Expert Opinion

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Drug delivery strategies for cathepsin inhibitors in joint diseases

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Cathepsins play important roles in the development of joint and bone diseases such as osteoporosis, rheumatoid arthritis (RA) and osteoarthritis (OA). Cathepsin inhibitors are presently in development and clinical testing for use as novel disease-modifying drugs for the improved treatment of osteoporosis. They may also be applicable for the treatment of joint diseases. However, some barriers still hamper their clinical applications in these indications. Based on pathophysiological features of RA and OA, the authors discuss six potential drug delivery strategies for the effective delivery of cathepsin inhibitors or other antiarthritic drugs to the arthritic joint tissue. Successful application of these strategies may significantly contribute to a more effective and safe treatment of RA and OA.

Keywords: cartilage, cathepsin, drug delivery, inhibitor, joint, osteoarthritis, osteoporosis, rheumatoid arthritis, subchondral bone

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1. Introduction

The human musculoskeletal system comprises of bones, cartilage, muscles, tendons, ligaments and so on, which provide physical support for the entire body and protection to all vital organs. Joints connect all moveable and non-moveable components within the skeleton and undergo significant loading and wearing stress. Articular joints and the disc of the vertebrae are the sites of numerous degenerative, acute and chronic diseases, which have a dramatic impact on national health systems [201]. Among > 100 different types of arthritic joint diseases, two of the most common types are rheumatoid arthritis (RA) and osteoarthritis (OA). Both diseases are outstanding in their significance on human health and affect 0.5 – 1.5% of the western population in the case of RA, and potentially every elderly person for OA. Both diseases are characterised by significant damage to articular cartilage and subchondral bone caused by, among others things, high activities of proteolytic enzymes, including cathepsins [1]. Because of the role of cathepsins in the joint destruction processes, much effort has been devoted to the development of specific protease inhibitors for the improved treatment of RA and OA. Previously, the authors, and colleagues, had discussed the advantages of targeting cathepsin K inhibitors to subcellular compartments, such as lysosomes, and the resorption lacunae of osteoclasts, using a polymeric carrier [2]. The present review will briefly debate the role of cathepsins in the pathology of joint diseases and will focus on the potential applications of various drug delivery strategies that would give tissue specificity to cathepsin inhibitors.

2. The joints and associated diseases

Human joints are unique structural units of the skeleton and may be classified into three types:

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- synovial or diarthrodial joints (e.g., joints in the four extremities)
- amphiarthroses (e.g., pubic symphysis, intervertebral discs of vertebral bodies, the distal tibiofibular articulation and the sacroiliac joint articulation with pelvic bones)
- synarthroses, which are found only in the skull (suture lines) [3]

Both synovial and amphiarthroses joints can be affected in RA or OA development.

2.1 The structure of articular cartilage

Articular cartilage is a specialised connective tissue that covers the surfaces of diarthrodial joints. A mature articular cartilage is a heterogeneous tissue with four distinct regions: the superficial tangential (or gliding) zone, middle (or transitional) zone, the deep (or radial) zone and the calcified cartilage zone located immediately below the tidemark and above the subchondral bone. Chondrocytes are the only cellular component of adult hyaline articular cartilage and are responsible for maintenance of the cartilage matrix. The cartilage extracellular matrix is composed of a network of collagen fibrils, which confers tensile strength, and another interlocking network of negatively charged proteoglycans, which provides compressive stiffness through osmotic pressure [3]. The unique structure resembles an interpenetrating network hydrogel [4] that is familiar to polymer scientists. Clearly, the degradation of either of the two networks would weaken the overall mechanical properties of the cartilage matrix.

The major collagen in articular cartilage is type II collagen, which accounts for > 50% of its dry weight. It is a homotrimer made of three identical polypeptide chains called α_1(II). These collagen triple helices interact with each other to form the collagen fibrils in a tightly packed quarter-staggered array, which is highly stable. Each of the fibrillar collagens also has a nonhelical peptide sequence at each end. They are known as telopeptides and have been widely used as diagnostic markers of collagen metabolism [5,6].

As the second component of cartilage, aggrecan is a multidomain proteoglycan with several well-characterised functional regions. The core protein of aggrecan comprises of two N-terminal globular domains, G1 and G2, separated by the E1 domain, followed by a more extended E2 domain, and terminated by the third globular domain, G3. The negatively charged groups in aggrecan are keratan sulfate and chondroitin sulfate in the E2 domain. The large amount of negative charges in this domain contributes to the stiffness of cartilage [7].

2.2 Rheumatoid arthritis

RA is a chronic, systemic, inflammatory disease, which may lead to the destruction of joints. Many consider it to be an autoimmune disorder, although its exact cause is unknown. The primary target of the disease is the synovial tissue. The inflamed synovium invades and damages articular bone and cartilage, leading to significant pain and loss of function [8-10].

In RA joints, the cartilage is often covered with a highly vasculated tissue, called the pannus, which has certain features of neoplastic invasive growth [9]. A matured pannus has been shown to have macrophage-like and fibroblast-like cells penetrating the cartilage [11]. On the molecular level, the damage to the cartilage is mainly mediated via proteolytic activities in the invasive pannus tissue. Damage to the cartilage may also come from proteolytic enzymes from chondrocytes or the synovial fluid. Accumulation of these cellular insults would eventually trigger fragmentation and fibrillation of the cartilage under external mechanical impact. In addition to cartilage, subchondral bone is also subjected to damage by abnormal bone-resorbing protease activities induced by inflammatory cytokines in RA development [12].

Current RA treatments may be classified into two approaches: symptomatic treatment with NSAIDs and disease-modifying antirheumatic drugs (DMARDs) [12]. NSAIDs are mainly directed to the prostaglandin pathway with COXs as their molecular targets [13]. They are analgesics with little or no effect on disease progression. Long-term use of NSAIDs may lead to gastric and severe cardiovascular complications, as evident by the recent withdrawal of the selective COX-2 inhibitor rofecoxib (Vioxx™, Merck) and valdecoxib (Bextra™, Pfizer) from the market [202,203]. Methotrexate (MTX) is still the most commonly used DMARD. The newly developed biological DMARDs (e.g., infliximab and anakinra) are designed to target inflammatory cytokines such as TNF-α or ILs. However, systemic blockage of inflammatory cytokines with antibodies and antagonists may lead to severe risks of infections [14-16]. Glucocorticoids are also very effective in the treatment of RA and are considered by many as DMARDs [17]. Their systemic side effects (e.g., secondary osteoporosis) have limited their clinical application.

