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Cathepsin K inhibitor–polymer conjugates: potential drugs for the treatment of osteoporosis and rheumatoid arthritis

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Abstract

The role of the newly discovered cysteine protease, cathepsin K, in osteoporosis and rheumatoid arthritis is reviewed. The current development of cathepsin K inhibitors and their targeted delivery using synthetic polymer carriers are discussed. Future challenges and possible strategies to improve these delivery systems are addressed.

Keywords: Cathepsin K; Inhibitor–polymer conjugates; Semitelechelic HPMA polymer; mPEG; Antiresorptive; Resorption lacuna

1. Introduction

Osteoporosis, is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures, especially of the hip, spine, and wrist. Presently in the United States, 10 million individuals are estimated to already have the disease and almost 34 million more are estimated to have low bone mass, placing them at increased risk for osteoporosis. The social and economical impacts of such disease to the society are substantial (National Osteoporosis Web Site, 2004).

For a healthy individual, the structure and density of the skeleton is well maintained mainly by two types of cells: the bone-forming cells called osteoblasts and bone-resorbing cells called osteoclasts. However, for various reasons, the population and activity of osteoclasts can become greater with respect to osteoblasts, which eventually leads to osteoporosis (Teitelbaum, 2000).

Bone mainly consists of hydroxyapatite and type 1 collagen (Marks and Odgren, 2002). Dissolution of the inorganic phase of the bone precedes the organic matrix degradation. Bone demineralization involves acidification of the isolated extracellular microenvironment, a process mediated by a vacuolar H^+-adenosine triphosphatase (H^+-ATPase) in the ruffled border. Subsequently, the organic component of the bone may be digested by proteases (Väänänen and Zhao, 2002).

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For many years, lysosomal enzymes, such as cathepsins B or L (Goto et al., 1994) and metalloproteinases (Everts et al., 1999), have been suggested to be important in the bone resorption by osteoclasts. Only a few years ago, the novel lysosomal enzyme, cathepsin K, was discovered and was shown to play a critical role in the bone resorption process.

A 24-kDa cysteine protease of the papain superfamily, cathepsin K, was first discovered in a rabbit osteoclast cDNA library and named OC-2 (Tezuka et al., 1994). The human equivalent of the protein was subsequently cloned by several groups and named cathepsin O (Shi et al., 1995), K (Inaoka et al., 1995), X (Li et al., 1995), and O2 (Brömme and Okamoto, 1995). By recommendation of the International Union of Biochemistry (IUB), the protease is now designated cathepsin K. It shares the highest homology of DNA and amino acid sequence with cathepsins L and S (Brömme and Okamoto, 1995).

In osteoclasts, cathepsin K is expressed at a high level, while cathepsins B, L, and S are expressed at relatively low or undetectable levels (Tezuka et al., 1994; Brömme and Okamoto, 1995; Brömme et al., 1996a; Drake et al., 1996). Deficiency of cathepsin K activity in osteoclasts induces pycnodysostosis, a rare inherited disorder with an osteopetrotic phenotype (Gelb et al., 1996; Johnson et al., 1996). Specific inhibition of cathepsin K expression with antisense oligonucleotides has been shown to produce a 40–50% reduction in bone resorption (Inui et al., 1997). Currently, cathepsin K is the only known mammalian cysteine protease capable of cleaving native type 2 collagen in its triple helix region at multiple sites (Kafienah et al., 1998; Garnero et al., 1998). These findings clearly demonstrate the unique physiological role of this enzyme in organic matrix degradation during the bone resorption process.

Not surprisingly, cathepsin K has also been shown to be able to cleave native type 2 collagen in its helical region (Kafienah et al., 1998; Hummel et al., 1998). Most likely, cathepsin K is also involved in cartilage breakdown in the joints of rheumatoid arthritis patients. The expression of cathepsin K in rheumatoid arthritis-derived synovial fibroblasts has been observed and correlated with the severity of the disease (Hou et al., 2001). It is also possibly involved in the rheumatoid arthritis-related osteoporosis.

