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Disease-modifying anti-osteoarthritic drugs: current therapies and new prospects around protease inhibition

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Abstract

Although osteoarthritis is commonly found in the elderly, the pathophysiological mechanisms of this degenerative disease are still poorly understood. Among the many factors leading to cartilage degradation, the proteolytic activity of a panel of enzymes seems to play a major role, leading to the cleavage of collagen and proteoglycans, the two main components of cartilagenous matrix. Aspartic, cysteine, serine and metalloproteases have been detected in or around the osteoarthritic articulation and their enzymatic activity is reviewed here. The cartilage-sparing properties of the respective inhibitors are listed, giving rise to the hypothesis that some of these compounds could be developed as chondroprotective agents.

Keywords: Osteoarthritis; Protease inhibitors; Proteases

1. Introduction

Osteoarthritis (OA) is probably the most common joint disorder in the world, and radiographic evidence of OA is found in the majority of people aged 65 years and above. Despite this important health impact, the causes of the pathology remain unclear, and effective preventive strategies still remain a distant goal. The key pathological feature is focal destruction of articular cartilage, associated with changes in subchondral bone. Consequent reparative mechanisms at the bone level include bony sclerosis and osteophyte formation [1]. The present contribution will first rapidly analyse the few physical and pharmaceutical possible treatments currently available; we will further concentrate on the pathophysiological role of a series of proteases and their exact implication in the degradation of cartilage function.

2. Structure of articular cartilage

Cartilage is a conjunctive tissue whose structure is specifically adapted to the effects of load, by virtue of the unique elasticity and compressibility conferred by its molecular constituents. A network of type II collagen fibrils provides tensile strength and stiffness, and the interstices are occupied by a hydrated proteoglycan gel. Water is released at the articular surface when compressive loading is applied, and the process is reversible when removing the load. This efficiency is impaired with OA, with fibrillation and ulceration of cartilage being the most dramatic structural changes. Proteoglycan (PG) content will decrease as a result of degradation by different proteases, although repair processes tend to limit the ongoing general degradation [2]. It is noteworthy that the cartilaginous tissue is characterized by an absence of innervation and vascularization, which makes the selective delivery of active substances a challenge for the medicinal chemist.

3. Current therapies available

A large number of recommendations are given to the OA patient by the physician: reduce body weight, use a stick when walking, do regular physical exercise to maintain joint motion and muscle strength. In terms of drug therapies, by far the most used class of drugs are the non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and ibuprofen making up the bulk of alternative treatments [3]. The recent marketing of COX-2 inhibitors, which spare the gastrointestinal tract while maintaining potent anti-inflammatory and analgesic activities, may change the prescription profile in the near future.

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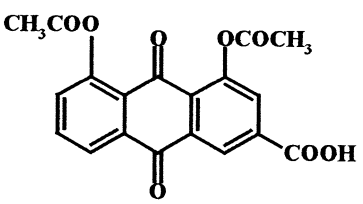
The second class of drugs used for OA are the so-called 'disease-modifying agents' (DMAs). The first category includes hyaluronic acid, which exerts its effect by supplementing the existing synovial fluid and improving its rheological properties: this articular lubricant is administered via intraarticular injection [3]. The second category of DMAs is composed of drugs that inhibit cartilage degeneration, and may be classified as chondroprotective agents. These include Diacerhein (**1**) (Artrodar®), which has been described as an IL-1β production inhibitor [4]; glucosamine (**2**) (Anatril®) and glycosaminoglycan sulfate (Rumalon®) are rapidly incorporated in the proteoglycans of the cartilage matrix and appear to increase protein synthesis and decrease proteolytic activity [5].