2.3 Osteoarthritis

In contrast to RA, OA is a noninflammatory degenerative joint disease occurring mostly in older persons and is characterised by the degeneration of articular cartilage, hypertrophy of bone at the margins and changes in the synovial membrane. It is accompanied by pain and stiffness, particularly after prolonged activity [18].

OA is a slowly developing degenerative breakdown of cartilage with only episodic spurs of synovitis. Although there is usually no pannus formation, the proteolytic enzymes secreted by chondrocytes in the cartilage and those present in the synovial fluid seems to breakdown collagen fibrils and remove aggrecan from the cartilage [19].

In contrast to RA, no drugs are available with proven disease-modifying efficacy in OA. The only registered systemic oral drug therapy for OA is the symptomatic treatment

using analgesics or anti-inflammatory agents, such as COX-2 inhibitors.

3. Proteolytic enzyme activities in rheumatoid arthritis and osteoarthritis joint degeneration

3.1 Proteases in joints

Many enzymes have been known to be involved in the pathological development of RA and OA. In addition to COXs [20], which are critical in prostaglandin-related pathways, proteases such as metalloproteinases (matrix metalloproteases [MMPs], a disintegrin and metalloproteinase with thrombospondin type I motifs [ADAMTS]) and cathepsins have all been documented for their damaging roles in arthritic diseases [21-25]. Cathepsin K in particular is known for its strong proteolytic activities against helical collagens of type I and II [26]. Cathepsin K is highly expressed in osteoclasts [27] but it is also found in macrophages and fibroblasts of RA and OA joints [24,28]. Cathepsin S, on the other hand, is known for its significant role in antigen presentation, which is important for RA development [24,29]. Furthermore, cathepsin S is the only cathepsin stable at neutral pH value that would allow a potent extracellular activity. For example, Reddy *et al.* [30] demonstrated the secretion of active cathepsin S activity by macrophages and the degradation of matrix proteins such as elastin. However, it should be noted that extracelluar matrix proteins, such as collagen fibrils, are effectively phagocytosed and subsequently degraded intracellularly [22,31]. The reader is referred to [32] for a detailed discussion of cathepsins in arthritis.

3.2 The degradation of articular cartilage in rheumatoid arthritis and osteoarthritis

Type II collagen fibrils, the major constituents of articular cartilage, are highly resistant to general proteolysis and require specific proteases for their degradation. MMPs have been considered as critical proteases responsible for the cartilage collagen degradation [33-35]. They typically cleave type I and II collagens at a distinct peptide bond within the helical region and generate ¾ and ¼ fragments at neutral pH [1]. On the other hand, cathepsins of the cysteine protease family (except cathepsin S) require an acidic microenvironment for their activity and are thought to be active only within lysosomes for postprocessing of collagen fragments. Cathepsins B and L have been known to cleave within the nonhelical telopeptide region of collagens [36,37] whereas cathepsin K is capable of cleaving at multiple sites within the triple helix of types I and II collagens [38,39]. It is important to point out that the current understanding of the roles of cathepsins, especially that of cathepsin K, in the degradation of cartilage may be largely underestimated. Due to hypoxia and consequent anaerobic metabolism, joint tissue acidosis has been reported in both RA and OA patients [25,40-42]. Given the strong collagen fibril degradation activity of cathepsin K, it is very likely that cathepsin K (and other cathepsins) may be secreted and participate in the extracellular degradation of cartilage collagen fibrils in RA and OA joints.

Aggrecan is the second main component in cartilage and its degradation leads to the loss of negatively charged glycosaminoglycans, which reduces the stiffness of cartilage. It is recognised that both MMPs and aggrecanases (ADAMTS) are major aggrecan-cleaving proteases [43-45]. In addition, cathepsins B [46], G [47], K [48] and L [49] have all been suggested to be effective in aggrecan degradation. Among them, cathepsin S is the only cysteine protease that is active at acidic, neutral or even slightly alkaline conditions, and has a potent proteoglycan-degrading activity. It is very efficient in hydrolysing aggrecan at neutral and acidic pH [48].

3.3 Bone and bone turnover in rheumatoid arthritis and osteoarthritis

Bone is a mineralised connective tissue. In the case of bovine cortical bone, mineral content (mainly apatite) accounts for ~ 69% of the weight of fresh bone, the organic matrix makes up ~ 22% and water represents the remaining 9%. Of the organic matrix ~ 90% is type I collagen. Other noncollagenous proteins, such as osteocalcin, sialoprotein and osteopontin, constitute the remaining ~ 10% [50]. As a complex living tissue, bone mainly contains three different types of cells: osteoblasts, osteoclasts and osteocytes. Osteoblasts are bone-forming cells that originate from local osteogenitor cells and are responsible for the production of the bone matrix. Osteoclasts are large, multinucleated bone-resorbing cells that originate from various haemopoietic tissues. As the most abundant bone cell type, osteocytes are mature osteoblasts located in the bone matrix [51].

Normally, bone resorption by osteoclasts and bone formation by osteoblasts are well balanced to maintain the function of the skeleton. However, under pathological conditions (e.g., RA and OA) such balance is disturbed. Bone turnover at the bone-cartilage interface is greatly accelerated in the early phase of OA. Osteophytes (bone spurs) are often observed at nonweight-bearing zones. In the late stage of the disease, radiographical visible cysts can also be observed [19]. One of the hallmarks of RA is subchondral bone destruction. The pannus tissue not only damages cartilage but also invades cortical or subchondral bone [9]. Bone resorption, however, is mostly mediated by osteoclasts. It is believed that various cytokines released during disease development help to recruit osteoclasts to the sites of destruction. As osteoclasts start the bone resorption process, they will secrete cathepsin K to cleave the type I collagen triple-helix fibrils and other bone proteins [52]. Other cathepsins and MMPs may also play certain roles in the bone resorption process [53,54].