2. Cathepsin K inhibitors

Since being implicated in the resorptive activity of osteoclasts, cathepsin K has been considered a novel target for the development of antiresorptive therapeutics for the treatment of osteoporosis. Similar drugs may also be developed for the treatment of rheumatoid arthritis. Because of this market potential, the pharmaceutical industry has been actively involved in the development of cathepsin K inhibitors.

Many low molecular weight cathepsin K inhibitors have been synthesized and extensively investigated (Yamashita and Dodds, 2000; Brömme et al., 1996b). It has been shown that the P_2 position is very critical for cathepsin K substrates. Sequences, such as Gly-Pro-Arg, or compounds mimicking such structure may improve the specificity of cathepsin K inhibitors (Lecaille et al., 2002). A peptidomimetic strategy and introduction of conformational constraint, such as heterocyclic moieties, have improved the oral bioavailability of the inhibitors (Marquis et al., 2001; Veber et al., 2001). Animal studies demonstrated strong antiresorptive activity of some inhibitors (Stroup et al., 2001).

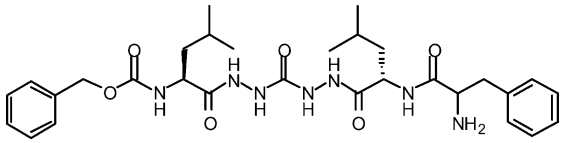
However, clinical applications of synthetic cathepsin K inhibitors have been problematic due to a lack of tissue specificity (Zaidi et al., 2001). Cathepsin K is not only expressed in osteoclasts, it has been observed in thyroid, ovary, colon, etc. as well (Brömme and Okamoto, 1995). Although it remains unclear, some studies have indicated possible involvement of cathepsin K in these organs' normal function (Tepel et al., 2000; Oksjoki et al., 2001; Jokimaa et al., 2001). In a pathological condition, such as rheumatoid arthritis, cathepsin K has been observed in the synovial fibroblasts of the arthritis joints. Apparently, the administration of low molecular weight cathepsin K inhibitors may not only inhibit the enzyme activity in osteoclasts and synovial fibroblasts, but interfere with the other physiological functions as well. Therefore, a drug carrier, which could deliver cathepsin K inhibitor to specific sites in the bone or arthritis joints would be of great advantage.

The localization and activation of cathepsin K has been investigated using cytochemical and immunohistochemical assays (Xia et al., 1999; Dodds et al., 2001). In osteoclasts, cathepsin K was found to be expressed and processed intracellularly prior to cell

attachment and bone resorption. The activation of procathepsin K is believed to be mainly an autocatalytic process within lysosomes (McQueney et al., 1997; Rieman et al., 2001). Once resorption had started, a high level of cathepsin K was observed in both the resorption lacuna and the intracellular vesicles. In the osteoclasts of patients with cathepsin K deficiency, undigested collagen fibers were observed deposited within the endosomal–lysosomal compartment, which indicated the possible participation of cathepsin K in the terminal breakdown of collagen fibers during transcytotic transport of the bone resorption products (Everts et al., 1985). Recently, the expression of cathepsin K in rheumatoid arthritis-derived synovial fibroblasts was found to be mainly in the lysosomal compartment. Specific inhibition of cathepsin K prevents the intracellular degradation of collagen, leading to an accumulation of undigested fibers within subcellullar compartments (most likely lysosomes) (Hou et al., 2001). Theoretically, these experimental observations suggest that lysosomes represent an ideal subcellular target for the delivery of cathepsin K inhibitors.

