PREVENTION OF CARTILAGE BREAKDOWN BY MATRIX METALLOPROTEINASE INHIBITION—A REALISTIC THERAPEUTIC TARGET?

Many existing therapies for the rheumatic diseases relieve the pain and swelling associated with the disease, but have little effect on the underlying destruction of tissue and the corresponding loss of function. Although Voltaire cynically stated that ‘The art of medicine is the ability to keep the patient entertained whilst the disease runs its inevitable course’, rheumatologists have long expressed the need for drugs that prevent joint damage. The second-line agents currently in use, so-called disease-modifying drugs, have mainly been adapted for the rheumatic diseases from other specialities and the mechanism and/or target of action is not always clear. Although some of these drugs do slow the progression of joint destruction [1], recent developments have increased our understanding of the different disease mechanisms involved and led to the identification of new targets. There is a clear need for new therapeutic approaches that block precise steps in the disease processes and some of these are in the early stages of development [1].

The destruction of connective tissues such as cartilage and bone is the final stage of many diseases of the joint. Both the major proteins of cartilage, proteoglycan and collagen, are degraded. In normal human cartilage, proteoglycan pulls water into the tissue and swells against a type II collagen fibrillar network. This structure gives cartilage its tensile strength whilst allowing it to resist compression. Whilst proteoglycan can be readily replaced by the chondrocytes in cartilage, the loss of the collagen network leads to irreversible damage. This makes collagen breakdown a key therapeutic target [2]. Of the four main classes of proteinases that can degrade proteins, the serine and metalloproteinases act extracellularly at neutral pH and could therefore be responsible for the breakdown of the extracellular matrix [3].

Collagen can be specifically cleaved by the collagenases, members of a family of zinc-containing enzymes called the matrix metalloproteinases (MMPs). MMPs can degrade proteins of the extracellular matrix and often require specific stimuli to upregulate their production [4]. The pro-inflammatory cytokines upregulate many MMPs which are synthesized as pro-enzymes and require activation before substrate can be degraded [5]. The active forms of each enzyme can be inhibited by tissue inhibitors of metalloproteinases (TIMPs), inhibitors that specifically block the action of this family of proteinases [6]. If the amount of active MMPs exceeds the locally available TIMPs, then connective tissue turnover is the result.

There are three human collagenases (MMP-1, MMP-8 and MMP-13) and there is good evidence that these enzymes are closely involved in the turnover of collagen in joint tissues in health and disease. In situ hybridization techniques have been used to measure collagenase gene expression in synovial and cartilage tissue from diseased joints [7–10]. Other studies have measured MMP protein in these tissues by immunohistochemistry [11]. MMPs have been extracted from diseased tissues and assayed by activity or using immunooassays [12, 13]. Finally, the levels of MMP protein have been measured in synovial fluids and serum samples from patients [14, 15]. If these proteinases are involved in tissue destruction, then they could represent a new therapeutic target that would allow the prevention of tissue destruction. Any attempt to prevent tissue damage by targeting the MMPs could follow a number of different strategies. These include blocking the production of MMPs, inhibiting the enzymes responsible for activation of proMMPs, increasing the amount of TIMPs available, thus blocking MMP activity, or directly inhibiting the activated enzymes (reviewed in [16], [17]).

The direct inhibition of MMPs as a therapeutic approach has been followed for a number of years. 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. Greater activity is seen with some modified tetracyclines [18, 19] and collagenolysis is inhibited in vivo and in vitro [20]. Recent data from animal models suggest that the levels of collagenase and gelatinase are reduced, but with no overall reduction in bone and joint damage [21]. Patient studies have resulted in contradictory findings, although they do suggest that, in combination with NSAIDs, these compounds protect joints, and further studies are in progress with newer derivatives of these compounds [18, 22]. The obvious and clear advantage of this class of compounds is that they are already in clinical use and could give useful data on the inhibition of MMPs as a therapeutic strategy. The disadvantages are that they have a relatively low potency and, as they are licensed for a considerable period of time, the cost of extensive and lengthy trials will be difficult to find.

Other more targeted approaches have been followed. MMPs are related to the metallopeptidase angiotensin converting enzyme (ACE) and synthetic inhibitors have been made that block the action of ACE. These inhibitors have a chelating group (hydroxamate) attached to a peptide mimic of the sequence of amino acids cleaved by the enzyme in angiotensin I. When these inhibitors were screened against MMPs, it was discovered that a stereoisomer, inactive against ACE, gave significant inhibition. This early lead allowed the
synthesis of inhibitors that mimicked the cleavage site in collagen attached to a chelating group that had activity in the micromolar range [23–25]. Other approaches to developing specific inhibitors have attempted to copy the peptide sequences in the propeptide region of MMPs or to mimic sites cleaved during the activation of MMPs or in certain inhibitors such as z-2-macroglobulin.

These early inhibitors, although relatively potent with activities in the nanomolar range, were often not active in vivo after oral dosing as they were metabolized. Considerable effort was made to overcome these problems and a number of inhibitors are now available that are biologically active and potent [26–28]. The design of orally active inhibitors is still largely empirical; removal of peptide bonds prevents proteolysis and limiting the number of hydrogen bond donors increases absorption [29], presumably by lowering the energy required to pass from the aqueous to the lipid environment of the gut wall membrane. Effective inhibitors will need to remain biologically active and penetrate the cartilage matrix.