3.4 The role of cathepsin S in antigen presentation

RA is considered as an autoimmune disorder. The dendritic cell-mediated immune response against as yet unknown antigen(s) during the development of RA is essential [55]. For the initiation of the major histocompatibility complex (MHC) class II-mediated immune response towards an antigen, cathepsin S has been suggested to be essential in the presentation

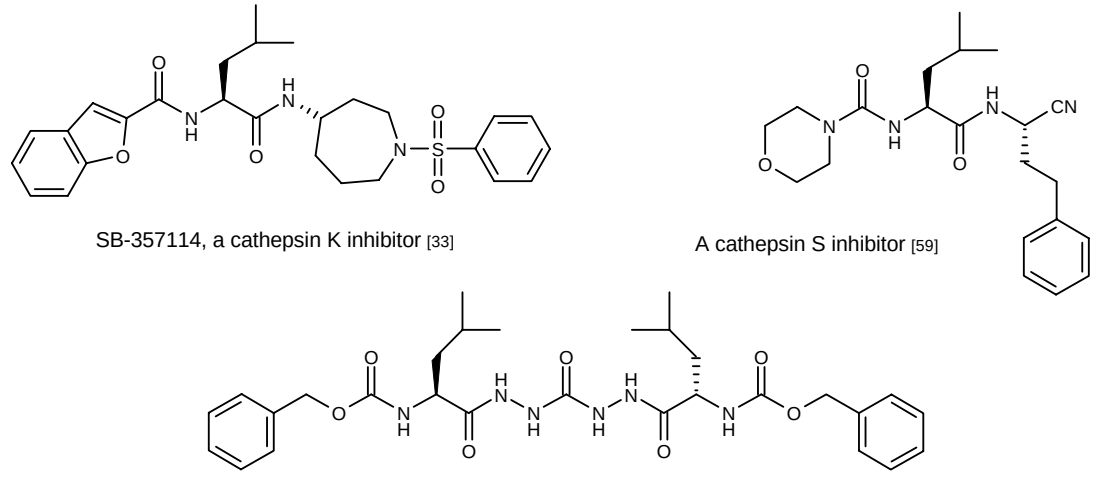

Figure 1. The structures of representative cathepsin K and S inhibitors.

process. Prior to the binding to and subsequent presentation of an antigen by the MHC class II complex, the so-called invariant chain has to be removed by proteolytic degradation. The invariant chain acts as a chaperone for the transport of the MHC class II complex to the endosomal compartment and blocks the binding grove in the MHC class II complex to a prospective antigenic peptide. Cathepsin S, which is predominantly expressed in antigen-presenting cells, specifically degrades the invariant chain to the so-called class II-associated invariant chain peptide (CLIP) peptides, which is replaced by an antigen peptide. Mice deficient in cathepsin S have been shown to exhibit a reduced susceptibility to collagen-induced arthritis and thus underline the critical role of cathepsin S in RA [56]. A more detail description of this process has been reviewed by Honey *et al.* [57].

4. Delivery strategies for cathepsin inhibitors in the treatment of rheumatoid arthritis and osteoarthritis

It is very clear that cathepsins, in addition to MMPs, have been heavily involved in the degeneration of type II collagen fibrils and aggrecan in articular cartilage, and in the high turnover of bones in RA and OA joints. The inhibition of their proteolytic activities will surely slow down or possibly even halt the tissue damage and disease progression. Therefore, inhibitors of these proteases have been considered as novel disease modifying drugs for future RA and OA treatment. Furthermore, inhibitors for cathepsins, especially cathepsin S, will reduce the dendritic cell-mediated autoimmune response and subsequently modify the development of RA.

As a logical first approach, any therapy that would use endogenous protease inhibitors (e.g., cystatins for cysteine proteases) directly or boost their expressions at the diseased sites could potentially reduce the local proteolytic damage [58]. However, due to various problems intrinsic to protein-based drugs (e.g., high production costs, potential antigenicity, application by injection), the focus of major pharmaceutical companies is on the development of low molecular weight (MW), orally available, synthetic reversible inhibitors [32]. So far, the most promising low MW drug candidates are inhibitors for cathepsins K and S. These inhibitors have been intensively reviewed in recent years [59-63]. Figure 1 shows chemical structures of a few representative inhibitors for cathepsin K and S.

Although much progress has been made in the development of highly potent and selective inhibitors for cathepsins, there are still several challenges for the clinical application of these inhibitors.

First, it is well understood that cathepsin K is the key protease that dissolves type I collagen fibrils in the bone turnover process. But it is not clear which protease dominates the process for the degradation of articular cartilage. MMPs and cathepsins have all been suggested to participate in this process. It is most likely that they are redundant in type II collagen fibril and aggrecan cleavage. Whereas cathepsin K inhibitors for the treatment of osteoporosis may soon flourish, the development of novel specific inhibitors of cathepsins K or S for RA and OA treatment is still questionable due to protease redundancy. Specific inhibition of one of the proteases involved will not alter the disease progression. Several MMP inhibitors have failed in their clinical evaluation for the treatment of arthritis [64,65]. One of the suggested reasons was the overestimation of the importance of MMPs in the multi-protease-mediated joint degradation process. For example, cathepsins were not considered. Whether cathepsin inhibitors alone will lead to a therapeutic disease modification remains to be demonstrated in clinical trials. On the

