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Cathepsin K inhibitors for osteoporosis and potential off-target effects

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Abstract

Cathepsin K is a highly potent collagenase and the predominant papain-like cysteine protease expressed in osteoclasts. Cathepsin K deficiencies in humans and mice have underlined the central role of this protease in bone resorption and thus rendered the enzyme an attractive target for anti-resorptive osteoporosis therapy. Within the last decade, great efforts have been made in developing highly potent, selective, and orally applicable cathepsin K inhibitors. Some of these inhibitors have passed preclinical studies and are presently in clinical trials at different stages of advancement. The development of the inhibitors and preliminary results of the clinical trials revealed problems and lessons concerning the *in situ* specificity of the compounds and their tissue targeting. In this review, we will briefly summarize the history of cathepsin K research and will discuss the current development of cathepsin K inhibitors as novel anti-resorptives for the treatment of osteoporosis. We will also discuss potential off-target effects of cathepsin K inhibition and alternative applications of cathepsin K inhibitors in arthritis, atherosclerosis, blood pressure regulation, obesity, and cancer.

Keywords

cathepsin K; inhibitor; osteoporosis; arthritis; bone; cartilage

1. Introduction

Osteoporosis affects about 10% of the population in Europe, Japan and North America and its incidence rate is expected to grow with increasing life expectancy. It is prevalent in both genders although women experience bone loss about 15-20 years earlier than men due to hormonal changes during and after menopause (www.iofbonehealth.org/health-professionals/about-osteoporosis/epidemiology.html). The loss of estrogen has been positively linked with bone loss and thus estrogen hormone replacement therapy (HRT) is a major bone protective treatment for women. It was introduced more than 20 years ago and has had a consistent effect on bone mineral density accompanied by a trend in reducing vertebral and non-vertebral fractures [1]. However, epidemiological studies revealed increased incidence rates in breast cancer, heart attacks, stroke, and blot clot formation, prompting the Food and Drug Administration (FDA) to order safety warnings and to recommend against the use of HRT as a general preventive osteoporosis therapy [2]. Besides HRT, bisphosphonates have been developed as the main stack of modern anti-resorptive therapy. Bisphosphonates are generally taken once a week

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and they accumulate on the bone surface. Newer developments may require only once a year applications of the drug. The mechanism of action has been linked to the induction of apoptosis in osteoclasts and thus the reduction of osteoclast-mediated bone resorption [3]. Recent studies, however, have indicated that bisphosphonates may actually increase the total number of osteoclasts. These osteoclasts have been described as hypernucleated, detached and proapoptotic and thus are likely dysfunctional [4]. Bisphosphonates are also used in anti-cancer therapies to reduce bone-metastasis-associated bone resorption. In contrast to the conventional oral osteoporosis treatment regime, bisphosphonates for cancer are given intravenously and at significantly higher doses. It was here where the most severe side effects such as jaw osteonecrosis occurred [5]. Between 1 and 7% of cancer patients on bisphosphonates experience debilitating jaw necrosis [6,5]. Studies indicated that bisphosphonates at high doses interfere with angiogenesis in bone and thus affect the blood supply leading to tissue necrosis [7]. This and anecdotal reports of jaw and hip necrosis events in people on oral osteoporosis therapy raised the awareness for the need of alternative anti-resorptive therapies.

Other common and novel therapies include selective estrogen receptor modulators (Reloxifene), calcitonin, and PTH, which each have been extensively reviewed elsewhere [8-12]. Emerging pharmacologic therapies for osteoporosis include novel estrogenic compounds and PTH derivatives, RANKL antibodies such as Denosumab, c-src kinase inhibitors, chloride channel inhibitors, novel bisphosphonates (see for recent review: [9]), and last but not least, cathepsin K inhibitors, the subject of this review.

