Novel Biologic Therapies—Metalloproteinase Inhibitors

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1. Introduction

Recent developments have increased our understanding of the different disease mechanisms involved in equine traumatic arthritis and osteoarthritis (OA). This knowledge has been recently reviewed and has led to the identification of new targets for therapy. It is felt that there is a clear need for new therapeutic approaches that block precise steps in the disease processes and there has been development of these in recent years.1

The destruction of articular cartilage and bone is the final stage of OA and traumatic injuries to the joint. As previously reviewed, aggrecan and collagen, the two major structural components of cartilage, are degraded. In normal cartilage, aggrecan attracts and holds water within the tissue, which swells against a type II collagen fibrillar network. This organization of the extracellular matrix of articular cartilage supplies tensile strength while allowing resistance to compressive forces. Aggrecan can be relatively readily replaced by the chondrocytes in cartilage, but degradation of the collagen network is believed to be a permanent change leading to irreversible damage. It has therefore been implied that collagen breakdown is the key therapeutic target.2

It is reasonably well accepted that the initial degradation of collagen is associated with a group of collagenases belonging to the matrix metalloproteinase (MMP) family, in particular, MMP-1, MMP-8, and MMP-13 collagenase-1, -2, and -3, respectively). Another member of the MMP family (MMP-3 or stromelysin-1) has been shown to degrade proteoglycans and to activate other MMPs, including the aforementioned collagenases. The MMPs often require specific stimulation, such as by the proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), to upregulate their production.3 The MMPs are synthesized as inactive proenzymes and require activation before their substrates can be degraded. Once activated, these enzymes can be inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs),4 which are also generated by chondrocytes, and by the broad spectrum enzyme inhibitor α2-macroglobulin (α2M).

Any attempt to prevent tissue damage by targeting MMPs could follow a number of different strategies. These include blocking the production of MMPs (e.g., glucocorticoids, cytokine inhibitors), inhibiting those factors responsible for activation of proMMPs or directly inhibiting the activated enzymes by either increasing the amount of TIMPs available or adding exogenous MMP inhibitors.5,6

2. Inhibition of Metalloproteinases as a Therapeutic Approach

The direct inhibition of MMPs has been studied for a number of years as a direct therapeutic approach to arthritis. The antibiotic tetracycline shows some activity as an inhibitor of collagenase. Direct inhibitory activity is noted primarily against MMP-8 and the collagenases also autodegrade in the presence of this class of compound.1 Greater activity has been reported with some chemically modified tetracyclines (CMTs),7 and although data from animal models suggest that the levels of some MMPs are reduced, there was no overall reduction in bone and joint damage.8 The CMT doxycycline (Periostat®) has been recently approved for use in the treatment of adult periodontitis. The advantage of tetracyclines is that they are in clinical use and have been able to provide some data on inhibition of MMPs as a therapeutic strategy. However, the disadvantages are that they are all of relatively low potency, and being already licensed, research funding to validate their use could be difficult.
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Peptide-Based Inhibitors

Numerous peptide-based inhibitors were initially synthesized, including hydroxamic acids (batimastat, marimastat, Ro32-3555), carboxylic acids, phosphorus-containing inhibitors, and sulfur-containing inhibitors (D2163, D5410), however problems were experienced with these in clinical trials. Hydroxymate inhibitors were relatively potent with activities in the nanomolar range but were often not active in vivo after oral dosing because they were rapidly metabolized. Improvements have been made but effective inhibitors will need to remain biologically active, both targeting and penetrating the cartilage matrix at sufficient concentrations to inhibit the putative enzymes.

Nonpeptidyl Inhibitors

These include BAY12-9566, which is the MMP inhibitor that we are studying, as well as the CMTs (doxycycline) and possibly the biphosphonates (alendronate, clodronate, etidronate).

Naturally Occurring Inhibitors

As implied above, there are naturally occurring MMP inhibitors including the TIMPs as well as α2 macroglobulin and N-3 fatty acids (fish oils). Recent work has demonstrated that N-3 fatty acids as found in fish oils will inhibit MMPs and aggrecanase, a recently discovered and key enzyme in the degradation of aggrecan.9,10

There are a number of issues to consider when evaluating MMP therapy. The first is deciding which MMP(s) to target. Broad spectrum inhibitors will tend to inhibit many MMPs (potentially including those yet to be discovered) plus enzymes that belong to closely related classes. Moreover, broad spectrum inhibitors may lead to undesirable side effects, as observed in clinical trials with marimastat. It is a broad spectrum MMP inhibitor with which people were getting very sore shoulders (thought to be related to disruption of normal remodeling with excessive fibrosis). The alternative is to utilize highly specific inhibitors if the destruction of tissue in a particular disease is caused by one or two identifiable MMPs.