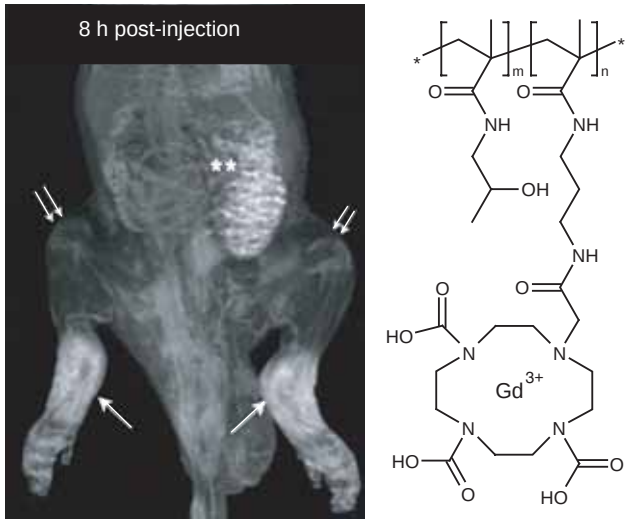

Figure 2. The chemical structure and preferential deposition of HPMA copolymer-DOTA/Gd^{3+} in inflamed arthritic joints of adjuvant-induced arthritic rats. A very high accumulation of the compound is observed in ankle joints (single arrow) and low accumulation in knee joints (double arrow) 8 h post-injection of the polymer. The injection site of anaesthetic agents (**) is also highlighted due to the retention of the aqueous solution.
DOTA: 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetra(acetic acid); Gd: Gadolinium; HPMA: N-(2-Hydroxypropyl)methacrylamide.

other hand, early experiments using cathepsin inhibitors with a broad specificity in rodent models of RA were very promising [66,67].

Second, potency and selectivity of an inhibitor at the molecular level are not sufficient to guarantee a desirable efficacy and safety profile. For a low MW inhibitor to reach therapeutic efficacy, a sufficient dose in the affected joint tissue has to be achieved, which may require significantly higher overall systemic dose applications that in turn could potentially trigger an undesirable toxicity reaction. In fact, toxicity issues have been suggested as the critical factors that contribute to the failure of MMP inhibitors [65]. Clearly, specificity to the targeted protease and specificity to the affected joint tissue will both be highly desirable in the development of inhibitors for cathepsins.

Drug delivery technologies have been developed for several decades [68]. Many delivery tools have been designed and successfully evaluated in clinical applications. The concept can be traced back almost a century to Paul Ehrlich, who first envisioned the 'magic bullet' that would direct a therapeutic agent coupled to a homing device to the sites to be treated for enhancement of efficacy and reduction of toxicity [69]. The key is how to design the homing device that would recognise the diseased sites but spare the normal tissues. The authors believe that RA and OA joints have some unique pathophysiological features that may be recognised by drug delivery systems, and delivery technologies may eventually help to solve some of the major problems encountered in the clinical applications of cathepsin inhibitors.

4.1 Selective drug delivery to inflamed rheumatoid arthritis joints using colloidal carriers

The RA joint is characterised by synovial membrane inflammation (synovitis). The histopathological appearance of the synovium of RA is marked with significant angiogenesis and influx of inflammatory leukocytes that lead to damage of the joint tissues [12]. The inflamed synovial lining, especially the pannus tissue, resembles neoplastic tissues in many ways. These include the leaky nature of blood capillaries, which leads to abnormal serum protein infiltration into the synovium and high protein contents in the synovial fluid (SF) of RA patients when compared with normal individuals [70]. At different stages during disease development, the leakiness of the vasculature may change significantly [71,72]. The observed damage and depletion of lymphatics in RA joints may retard the clearance of the macromolecules, such as serum proteins from the synovium [73,74]. In solid tumours, similar pathophysiological characteristics are being recognised as enhanced permeability and retention (EPR) effect [75,76]. In other words, macromolecules and other colloidal vesicles (e.g., proteins, synthetic water-soluble polymers, micelles, and liposomes) may selectively leak out of the fenestrated capillaries and reside at the diseased sites for prolonged times due to the poor lymphatic drainage. Based on this principle, many colloidal drug delivery systems have been developed for improved cancer chemotherapy with many in clinical trials [77-79].

Because of certain similarities between RA joints and solid tumours the authors hypothesised that colloidal drug delivery systems, such as *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, may be able to preferentially accumulate to the RA joints after systematic administration and, therefore, may be able to selectively deliver drugs, such as inhibitors of cathepsins to RA joints. To prove this, they synthesised a HPMA copolymer containing 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(acetic acid) (DOTA) and chelated the copolymer with gadolinium (Gd^{3+}) ions. Such chelation structure is a contrast signal-enhancing agent, which could allow them to noninvasively follow the distribution of a labelled polymer using the magnetic resonance imaging (MRI) technique [80]. After intravenous administration of the DOTA-Gd^{3+} labelled HPMA copolymer to adjuvant-induced arthritic (AIA) rats, the polymer stays in the circulation for a relatively longer period of time and gradually accumulates into the inflamed joints over 8 h (Figure 2). At the same time, it will also gradually clear from the body via the kidneys. Although there is an initial deposition into the liver, it is minimal and quickly redistributed and cleared from the organ. In contrast, the clearance of the polymer from the arthritis joints is rather slow. After 48 h, residues of the polymer are still visible in the arthritic joints. Interestingly, in conjunction with histological analyses, the authors noticed that the accumulation of the

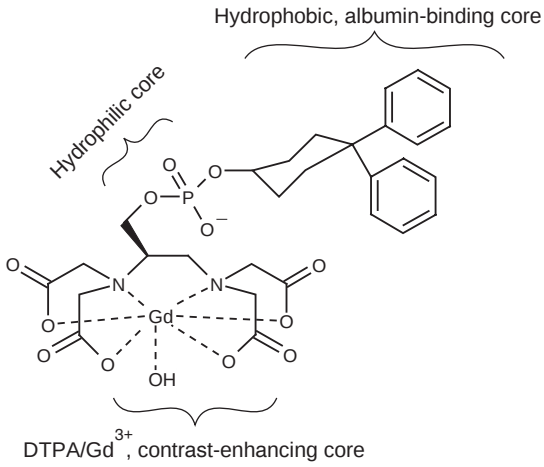

Figure 3. Structure of blood pool magnetic resonance imaging contrast agent MS-325, an example of albumin-binding delivery.
DTPA: Diethylenetriamine penta-acetic acid.