2. Cysteine proteases and cathepsin K in bone resorption

The main feature of osteoporosis is bone loss mediated by osteoclasts and insufficient rebuilding of bone matrix by osteoblasts. Bone consists of up to 90% type I collagen fibers, which provide the scaffold for mineral apposition. During osteoporosis bone loses its mineral as well as organic matrix content due to the activity of osteoclasts. Historically, collagenases of the matrix metalloproteinase (MMP) family have been considered as the main culprit proteases for the degradation of collagen as they were thought to be the main proteases capable of cleaving triple helical collagen [13,14]. However, MMPs are active at neutral to slightly alkaline pH values whereas at the site of bone resorption, within the so-called resorption lacuna, acidic pH conditions prevail. Thus, acidic lysosomal hydrolases were proposed to act as the main collagen degrading proteases as early as in 1968 by Vaes [15]. Cysteine proteases, which represent a major group of the lysosomal protease repertoire, were implicated for the first time in 1980. Delaisse and coworkers from the Vaes laboratory demonstrated that cysteine protease inhibitors such as leupeptin can inhibit bone resorption in tissue culture and later in isolated osteoclasts [16-18]. The first cysteine protease inhibitors used were peptide aldehydes such as leupeptin and antipain which are also active against tryptic serine proteases and later were followed by the more specific peptide epoxides (E64) and peptidyl diazomethanes [19,20]. During the 1980s, only cathepsins B, L, and H were known. Cathepsins B and L were thought to be the main actors as both enzymes were known to cleave in the telopeptide region of triple helical collagens [21,22]. As both enzymes are ubiquitously expressed they earned little interest as pharmacological targets. This situation changed dramatically when a novel cathepsin-related cDNA was cloned from rabbits in 1994 which was shown to be predominantly expressed in osteoclasts [23]. This triggered the cloning of the appropriate human orthologue which was initially named cathepsin O, then cathepsin O2, and finally cathepsin K [24-26]. Cathepsin K (O2) was expressed and characterized as a recombinant enzyme in 1996 [27,28]. The protease exhibits a potent collagenase activity towards the main connective tissue collagens type I and II, and immunohistochemical analyses revealed a predominant but not exclusive expression in osteoclasts [27,25,28-30]. One year later, the genetic cause of pycnodysostosis, a rare bone

dysplasia with an osteopetrotic-like phenotype, was identified as cathepsin K deficiency [31]. From this time on it was clear that cathepsin K represented a critical bone resorbing protease and the race was on to develop highly selective cathepsin K inhibitors for the treatment of osteoporosis.

3. Cathepsin K-expression profile and substrate specificity

Human cathepsin K is a 329 amino acid long protein consisting of an N-terminal 15 amino acid long signal sequence, a 99 amino acid long propeptide, and a 215 amino acid long catalytic unit [25]. It shares about 60% protein sequence identity with cathepsins L, S, V and less than 35% with cathepsins F, O, B, H, and W (for review: [32]). Cathepsin K is expressed predominantly in osteoclasts and various other multinucleated cells such as giant foreign body cells and Langhans cells ([25,27,26]; unpublished DB). To a lesser degree it is found in macrophages, synovial fibroblasts, and fibroblasts at locations of wound healing or inflammation, chondrocytes, various epithelial cells of the human fetus, adult lung airway epithelium, thyroid epithelium, and possibly at low concentrations in smooth muscle cells [33-36].

Its predominant expression in osteoclasts was demonstrated on the mRNA level with expression levels about 100-times higher than those of cathepsins L and S [25]. Random sequence analysis of EST clones revealed that 4% of osteoclastic cDNA represents cathepsin K and that it constitutes about 98% of the total cysteine protease ESTs in these cells [26]. The enzyme is sequestered into lysosomes and can be secreted into the extracellular environment. It is specifically secreted into the resorption lacuna underneath actively resorbing osteoclasts where it is responsible for the degradation of the collagen type I dominated organic bone matrix [37]. Highly relevant to osteoporosis, cathepsin K and cysteine protease-associated bone resorption by osteoclasts is down-regulated by estrogen [38-40]. Moreover, the osteoclastogenesis factor RANKL, appears to directly up-regulate cathepsin K expression [41].

Recombinant human cathepsin K exhibits an exceptionally high collagenase activity towards triple helical collagens [29,30]. In contrast to other human cathepsins it is capable of cleavage within the telo as well as triple helical domains of collagens. Unique among all known mammalian collagenases, cathepsin K cleaves at multiples sites within the triple helices of collagens [29,30]. In contrast, collagenases of the MMP family specifically cleave only one single bond and thus generate two collagen fragments: an N-terminal 3/4 fragment and a C-terminal 1/4 fragment (Figure 1A) [42].