3. Clinical Trials in Humans

Currently, a number of compounds are being developed for the treatment of rheumatic diseases. The initial trials of MMP inhibitors involved patients with rheumatoid arthritis (RA) as the destruction of tissue is more rapid here than in OA. It has been recommended that patients should be treated in the early stages of disease rather than in an established disease where most tissue destruction has already occurred.1 Different mechanisms are likely to be responsible for the modulation of these enzymes in RA compared to OA. The destruction of collagen in OA is more focal and also the metabolic rate for the tissue as a whole is increased. This makes it more difficult to treat by specifically blocking degradation as there are also focal areas of excess synthesis with the formation of osteophytes. Identification of a single MMP as a factor in these clinical diseases would enable development of a highly specific inhibitor and its testing. This would presumably help to avoid some of the side effects described above for the broad spectrum inhibitors.

 Obviously, before MMP inhibitors can be used routinely as drugs, the musculoskeletal side effects need to be eliminated. We need to ensure that treatment is not associated with fibrosis and that it does not interfere with wound healing. MMP inhibitors could have an effect on joint inflammation by blocking the release of inflammatory proteins from the cell surfaces, but it is likely that MMP inhibitors will have to be combined with therapies that also target different stages of the disease process such as pain and swelling. Moreover, sensitive methods will need to be available to screen the response to treatment, and the use of biochemical markers of skeletal tissue metabolism has been proposed to aid in monitoring this response.11

4. Experience with MMPs in Veterinary Medicine

Although we have done in vitro work with the MMP inhibitor BAY12-9566 using equine and canine articular cartilage explants in an IL-1 degradation model, our in vivo work is restricted to the dog. However, it gives us insight into the potential of MMP inhibition in the treatment of articular cartilage degradation as occurs in arthritis.

In evaluating the effectiveness of the MMP inhibitor BAY12-9566, we have evaluated its ability to inhibit glycosaminoglycan (GAG) release from articular cartilage explants on exposure to 10 ng/ml of IL-1α, as well as measuring collagenase-cleaved type II collagen release using the COL2-3/4C_short12 and 234CEQ assays.13 We demonstrated that the 50% inhibitor concentrations (IC50s) of the nonpep-
The study suggests a significant role for stromelysin-1, the gelatinases and/or a membrane bound MMP, MT1-MMP, in the generation or release of cleaved type II collagen from IL-1 stimulated articular cartilage. This is based on the observations that the IC$_{50}$ for cleaved collagen release (7 nM) was approximately 300- to 10,000-fold lower than the inhibitory constants of BAY12-9566 for MMP-1, -8 and -13 and only 2- to 70-fold lower than IC$_{50}$ for MMP-2, MMP-3, MMP-9 and MT1-MMP. Stromelysin-1 (MMP-3) has been shown to significantly enhance collagenase activity. Some inhibition of MMP-3 may lead indirectly to lower levels of cleaved triple helical type II collagen through a subsequent reduction in collagenase activity. MT1-MMP can digest native fibrillar type II collagen and can induce progelatinase and procollagenase activation cascades. The IC$_{50}$ for proteoglycan degradation (140 nM) approximates the IC$_{50}$ for MMP-3, supporting involvement of this enzyme in aggrecan catabolism, although inhibition of the recently characterized proteinase “aggrecanase” by this MMP inhibitor cannot be ruled out. One must also consider the potential for more concentrated tissue levels of the inhibitor due to the higher protein-binding characteristics of BAY12-9566.

5. Summary

There are a number of requirements of MMP inhibitors for in vivo efficacy. These include:

- Oral bioavailability (peptide-based inhibitors are rapidly degraded and inactivated in the GI tract)
- Targeting and penetration of cartilage
- Nonantigenic (nonimmunogenic)
- Nontoxic (possess a short half-life)
- Broad-spectrum (increased side effects) vs MMP-specific (which MMPs are important?)
- Concomitant therapy (NSAIDs, analgesics)
- Noninvasive monitoring of therapeutic response (markers and imaging)

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References