3. Cathepsin K inhibitor–polymer conjugates

The subcellular location of cathepsin K suggests the potential for the development of a new class of cathepsin K inhibitors–conjugates of low molecular weight inhibitors and water-soluble polymeric carriers. Attachment of low molecular weight compounds, e.g. anticancer drugs, to macromolecules has been shown to render them lysosomotropic (De Duve et al., 1974). Therefore, conjugation to water-soluble polymers will result in internalization of cathepsin K inhibitors via the endocytic pathway (for study of endocytosis in osteoclasts, see Palokangas et al., 1997) and bring them into contact with the target enzyme. This unique feature of macromolecular therapy has been demonstrated by its successful application in cancer chemotherapy (Kopeček et al., 2000). Furthermore, incorporation of bone-targeting moieties into the cathepsin K inhibitor–polymer conjugates may greatly enhance drug accumulation in hard tissues (Pierce and Waite, 1987; Fujisaki et al., 1995) and prevent interference with the normal function of the enzyme in other organs. In addition, the enhanced vascular permeability of rheumatoid arthritis patients may result in enhanced accumulation of the conjugates in arthritis joints (Maeda, 2001; Fava et al., 1994). Other advantages of polymer conjugation may include improved pharmacokinetic parameters (e.g. AUC and MRT) and better water solubility of the hydrophobic inhibitors.



Scheme 1. Structure of cathepsin K inhibitor **I**.

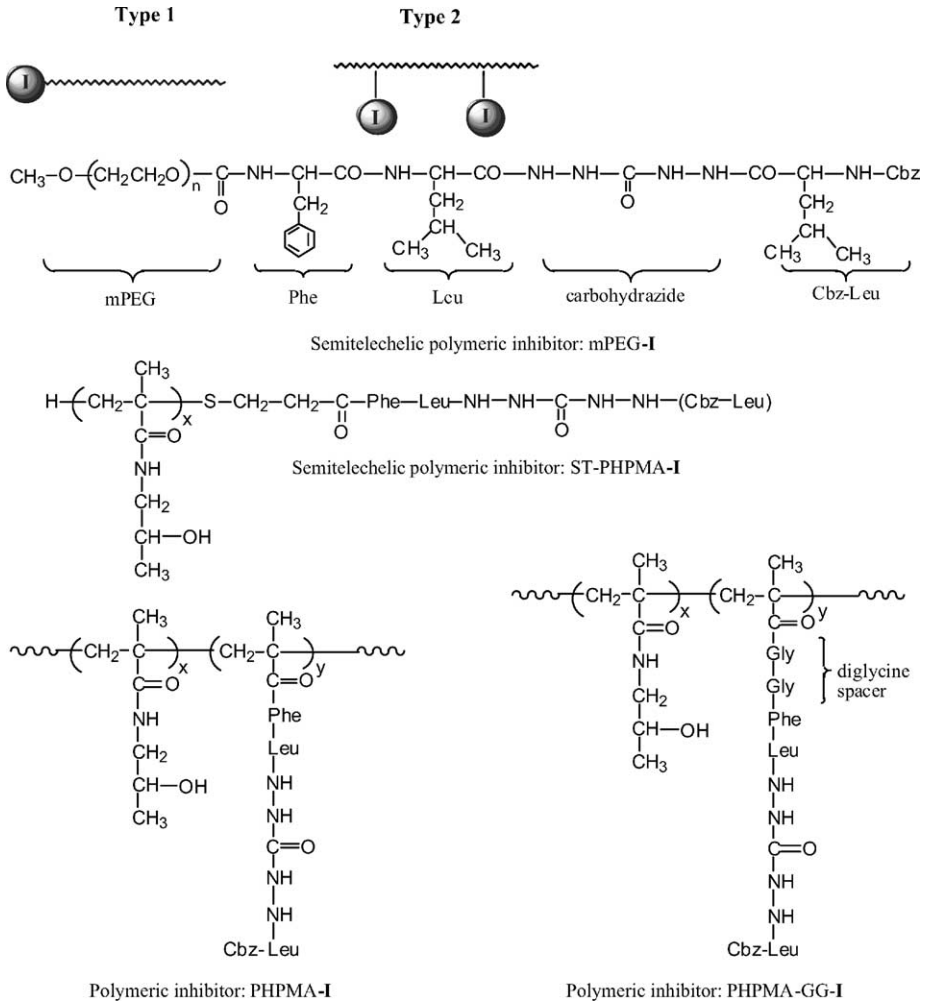
To develop inhibitor–polymer conjugates, it is important to clarify whether the polymer-bound inhibitor will still retain its inhibition activity compared to the free inhibitor.