Further improvement of MMP synthetic inhibitors was possible after the crystal structures of the active site cleft of collagenase-1, collagenase-2 and stromelysin-1 became available. Potent inhibitors with specificity for individual enzymes are now possible. For example, a series of inhibitors were made for MMP-2 that were shown to be highly specific for this enzyme [30] and some inhibitors have been shown to be specific for MMP-13 [31].

A number of issues remain controversial. It is not known what type of MMP inhibitor should be tested first. Broad-spectrum inhibitors tend to inhibit all MMPs (including any not yet discovered) plus enzymes that belong to closely related classes. Thus, in early trials, in addition to sparing joint tissue destruction, these synthetic inhibitors also reduced joint pain and swelling. This effect was mediated by inhibition of the tumour necrosis factor alpha (TNF-α) convertase which belongs to a different but closely related family [32, 33]. However, broad-spectrum inhibitors may lead to undesirable side-effects (see below). Alternatively, highly specific inhibitors could be used if the destruction of tissue in a particular disease was caused by an individual MMP.

It is not yet known which will be the most appropriate collagenase to target specifically. MMP-1 is widely distributed, MMP-13 is found in chondrocytes and osteoblasts. MMP-8 is stored in neutrophils. MMP-1–TIMP-1 complexes can be found in rheumatoid synovial fluid, indicating that MMP-1 is activated within the rheumatoid joint [15]. Convincing evidence for a role of MMP-13 in cartilage degradation in vitro was presented where synthetic inhibitors targeted to MMP-13 were effective at concentrations where MMP-1 was not inhibited [29]. MMP-1 is not found in rodents, and yet cartilage and bone degradation proceeds in rodent models of arthritis. MMP-13 also has a much wider substrate specificity and can effect further cleavage of collagen at the N-terminus of the triple helix. Some workers have suggested that as MMP-13 cleaves Type II collagen most efficiently of all the collagenases, then this enzyme is responsible for normal turnover whilst MMP-1 might be especially damaging in disease. Others suggest that MMP-13 is responsible for the majority of damage caused in the arthritides. There is no doubt that there are differences in specificity, location and control mechanisms with these two collagenases. Chondrocytes are also able to produce MMP-8 in addition to MMP-1 and MMP-13, and recent work has shown that this collagenase is also found in osteoarthritistic cartilage tissue [34] in addition to being stored in neutrophils. Mitchell et al. [10] showed that out of six osteoarthritic (OA) cartilages, five had MMP-1 present, four had MMP-13 and some samples had one collagenase with no trace of the other. It is likely that all three collagenases are important, but to differing degrees in different diseases.

As synthetic MMP inhibitors block the metastatic traffic of tumour cells, angiogenesis, and tumour invasion in animal models, then the early clinical trials were conducted in patients with various cancers [35, 36]. Whilst proving to be partially effective in blocking the progression of different tumours, treatment was associated with joint pain, particularly in the shoulder and the hand, in a time- and dose-dependent way in about a third of patients on the highest dose. These symptoms would disappear if treatment was stopped for a period of time. One explanation for the occurrence of these side-effects is that the MMP inhibitors are blocking the normal turnover of the connective tissues surrounding the joint. These symptoms can be reproduced in animal models (both rat and marmoset) and new compounds under development can now be screened in rodent models to eliminate compounds with this side-effect profile. Whether it is sufficient just to screen these compounds in rodent models is debatable since the MMP profile of humans and rodents is obviously different in relation to the collagenases.

Currently, a number of compounds are being developed for the treatment of the rheumatic diseases. The initial trials of MMP inhibitors will take place in rheumatoid arthritis (RA) as here the destruction of tissue is more rapid than in OA. Patients should be treated in the early stages of disease rather than in established disease where most tissue destruction has already occurred. 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. If direct evidence of the involvement of a single collagenase were to emerge in any of the rheumatic diseases, then highly specific inhibitors could be tested in this disease. This might avoid the side-effects seen previously and at least one
lead compound targeted for the arthritides has been abandoned because of similar side-effect profiles.

Before these compounds can be used routinely as drugs, we need to eliminate the musculoskeletal side-effects and ensure that treatment is not associated with fibrosis or interferes with wound healing. MMP inhibitors may have an effect on joint inflammation by blocking the release of inflammatory proteins from cell surfaces, but it is likely that MMP inhibitors should be combined with therapies that also target different stages of the disease process such as pain and swelling and preventing the trafficking of inflammatory cells into the joint [37]. New methods need to be developed to screen the response to treatment as the breakdown of cartilage is slow and a number of biochemical markers have been proposed that could aid in following the response to treatment [37]. If the release of connective tissue degradation products from the tissues is blocked by synthetic MMP inhibitors, this may have an effect on the chronicity of the disease if such products are responsible for continually stimulating the immune system in some joint diseases.

Effective inhibitors that are orally active are available and data from MMP structures are allowing specificity for individual MMPs to be introduced. We will await the results of the first trials of MMP inhibitors in patients with joint diseases with interest.

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References