

Mass vaccination has reduced the incidence of childhood infections in many countries. Vaccination campaigns are also associated with changes in the pattern and spatial synchrony of epidemics. Rohani et al. analyzed weekly incidence data for measles and whooping cough in England and Wales from 1944 to 1994, during when vaccination campaigns led to changes in the dynamics of the diseases. Although vaccination disrupted pre-existing synchrony in measles epidemics, it had the opposite effect on whooping cough and induced synchrony where none previously existed. Models suggest that differences in the incubation periods underlie the different progress of the two infections. These results have implications for the timing of pulsed mass vaccination for whooping cough.

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