In a recent study (Wang et al., 2002), we synthesized novel polymeric conjugates of a cathepsin K inhibitor, 1-(*N*-benzyloxycarbonylleucyl)-5-(phenylalanylleucyl)-carbohydrazide (**I**, Scheme 1) with α-methoxy poly(ethylene glycol) (mPEG), semitelechelic poly(*N*-(2-hydroxypropyl)methacrylamide) (ST-PHPMA), and HPMA copolymers containing reactive side chains. The structure of **I** was modified from a potent selective cathepsin K inhibitor, 1,5-bis(*N*-benzyloxycarbonylleucyl)carbohydrazide (Thompson et al., 1997). To retain the binding characteristics of the original structure, a phenylalanine was used to replace the Cbz group. An amino group was then introduced into the inhibitor structure as an attachment point for polymer conjugation.

Two types of conjugation strategies were applied. ST-PHPMA and mPEG were used in the synthesis of type 1 conjugates, while HPMA copolymers containing reactive *p*-nitrophenyl ester side chains were used in type 2 conjugates (Scheme 2).

Table 1
Characterization of inhibitors

Inhibitor	Type	Molecular weight	# **I**/chain
I	–	597.71	–
Boc-**I**	–	697.85	–
mPEG-**I**	1	2700	1.0
ST-PHPMA-**I**	1	4230	0.6
PHPMA-GG-**I**	2	7700	1.1
PHPMA-**I**	2	15500	2.9



Scheme 2. Cathepsin K inhibitor polymer conjugation strategies and the chemical structures of the conjugates.

In order to test the potency and selectivity of the inhibitors and inhibitor–polymer conjugates (Table 1), cathepsin K, cathepsin L, cathepsin B, and papain were used in the enzyme inhibition study.

Kinetic analysis revealed that free inhibitor **I** possessed an apparent second-order rate constant against cathepsin K ($k_{obs}/(I) = 1.3 \times 10^6\,M^{-1}\,s^{-1}$) similar to that of unmodified 1,5-bis(Cbz-Leu)carbohydrazide, while **I** conjugated to the chain termini of mPEG and ST-PHPMA-COOH showed a slightly lower value (approx. $5 \times 10^5\,M^{-1}\,s^{-1}$). The $k_{obs}/(I)$ value for **I** attached to the side chains of HPMA copolymers (PHPMA-GG-**I** and PHPMA-**I**) was about $3 \times 10^4\,M^{-1}\,s^{-1}$. When tested against cathepsin L, inhibitor **I** and all its polymer conjugates produced $k_{obs}/(I)$ values of 1–2 orders of magnitude less than those determined for cathepsin K, while for cathepsin B and papain, the values were 2–4 orders of magnitude lower. Apparently, the inhibition potency of these cathepsin K inhibitor–polymer conjugates depended on the mode of inhibitor **I** attachment. Attachment to polymer chain termini would result in minimal steric hindrance of the polymer chain on the formation of inhibitor–enzyme complexes. On the other hand, attachment to side chains of HPMA copolymer likely resulted in steric hindrance leading to a decrease in inhibitor potency when compared to the free (unbound) inhibitor. If a sufficiently long side chain was