DOTA-Gd^{3+} labelled polymer to the diseased sites is related to the severity of the inflammation. High accumulation is observed at sites with severe inflammation, such as the ankle joints (Figure 2, single arrow). Much lower accumulation is seen at sites with mild inflammation, such as the knee joints (Figure 2, double arrow).

In order to investigate if such delivery strategy may have any therapeutic benefit, preliminary treatment studies of the AIA rats with HPMA copolymer-bound dexamethasone (pH-sensitive drug-releasing mechanism; see Section 4.3) were performed. Given its potent antiarthritic effect and severe side effects [17], one may suggest that if any therapeutic or safety benefit can be obtained with HPMA copolymer-bound dexamethasone, then the system will also benefit most of the other antiarthritic drugs, including inhibitors of cathepsins. The preliminary treatment study with this delivery system seems very encouraging. The conjugate showed prolonged anti-inflammatory effects with much stronger preservation of joint bone mineral density and articular cartilage compared with free dexamethasone [81]. A similar study has been carried out using polyvinvylpyrolidones (PVP) as a carrier for glucocorticoids. However, here the results only show marginal improvement in efficacy in animal models [82]. As the drug is linked to PVP via an ester bond, the reduced effectiveness of PVP–drug conjugates may be attributed to its very slow drug release. As the delivery system can only reside in arthritic joints for a short time, slow release can only lead to low local free drug concentrations, and, consequently, low therapeutic efficacy. Similar to polymer delivery systems, the selective accumulation in arthritic joints would also work for other colloidal vesicles, such as micelles and liposomes. Liposomes have also been used in the delivery of prednisolone for the treatment of arthritic inflammation in joint [83]. Noninvasive radiography showed significant accumulation of liposomes in affected joints in an arthritis mouse model. The prednisolone-loaded delivery system was found to significantly reduce inflammation in joints of experimental animals. However, the delivery system also showed significant uptake in the spleen and liver, even with the application of stealth liposome design. This may eventually lead to toxicity issues that would obviate this approach from further development [84]. Albumin–MTX has been used in the treatment of solid tumours based on the EPR effect. The same research group recently extended the application of albumin–MTX to the treatment of inflammatory arthritis [85]. The albumin complex seems to more selectively accumulate in inflammatory joint tissues, and an improved therapeutic efficacy was observed. Due to the modified protein nature of the delivery system, however, there remains some concern about immunogenicity as well as prolonged efficacy. To reach an effective dose, a large amount of albumin carrier has to be given. The release of MTX from albumin seems to depend on the degradation of the protein inside the lysosomes.

In addition to selectively increasing the local concentrations of protease inhibitors in RA joints, all the colloidal carriers mentioned above could in theory deliver multiple inhibitors simultaneously, which would make cocktail therapies possible. However, caution must be taken regarding the treatment schedule with this proposed strategy. It is still not very clear whether there is a correlation between the leakiness of the vasculature and the disease development stage. This therapy may only be applicable when the joint vasculature is leaky. It may not aslo be possible to apply this delivery system to OA as it only has periodical synovitis and may not have a significant EPR effect as observed in RA. Apparently, more research is needed to explain in more detail the vasculature condition in RA development.

4.2 Albumin-binding drug delivery

As noted in the previous section, high infiltration of plasma albumin has been observed in RA joints due to the EPR effect. One may logically predict that if a therapeutic agent could preferentially bind to the protein, its deposition in arthritic joint may be greatly enhanced. In fact, albumin has been suggested as a 'one-way transport vehicle into sites of inflammation' [86]. Differing from the albumin–MTX study, such binding is not covalent but temporal, which makes it less likely to have immunogenic issues. Compared with the colloidal delivery system, such strategy would be more attractive to companies favouring low MW drugs. One of the most interesting examples of albumin-binding delivery is the development of a low MW albumin-binding blood pool MRI contrast agent MS-325 (AngioMARK™ or Vasovist™, EPIX Pharmaceuticals, Inc.) [87,88]. As shown in Figure 3, the structure of the contrast agent has a hydrophobic diphenylcyclohexyl group connected to the chelation core via a unique hydrophilic phosphodiester group. The hydrophobic part offers enough binding to albumin so that glomerular

filtration of the contrast agent can be delayed. The hydrophilic functional group balances the hydrophobicity of the whole structure and limits liver clearance of the molecule. Therefore, the contrast agent showed prolonged half-life in circulation with a bigger imaging window but could also be cleared from the body relatively faster when compared with the macromolecular contrast agent. In another study, a special peptide sequence (Ac-RLIEDICLPRWGCLWEDD-NH_2) has been found to be able to enhance the binding of proteins to albumin and subsequently improve the pharmacokinetics of protein therapeutics [89]. Hydrophilic antiarthritic drugs would probably have shorter half-lifes in circulation. Therefore, improvement of their binding to plasma albumin may not only help to target the drug to the arthritic joint, but may increase the half-life of the drug.

On the other hand, the authors also noticed that many drugs (e.g., the metabolite of leflunomide, A77 1726) bind too strongly to be released from albumin, which eventually would reduce the therapeutic efficacy, reside too long in the body and raise severe toxicity problems (long-term nonspecific release at normal tissues) [90]. It seems that a prodrug design including a more hydrophilic antiarthritic therapeutic core structure, a hydrophobic albumin-binding structure and a cleavage mechanism that would allow the therapeutic core of the prodrug to leave albumin, and the binding structure in the arthritic joint would be highly desirable. This is a big challenge for medicinal chemists in their original design of inhibitors for cathepsins and other antiarthritic drugs. However, such rational drug design would eventually lead to highly desirable pharmacokinetic and biodistribution profiles of these compunds.