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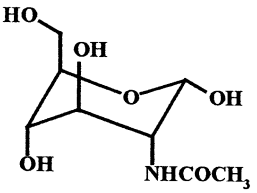

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4. Proteases in OA

The human OA cartilage is a rich source of proteases induced under a pathophysiological condition. Proteases released in the joint are at least partly responsible for the degradation of articular connective tissues. Inflammatory synovial fluids contain elevated levels of proteinases, such as elastase, collagenases, or stromelysin, and other proteases are secreted by cells of the synovium. Although the role of metalloproteinases in the process of cartilage degradation has been extensively studied within the last few years, little is known of the exact role of a large number of other proteases. In the present report, we describe the implication of several proteases that belong to the classical classes of tissue proteinases (Table 1).

4.1. Aspartic proteases

4.1.1. Cathepsin D

Little is known on the exact role of aspartic proteases in the catabolism of articular cartilage. Cathepsin D is so far the only member of this class of proteases detected in the joint. It is produced by the chondrocyte [6], and its proteolytic activity has been shown to be enhanced in OA synovium compared with normal synovium [7]. As with other proteolytic enzymes, cathepsin D degrades the PG core of the cartilaginous matrix [8], but it is also responsible for the processing of procathepsins B and L to their active forms [9]. Finally, by potentiation of cathepsin L activity, cathepsin D facilitates the transvascular migration of macrophages into the synovial tissue and the adhesion of synovial cells to cartilage, thus mediating the invasive destructive process in OA [10].

4.1.2. Cathepsin D inhibitors

The only report of cathepsin D inhibitors aimed at inflammatory disorders has been issued by Merck, describing the heptapeptide PyroGlu–(D)Phe–Pro–Phe–Phe–Val–(D)Trp [11]. Although primarily prepared for other purposes (particularly as cognition-enhancing drugs), several structures of cathepsin D inhibitors could be worthy of evaluation in the field of OA [12].

4.2. Cysteine proteases

4.2.1. Cathepsins B and L

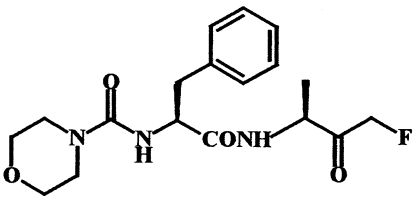
In contrast, much work has been devoted to the effects of cathepsins B and L, two lysosomal cysteine proteases, on cartilage degradation. Although involved in normal bone and cartilage turnover, both enzymes have been shown to be up-regulated in inflamed synovial cells and chondrocytes. They participate in the resorption of calcified tissues by osteoclasts, and in the degradation of type II collagen and PG [13]. Cathepsin B is part of a proteolytic cascade leading to the activation of matrix metalloproteinases (MMPs) [14], and it has recently been described as an alternative enzymatic pathway for articular PG, generating the aggrecan MMP cleavage neoepitope VDIPEN [15]. Thus, a combined therapy using cysteine and MMP inhibitors or double-headed inhibitors should be considered in order to obtain potent chondroprotective agents.

Table 1
Proteases implicated in OA

Aspartic	Cysteine	Serine	Metalloproteinases	Miscellaneous
Cathepsin D	Cathepsin B Cathepsin L Caspases–ICE	Trypsin Thrombin Urokinase Plasmin Elastase Cathepsin G	Collagenases Stromelysins Gelatinases Aggrecanases	PLA2 Tyrosines-Kinases

4.2.2. Cathepsins B and L inhibitors

Fluoromethyl ketones, exemplified here by compound **3**, are potent cathepsin B inhibitors [16]; they are able to reduce cartilage destruction and bone loss in an adjuvant-induced arthritis model after oral administration [17,18]. CA 074 (**4**), an epoxide-containing inhibitor, was found to inhibit IL-1-stimulated PG release from cartilage explants [19]. Finally, a large number of cathepsins B and L inhibitors have recently been patented, among which is the interesting cyano dipeptide **5** [20].