The predominant role of cathepsin K in collagen degradation became apparent when osteoclasts were treated with cathepsin inhibitors and large amounts of phagocytosed but undigested collagen fibrils were observed within the cells [35] (Figure 1B). This was exemplified by the finding that the autosomal recessive bone dysplasia, pycnodysostosis was caused by cathepsin K deficiency. Pycnodysostosis patients are characterized by various gross bone abnormalities (short stature, dysmorphic appearance, generalized osteosclerosis, dysplastic bones including hypoplasia of the distal phalanges, clavicles, craniofacial bones) and on the cellular level, osteoclasts and fibroblasts are packed with undigested collagen fibrils underlining the critical role of cathepsin K in collagen degradation [31,43,44]. Besides collagens, cathepsin K cleaves a variety of other bone- and cartilage resident proteins such as osteonectin, aggrecan, and IGF-1 [45-47,32].

The mechanism of triple helical collagen degradation by cathepsin K remains poorly understood. We have demonstrated that the collagenolytic activity of cathepsin K requires the interaction of the protease with certain glycosaminoglycans such as chondroitin and keratin sulfates [48,49]. Cathepsin K forms oligomeric high molecular weight complexes

with these bone and cartilage resident glycosaminoglycans. The complexes are thought to be necessary for the unwinding of triple helical collagens [50]. Cathepsin K is fully active as a gelatinase in the absence of glycosaminoglycans but requires them to exert its collagenase activity [51]. A crystal structure showing the binding of chondroitin sulfate to cathepsin K was recently published [50].

4. Cathepsin K inhibitors

The design of potent and selective active-site-spanning inhibitors for cathepsin K is now a highly competitive area of research and has been extensively reviewed elsewhere [52-59]. Therefore the following section is intended to provide an update from literature reports on recent inhibitors that have been designed and characterized pharmacologically *in vitro* and *in vivo*, and particularly on promising compounds that have advanced to clinical trials for the treatment of osteoporosis, osteoarthritis, and metastatic bone diseases associated with breast and prostate cancers.

Significant cathepsin K-related patent applications have been issued since its discovery fifteen years ago, particularly over recent years indicating the increased interest of several pharmaceutical companies to progress in the design and the development of efficient, selective, and safe compounds to inhibit cathepsin K for osteoporosis therapy and other disorders in which the enzyme is believed to be crucial (for review: [60]).

Major strategies such as high throughput screening, dynamic combinatorial chemistry and *in silico* screening have been exploited for the development of active site-directed inhibitors. Most efforts targeted the cysteine thiol moiety of cathepsin K with reactive electrophile “warheads” in order to reversibly inhibit or irreversibly inactivate its proteolytic activity (for review: [61]).

4.1. Criteria for a pharmacologically relevant cathepsin K inhibitor candidate

Ideally, cathepsin K inhibitors should be of low molecular weight, exhibiting minimal peptide character, bind reversibly and highly selectively without affecting other major cysteine cathepsin family members, particularly the closely related cathepsins L, S, and V (at least a 100-fold higher affinity, i.e. lower K_i or IC_{50} values). The major challenge of the inhibitor design also requires standard drug-like properties such as oral bioavailability with high pharmacological profiles (high membrane permeability, long plasma half-lives, slow elimination rates, no or low toxicity) for acute and chronic use. In the case of cathepsin K, inhibitors have to be delivered into the lysosomes and the resorption lacuna of osteoclasts (osteoporosis therapy) and to synovial fibroblasts for a potential rheumatoid arthritis therapy.

Briefly, early cathepsin K inhibitors were irreversibly acting compounds which inferred predictable side effects if used chronically (antigenic and immunologic complications by generating immunogenic haptens from covalently bound inhibitor-cathepsin adducts, significant off-target inhibition). Though pharmacologically not useful, these compounds were and are important research tools for the characterization of individual cathepsins. Examples are: E-64 and related epoxysuccinyl derivatives, ketones, diacyl-bis hydrazides, and vinyl sulfones [52,56,53].

Subsequently, most development efforts were and are concentrated on the synthesis of reversible inhibitors which include peptidyl aldehydes, amides, α -keto hetero-cycles, aliphatic ketones, and nitriles (for review, see [59]). As cathepsin K and most other cathepsins are lysosomal enzymes, inhibitors were designed to contain lipophilic and basic moieties to allow cell permeability and lysosomotropism. Once protonated within the acidic

subcellular organelles the inhibitors become membrane impermeable [62,61]. However, their increased accumulation in acidic lysosome/endosome may result in off-target inhibition of cysteine proteases other than cathepsin K. Therefore, the strategy shifted to the design of non-basic inhibitors which still maintain their potency and selectivity against individual cathepsins as well as their efficacy in cell-based assays [63,64]. Non-basic cathepsin K inhibitors appear to be safer as they preserve their selectivity over other related-cysteine cathepsins without altering their *in vivo* efficacy.