4.3 Delivery strategies based on the low pH of rheumatoid arthritis and osteoarthritis joints

In RA, the local inflammatory reaction in and around joint tissues promotes an acidic environment. This is partially due to the low levels of oxygen in the synovial tissue and fluid, which appears to induce a shift towards anaerobic glycolysis and lactate formation [40,41]. In some cases, pH values of SF have been reported to be as low as 6 [42]. Considering the buffer capacity of SF, a much lower pH value in the synovial tissue may be expected. In addition, there seems to be a direct correlation between the low pH of the joint tissues and indices of disease severity [91-93]. The low pH has also been associated with local osteoclast activity and bone destruction [94]. For OA, cartilage damage of the joint has been associated with a significant drop of pH at the articular cartilage surface, which may contribute to high activities of cathepsins in cartilage destruction [25]. As previously discussed, the understanding of the low pH value in arthritic joint is of paramount importance for the following reasons:

- It may help to re-evaluate the real contributions of active cathepsins in arthritic joint diseases. As most of cathepsins (except cathepsin S) involved in joint diseases require acidic pH for optimal activity, their role in cartilage degradation has been suggested as mainly postprocessing occurring inside lysosomes (acidic compartment). The finding of low pH in arthritic joint tissue indicates that they may also participate significantly in extracellular cartilage degradation.
- The diversity of the therapeutic effects of ionisable antiarthritic drugs, such as MTX, is known [8]. A lower pH in arthritic tissue could influence the solubility and partition coefficient of MTX and lead to heterogeneous drug deposition in the joint tissue. Different subcellular trafficking of MTX in tumour cells has also been observed [95,96]. If an inhibitor for cathepsins has an ionisable structure, the influence of pH of the tissue on its therapeutic effects has to be carefully monitored.
- Due to this pathological feature, one may implement pH-sensitive releasing mechanisms in drug delivery systems or prodrugs for site-specific drug release at the acidified arthritic joint.

pH-sensitive polymeric delivery systems using hydrazone, *cis*-aconityl, phosphamine, β-thiopropionate and so on have all been used for the acid cleavable site-specific delivery of a variety of drugs. As an example, Greenfield *et al.* reported linking doxorubicin to monoclonal antibodies via a hydrazone bond [97]. In this method, the 13-keto position of the compound was used as the site of attachment. This design allowed the release of unmodified doxorubicin in a pH range from 4.5 to 6.5. Structure–activity relationship studies were also performed to fine-tune the hydrazone structure for an optimal releasing profile. In addition to immunoconjugates, the hydrazone bond has been used in synthetic polymer drug carriers, such as HPMA copolymer–doxorubicin conjugates. As described above, the authors have also used hydrazone to conjugate dexamethasone to HPMA copolymer in the treatment of AIA rats. The results are very promising [81].

pH-sensitive liposomes have been widely studied for the site-specific delivery of various drugs [98]. Usually, they contain components such as phosphatidylethanolamine that destabilise liposomes at low pH. Other more sophisticated approaches include 'caged' liposomes using pH-labile *N*-maleylphosphatidylethanolamine derivatives [99] or alkylether bonds [100] and pH-sensitive peptides that would induce fusion with cellular membranes, thus mimicking viral invasion of cells [101,102]. One issue that needs to be emphasised is the hepatotropicity of regular liposomes, which could cause significant toxicity. As a counter measure, stealth liposomes may be made by sterically stabilising the vesicles with polyethylene glycol. The deposition of such liposome to the reticuloendothelial system may be greatly reduced [103].

Other colloidal carriers frequently used are micelles and some research has been done in the development of pH-sensitive micelles for anticancer drugs and gene therapy. Basically, two types of pH triggers have been developed based on different chemical structures. Polycations such as poly-L-histidine are hydrophobic and insoluble in water at neutral or

slightly basic pH. As a block copolymer containing poly-L-histidine forms micelles, it becomes pH sensitive. As the pH drops, the histidine imidazole groups become protonated, destabilising the micelle. Bae and colleagues have successfully employed this strategy for an effective tumour-targeted delivery of doxorubicin [104,105]. Another application of this type of micelles is in gene therapy. The negatively charged DNA molecules can be complexed with the polycation to form the hydrophobic core of the micelles. As the micelles encounter low pH, the complex will be disturbed and DNA will be released [106,107]. Another option is to form micelles using diblock copolymers with a pH-sensitive hydrophobic block. The conversion of the hydrophobic block into hydrophilic, under acidic pH, will destabilise the micelles and release the drug. Frechet's work on the development of pH-sensitive micelles for the delivery of doxorubicin is a good example of this approach [108]. He and his colleague used cyclic acetals of 2,4,6-trimethoxybenzaldehyde as the pH-sensitive core in their micelle design.

All above-described carriers can theoretically be used for the delivery of inhibitors of cathepsins as they all have their unique features. Liposomes are easy to make and to mass-produce; however, their payloads are usually hydrophilic, and their intrinsic hepatotropic nature (even with pegylated stealth liposomes [83]) may lead to severe liver toxicity. Micelles are usually used for the delivery of hydrophobic low MW drugs. The size of the micelles is usually much smaller and they may have a reduced tendency to accumulate in the liver. Both liposome and micelle preparations do not require chemical modification of the loaded compounds, which is an advantage that may be valued by pharmaceutical companies regarding regulatory issues. In most situations, however, drugs may have to be chemically conjugated to polymer carrier in preparation of polymeric drug conjugates. Although that may involve some temporal chemical modification of the inhibitors or other drugs, water-soluble polymeric delivery systems may provide lower deposition to the reticuloendothelial system depending on various factors, such as chemical nature, ζ-potential, hydrodynamic volume and so on [109]. Compared with the other two colloidal carriers, the release of the drug can be more precisely controlled via triggering structures that are sensitive to changes of pH, enzymatic degradation and modification [110]. When applied for the delivery of inhibitors of cathepsins in RA treatment, these three types of carriers may be considered as double-targeting delivery systems because of their preferred accumulation at the sites of inflammation and their acid-triggered inhibitor release.

Low MW pH-sensitive proinhibitors can also be designed to render the tissue-specificity of the compound to the RA or OA joints. However, to make them orally available, special dosage forms should also be developed to bypass the acidic stomach. pH-sensitive, colon-specific drug delivery systems may be used in such applications [111,112]. To render them arthrotropic in RA patients, their binding to and releasing from albumin may also have to be taken into consideration in the design of the inhibitor structures. However, other strategies have to be explored to specifically direct them to OA joints.