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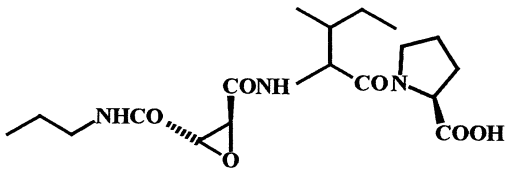

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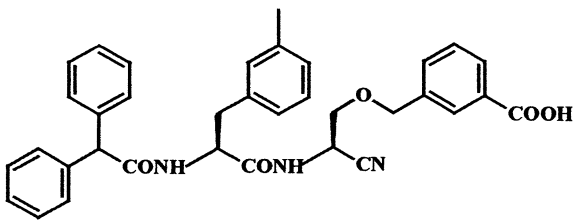

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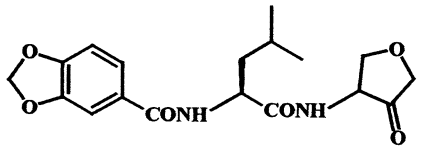
4.2.3. Cathepsin K

This cysteine protease is selectively expressed by osteoclasts, and inhibitors have been found to inhibit bone resorption [21]. Although these compounds are more devoted to treatment of osteoporosis than OA, the prime importance of subchondral bone resorption in early OA makes them interesting candidates for an experimental evaluation in OA. Several patents issued from Smith Kline Beecham described a large number of inhibitors, among which are derivatives **6** and **7** [22,23].

4.2.4. Interleukin converting enzyme (ICE)

ICE is a 45 kDa polypeptide, also called caspase-1 (cysteine aspartate-specific protease). It is responsible of the formation of IL-1β, but also, to a lesser extent, of IL-18. IL-1β is a key catabolic factor involved in joint tissue destruction; it is expressed and synthesized in OA



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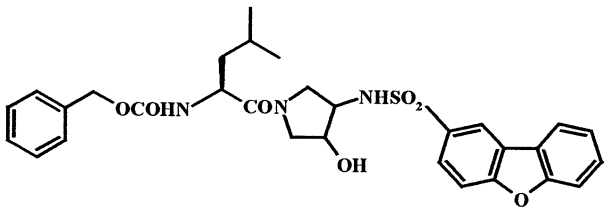

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synovium and chondrocytes as a 31 kDa precursor (pro-IL-1β), which is processed by ICE to the mature 17.5 kDa cytokine by cleavage between Asp^{116} and Ala^{117}. Extensive work by Pelletier's group has shown that ICE was expressed and synthesized in both human synovial membrane and cartilage, with increased number of ICE-containing cells in OA versus normal tissues [24]. Thus, ICE may promote OA progression by activating the proinflammatory cytokine IL-1β.

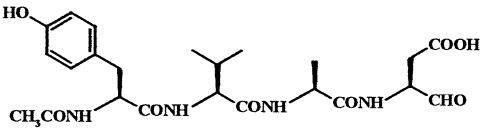
4.2.5. ICE inhibitors

The first inhibitors to be described were substrate-based designed tetra- or tri-peptides, such as the Merck compound L-709049 (**8**) [25] or the Novartis compound (**9**), which interacts with the enzyme through an acyloxymethyl ketone moiety [26]. Pseudo- and non-peptidic structures, such as VX-740 **10**, co-developed by Vertex and Aventis [27], and Merck compound **11** [28] respectively, have been reported more recently.

4.3. Serine proteases

4.3.1. Thrombin

Thrombin is the key enzyme of the coagulation cascade, since it cleaves soluble fibrinogen into the insoluble monomers of fibrin that will subsequently polymerize to form a clot. Because fibrin is commonly observed in arthritic joints, studies were undertaken to determine whether coagulation and fibrinolysis proteases were involved in cartilage degradation. All zymogens of the coagulation cascade are present in synovial fluid, especially thrombin, which was found to induce PG release from normal or OA human cartilage [29]. Moreover, thrombin stimulates MMP production, especially stromelysin [30]. To the best of our knowledge, no report describing a thrombin inhibitor as a cartilage-sparing agent has yet been published. The rapid development of potent and selective inhibitors active by the oral route should encourage in-depth evaluation of this class of inhibitors in OA.