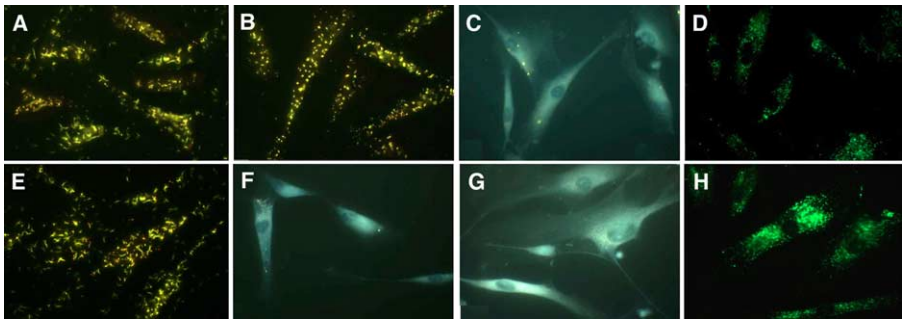


Fig. 1. Inhibition of cathepsin K activity in human synovial fibroblasts with mPEG-**I** and ST-PHPMA-**I**. (A) no inhibitor; (B) mPEG-**I**, 1 μM; (C) mPEG-**I**, 10 μM; (D) mPEG-FITC, 100 μM; (E) no inhibitor; (F) ST-PHPMA-**I**, 1 μM; (G) ST-PHPMA-**I**, 10 μM; (H) ST-PHPMA-FITC, 100 μM. The inhibition activities of the inhibitors and inhibitor–polymer conjugates were demonstrated by the reduction or disappearance of insoluble fluorescent product from cathepsin K hydrolysis of Cbz-Gly-Pro-Arg-MβNA (Nikon Eclipse E800, excitation 440–500 nm and emission above 510 nm). Control studies on polymer cellular entry were performed by incubating cells with mPEG-FITC and ST-PHPMA-FITC (D and H) in 5% CO_2 incubator at 37 °C for 5 h. After three washings with PBS, the intracellular fluorescent signal was detected by fluorescent microscopy. Concentrations refer to either **I** or FITC. Adapted from Wang et al. (2002).

to be used instead of the short Gly-Gly spacer, the inhibitor conjugate may retain most of its activity (Šubr et al., 1986).

The ability of mPEG-**I** and ST-PHPMA-**I** to inhibit cathepsin K activity in synovial fibroblasts was also evaluated (Fig. 1). Both polymer-bound inhibitors were able to inhibit cathepsin K activity completely (10 μM, Fig. 1C and G) in the synovial fibroblasts. However, they showed different inhibition activity at a lower inhibitor concentration (1 μM, Fig. 1B and F), which may be attributable to their different internalization rates (ST-PHPMA was internalized faster than mPEG, Fig. 1D and H). Presumably, they were internalized by endocytosis and were ultimately directed to the lysosomal compartment. The inhibitory activity in the synovial fibroblast assay correlated with their rate of internalization (Wang et al., 2002).

From this study, it appears that if cathepsin K inhibitor is conjugated to the polymer carrier by the proper attachment mode, most of the inhibitory activity will be retained. Any design of polymer-bound cathepsin K inhibitor that minimizes steric hindrance from the polymer would be expected to exert little influence with respect to the inhibitor potency and selectivity.

4. Perspectives

This initial study of cathepsin K inhibitor–polymer conjugates opened a gate for further successful development of a polymeric cathepsin K inhibitor delivery system. However, many aspects of such delivery systems still remain to be elucidated. Though the introduction of bone-targeting moieties would render the delivery systems osteotropic, a detailed bone histomorphological study is necessary to demonstrate the exact target site in hard tissue. Ideally, polymeric inhibitors would accumulate specifically in the active resorption site of the bone, where it would then be internalized by osteoclasts and then exercise its antiresorptive activity. Horseradish peroxidase has been observed internalized by the resorbing osteoclasts from the basolateral membrane and transferred into resorption lacunae (Palokangas et al., 1997). Most likely, the synthetic polymer would follow the same route. However, it needs to be demonstrated that the polymer-containing endosomes will merge with cathepsin K-containing lysosomes and that they would then be secreted into resorption lacunae. Such mechanistic studies can be carried out with a primary culture of isolated osteoclasts. Liposome drug carriers have been shown to preferably accumulate in the arthritis joint with enhanced efficacy (Corvo et al., 1999). Similarly, high molecular weight cathepsin K inhibitors could have the potential to passively accumulate in the synovial fluid of arthritis joints, due to their leaky vasculature. In the type of drug carrier described here, the clearance of non-degradable polymer from the joint fluid will have to be demonstrated.

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