4.4 Cartilage-targeting drug delivery

As described in Section 2.1, cartilage is a natural interpenetration network with type II collagen fibrils and aggrecan interlocking to each other. The presence of aggrecan with its very high amount of negative charges makes cartilage unique among all types of tissues and organs. This negatively charged tissue is the only one among all tissues and organs, and would certainly make an interesting tissue target for the delivery of inhibitors for cathepsins into the cartilage.

As a logical prediction, one may assume that positively charged molecules may be attracted to the negatively charged cartilage and have preferred accumulation to the tissue. Based on this principle, Madelmont and colleagues have done some preliminary work in cartilage-targeted delivery [113,114]. Basically, they synthesised a series of positively charged compounds with quaternary ammonium in the structure and hypothesised that the positive charge would recognise the negatively charged cartilage and direct the payload to the tissue. Structure–activity relationship studies were performed to fine-tune the quaternary ammonium structure for the best binding efficiency. They observed that such compounds would preferably accumulate in the articular cartilage of all skeletal joints. Based on a similar mechanism, promising X-ray contrast agents were also developed for a better imaging of cartilage [115]. This is a very inspiring strategy. If inhibitors for cathepsins can integrate a positively charged quaternary ammonium into the design, it would eventually bring them to exactly where they are needed. Although this approach is novel and very encouraging, precaution must be taken regarding this method.

- Besides cartilage, such compounds are also deposited in the liver and cleared by the kidney. Therefore, liver and renal toxicity issues have to be very carefully watched in the development of those compounds. In addition, positively charged colloidal delivery systems may also cause haemolysis.
- The cartilage-targeting strategy allows the molecules to be targeted to all cartilage tissues in the skeleton. But it will not be selective to diseased joints. In fact, the delivery system may even have less affinity to RA or OA joints because of the loss of aggrecan in the diseased cartilage. Therefore, it may be helpful if the cartilage targeting can be combined with other strategies to enhance the targeting specificity. As one possibility, a positively charged colloidal delivery system may be more specific in directing drugs to RA joints because of the EPR effect. The positive charge may help to prolong the retention time of the delivery system to allow complete release of the payload in the joint.
- The neutralisation of aggrecan negative charges by a positively charged delivery system may further weaken the mechanical strength of the cartilage. Therefore, it would be

ideal if the positive charge of the delivery system only temporarily resides in the cartilage and can eventually be cleared from the cartilage or degraded.
- Enhanced cell penetration of the positively charged delivery system may also raise the undesirable toxicity issue [116].

4.5 Bone-targeting delivery systems

Most OA patients are at advanced ages with very slow bone turnover in their skeletons [117]. However, as noted in Section 3.3, there is greatly enhanced bone turnover at the OA joints. Potentially, such pathophysiological features may be specifically recognised by bone-targeting delivery systems.

High bone-turnover sites are always characterised with freshly resorbed or formed bone surfaces with abundant blood supply. In some preliminary studies, it was found that bone-targeting HPMA copolymers using different bone-targeting moieties (D-aspartic acid octapeptides, bisphosphonates and so on) could recognise the skeleton and show significantly higher deposition at sites of high bone turnover (such as long bone growing plates in healthy young balb/c mice) in comparison with sites of lower bone turnover (e.g., periosteum and endosteum of the long bones) [118,119]. Such unique site recognition may occur for two reasons: the abundant blood supply and fenestrated vascularisation at the high turnover site allow more macromolecular carriers to selectively extravasate, and there is more efficient binding of the targeting moieties to the fresh bone surfaces. In addition to the treatment of OA, bone-targeting delivery systems may also be used to deliver cathepsin inhibitors to RA joints. The severe bone damage at the later stages of RA progression makes perfect targets for this delivery system. Apparently, the most likely drug candidates for bone-targeting drug delivery systems are cathepsin K inhibitors, which may slow down or halt further damage to the subchondral bone of RA- or OA-affected joints. In addition to other antiresorptive drugs, anabolic agents that promote bone formation, such as prostaglandin EP2 and EP4 receptors agonists, may also be delivered by the bone-targeting delivery system [120,121]. Most of these types of drugs have systemic toxicity limitations that could hamper their clinical application. It would be advantageous to deliver them specifically to the damaged bone site and generally spare other parts of the body. Another advantage of using a polymeric bone-targeting delivery system is that such carriers are lysosomotropic, which could help to direct cathepsin K inhibitors into lysosomes or into the osteoclast resorption lacuna [122]. This will help to achieve sufficient inhibitor concentrations in subcellular compartments. Nevertheless, such osteoclast subcellular targeting is unnecessary for the inhibition of cartilage damage, as cathepsins are often secreted into the extracellular matrix.

4.6 Cathepsin-activated prodrug approach

Extremely high proteolytic activities in RA and OA joints contribute directly to the progressive joint damage typically seen in these diseases. Therefore, the development of inhibitors for proteases, in particular for cathepsins, and various strategies for their effective delivery are critical. Whereas it is logical to develop inhibitors for enzymes such as cathepsins, the high proteolytic activities at the diseased joints may also serve as a trigger for prodrug activation and drug release from delivery systems.