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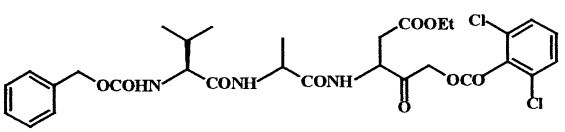

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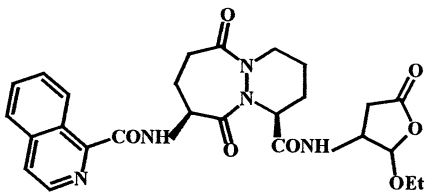

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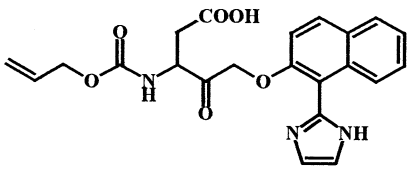

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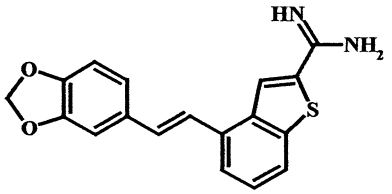
4.3.2. Fibrinolysis pathway

Plasminogen is processed to plasmin through proteolytic cleavage of tPa (tissue plasminogen activator) and uPa (urokinase). Modulation of the production of plasmin is realized by PAI-1 (plasminogen activator inhibitor). Plasmin itself is responsible for the degradation of the fibrin clot. uPa is produced by OA synovial fibroblasts, whereas tPa is secreted in endothelial cells of OA synovial membranes. Plasmin has been found to degrade basement membranes, to enhance PG release, and to participate in the mechanism of activation of MMPs. Interestingly, high levels of PAI-1 have been detected in arthritic fibroblast cultures compared with normal, suggesting that any drug modulating the PAI-1 levels may be of potential interest in the treatment of synovial inflammation [31].

4.3.3. Fibrinolysis modulators

Many urokinase inhibitors have been described in recent years. Compound **12** (B 623, Eisai) was one of the first one to be evaluated, giving an IC_{50} of 0.06 μM for urokinase inhibition [32]. Compound **13** is patented as an antagonist of the interaction of urokinase with its receptor uPaR [33]; although this compound is mainly indicated for cancer, it may be interesting to evaluate its potency in the field of OA. PAI-synthesis inhibitors, exemplified here by T 686 (**14**) [34], as well as tPa/PAI-1 interaction inhibitors **15** and **16** [35,36], are also potential candidates for modulating the fibrinolysis cascade at the joint level. Finally, it has very recently been published that the joint lubricant hyaluronic acid could actually inhibit the expression of uPa, uPaR and PAI-1 in human OA synovial fibroblasts [37].



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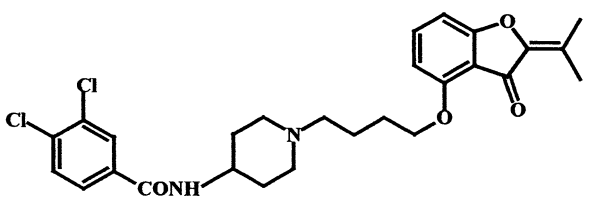

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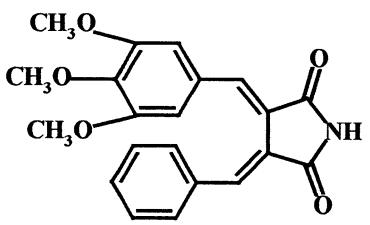

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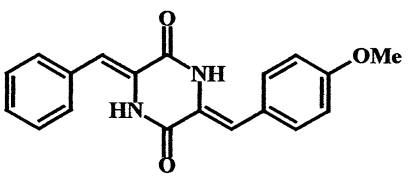