Polymeric drug delivery systems based on the cathepsin B activation mechanism have been extensively studied by Kopecek *et al*. as macromolecular chemotherapeutics [110,123]. For example, based on the EPR effect of solid tumours, polymer carriers will selectively accumulate to the neoplastic tissue. If specific antibodies or other low MW targeting moieties can be incorporated, active targeting will also be involved. As these polymeric delivery systems are being internalised by the tumour cells through endocytosis, they will accumulate in the lysosomal compartment containing proteases such as cathepsin B. A cathepsin B-specific spacer (Gly-Phe-Leu-Gly) that connects the drugs to the HPMA copolymer will then be cleaved to release the drug and cause death of the tumour cells. At present, there are several HPMA copolymer–drug conjugates in clinical evaluation for the treatment of various forms of cancer [124]. The anticancer drugs used include doxorubicin, paclitaxel, camptothecin and platinate. As discussed in Section 4.1, these macromolecular prodrug strategies may be used in the treatment of RA because of the preferred accumulation of macromolecular carriers in inflamed joints. The high protease activity in RA joints can be used to activate the macromolecular prodrug via the cleavage of peptide spacers. Many studies with low MW prodrugs that can be activated by cathepsin B have also been performed [125-127]. This small molecule prodrug approach itself, or in combination with other drug delivery strategies described above, may be applied in the development of novel treatment strategies for OA. At present, prodrugs or colloidal delivery systems that may be activated by cathepsins K and S have not been reported. However, because of their relatively high expression in RA and OA joints, prodrugs activated by cathepsin S or K may have more desirable safety profiles than those of cathepsin B.

One concern over cathepsin-activated polymeric prodrugs in the treatment of RA may be their relatively short residence time in the inflamed arthritis joints [80]. This may not allow sufficient activation of the prodrug conjugates. There are two options that may help to enhance the conjugate activation efficiency. Bone- or cartilage-targeting strategies may be implemented to prolong the residential time of the conjugates in arthritic joints, or novel spacers with faster cleavage kinetics may be required for the development of cathepsin-activated drug conjugates in the treatment of RA.

5. Summary

Although the aetiologies of RA and OA are still poorly understood, it is obvious that high proteolytic activities of enzymes including cathepsins are the direct cause of articular

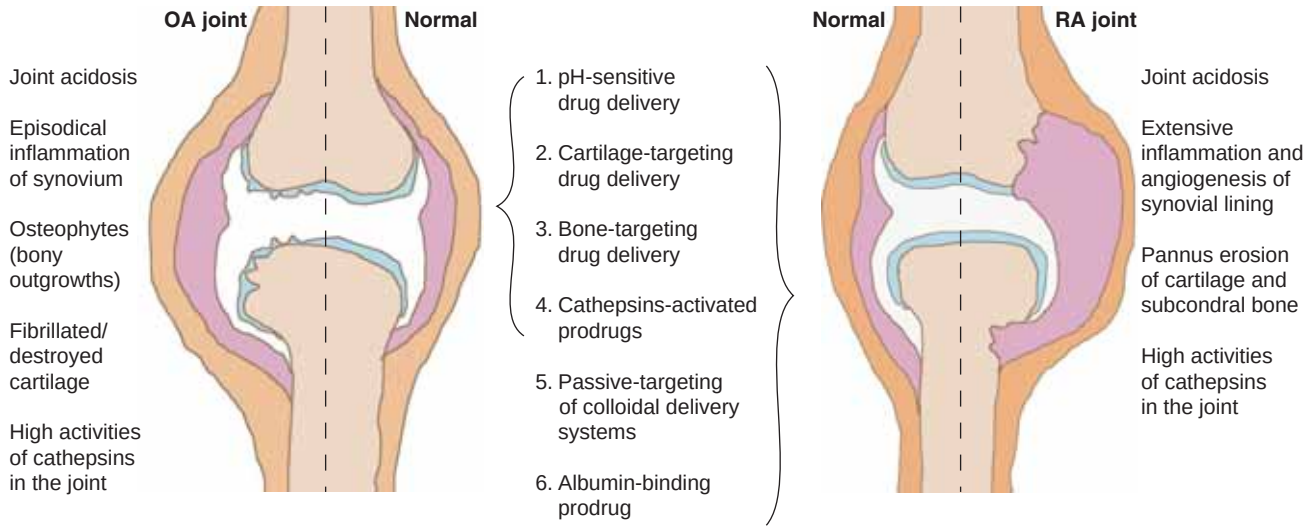

Figure 4. Pathological features and potential drug delivery strategies for improved treatment of RA and OA.
OA: Osteoarthritis; RA: Rheumatoid arthritis.

cartilage and subchondral bone damage. Substantial efforts have been devoted to the pharmacological development of highly potent and selective inhibitors of cathepsins K and S as disease modifying drugs for RA and OA. However, the lack of tissue specificity may eventually hamper their clinical application. Based on the unique pathophysiological features of RA and OA, several drug delivery strategies have been proposed that may be able to specifically direct the inhibitors of cathepsins to RA and OA joints (Figure 4). Some of the proposed methods have been successfully used for the treatment of other diseases, such as cancer. Preliminary treatment studies using colloidal delivery systems showed that they may also be applicable in RA. The authors believe that implementation of these strategies for the joint-specific delivery of cathepsin inhibitors will significantly enhance their therapeutic efficacy and may eventually contribute to a more efficient treatment of these diseases.

6. Expert opinion

It seems to be a golden rule that low MW, orally available drugs are the best drug candidates for chronic diseases, mainly for the reasons of patient compliance, cost of hospital visits and the long-term benefit of the manufacturer. Based on such rules, most of the drug delivery strategies (except low MW prodrugs) discussed above may never be considered for development by the pharmaceutical industry. However, the marginal therapeutic effects for most of the currently available RA and OA treatments plus their questionable safety profiles may cause the industry to reconsider the option of drug delivery. For a most effective treatment of RA, a two-target approach may be advisable. The combined inhibition of cathepsins S and K by individual, highly selective inhibitors, or a single inhibitor of less selectivity, may inhibit the inflammatory as well as matrix-degrading component of the disease at the same time. Cocktail delivery strategies as discussed in Section 4.1 would allow the delivery of two compounds at the same time, whereas a cartilage-directed delivery strategy would limit the toxic effect of a potentially less-selective drug to other organs.

It is true that most of the delivery methods discussed above would need an intravenous or intraperitoneal route of administration and potentially multiple visits to the hospital during the treatment. However, the delivery technologies also promise improved therapeutic effects and safety profiles. In other words, the progression of the arthritis may be controlled much faster with fewer adverse events by using drug delivery technologies than the free drugs. After achieving such results during out-patient treatment, the patients may then switch to low-dose regular low MW drugs to maintain the response.

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