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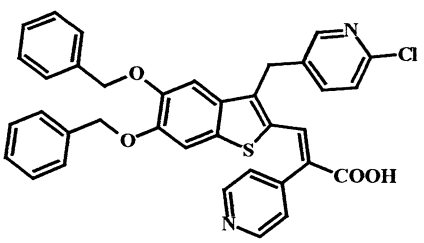

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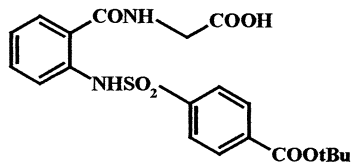
4.3.4. Elastase

Neutrophil elastase has a broad substrate specificity, including collagen and PG. During OA, the influx of activated polymorphonuclear neutrophils (PMNs) provokes an increase of elastase activity and a subsequent degradation of cartilage components [38,39].

4.3.5. Elastase inhibitors

Two elastase inhibitors have been specifically studied in the field of inhibition of cartilage degradation: ONO 5046 (**17**) has been found to protect cartilage from the direct effect of neutrophils [40], whereas MDL 101,146 (**18**) inhibited joint destruction (evaluated by histological assessment) in the rat collagen-induced arthritis model [39].



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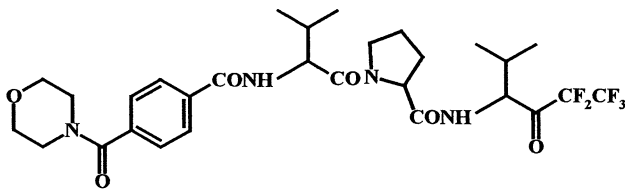

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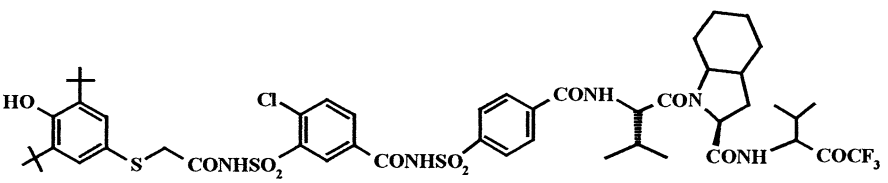
Protection of glycosaminoglycan loss induced by PMNs was also obtained when using antioxidants such as superoxide dismutase or catalase suggesting that reactive oxygen species may participate in joint destruction [41]. Compound **19**, a dual elastase inhibitor–antioxidant derivative (IC_{50} elastase: 26 nM; IC_{50} lipid peroxidation: 0.6–1.2 μM) may be of particular interest in order to obtain cartilage-sparing properties [42].



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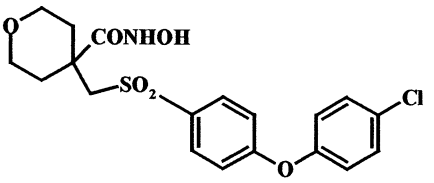
4.4. Metalloproteinases

It is not the purpose of this report to give an overview on MMPs in OA. However, it may be useful to emphasize the prime importance of MMP 13 in the degradation of OA cartilage [43]. Several selective MMP 13 inhibitors, such as RO 1130830 (**20**) [44], are currently under preclinical or clinical investigation.



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5. Conclusions

Besides the impressive amount of work that has been performed concerning the implication of MMPs in OA, little is known on the exact role of many other proteases. Development of potent and selective enzymatic inhibitors with oral bioavailability will help to define the exact implication of the different enzymes. Cathepsin B, in the cysteine family, and thrombin and the fibrinolysis proteases in the serine family seem to be the most valuable targets to inhibit in order to find new drugs able to limit or even suppress cartilage degradation. A dual cysteine–MMP inhibitor might also be worthy of interest, since both families of enzymes appear to be strongly implicated in the pathogenesis of OA.

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