No anti-cathepsin K drug has been FDA approved. However several inhibitors of cathepsin K are currently at various phases of clinical development for osteoporosis. The interested reader is referred to the following recent reviews [55,65-68]. Inhibitors, namely balicatib in Phase II (Novartis); relcatib in Phase I (GlaxoSmithKline), odanacatib in Phase III (Merck Frosst/Celera) as well as MIV-701/710 in Phase I/pre-clinical (Medivir AB), and an inhibitor from Amura Pharmaceuticals in pre-clinical evaluation will be described in more detail (Table 1). This list is not exhaustive and only comprises more advanced inhibitors.

4.2. Balicatib

Balicatib (AAE581) is the most advanced cathepsin K inhibitor in the Novartis pipeline. Balicatib is a basic peptidic nitrile compound and a potent human cathepsin K inhibitor *in vitro* (IC_{50} = 1.4 nM) with a high selectivity against human cathepsins B, L, and S (> 4,800-fold, > 500-fold and > 65,000-fold, respectively) [62]. Clinical studies showed a reduction of biochemical markers of bone resorption and an increase in bone mineral density in the spine, femur, and hips in ovariectomized monkeys over one year of treatment [69]. The compound was well tolerated in a phase I trial and had a dose-dependent suppression of cathepsin K, with 90% suppression at the 25-mg dosage. Moreover, besides its anti-resorptive activity, the compound appeared to support new bone formation on the outer surfaces of the bones in postmenopausal women, an advantage to bisphosphonates such as alendronate which inhibits bone resorption but slows bone formation as well [70]. However its lysosomotropic character resulted in its accumulation in lysosomes and in nonselective off-target effects which may explain the dramatically decreased selectivity in cell-based enzyme assays when compared to *in vitro* enzyme assays (10 to 100-fold loss in selectivity) [62]. This may also explain why this compound induces skin adverse events since other cathepsins B and L are highly expressed in lysosomes of skin fibroblasts. Moreover, cathepsin K may play an important role in the homeostasis of dermal extracellular matrix [71]. Since cathepsin K-knockout mice are more predisposed to develop bleomycin-induced lung fibrosis, [72], excessive collagen deposition could be related to cathepsin K inhibitor-induced morphea. Phase II trials for balicatib have been discontinued, reportedly due to cutaneous lesions such as pruritus, skin rashes and rare morphea-like skin changes [69,73].

Besides the nitrile-based inhibitors, Novartis is developing arylaminoethyl amides derivatives as potent cathepsin K inhibitors with potencies in the low nM range [74,75]. These small, apparently non-covalent, compounds do not carry an electrophilic warhead that could react with the active cysteine residue. However discrepancies in their mode of inhibition have been reported [65]. Their selectivity for cathepsin K over cathepsins L and S depends on the nature of the lipophilic P1' substituent (> 10,000-fold when P1' is an isobutyl residue), since the S'1 subsite is able to accommodate larger groups. Recently, novel scaffolds of purine nitrile cathepsin K inhibitors have been designed with non-peptidic cyano pyrimidine moieties, focusing particularly on the P3 residue [76]. The IC_{50} values of some derivatives towards cathepsin K were in the nanomolar range and they did not inhibit cathepsins L and S.

4.3. Relacatib

The non-basic 7-methyl-substituted azepanone analogue [77], also known as SB-462795 or relacatib, is a highly potent human cathepsin K inhibitor ($K_{iapp} = 41$ pM) developed by GlaxoSK with good oral bioavailability (89%) and favorable pharmacokinetic characteristics. However, its selectivity is rather low as it inhibits human cathepsins L and V ($K_{iapp} = 68$ and 53 pM, respectively) as well. It displays a selectivity against cathepsins S and B (39–300-fold selectivity) in enzyme assays [78]. Administration of relacatib to ovariectomized and control monkeys resulted in an acute and rapid reduction of bone markers, and this effect lasted for up to 2 days depending on the dose delivered.

After extensive *in vivo* safety and efficacy evaluations in monkey studies, and efficacy experiments on human osteoclasts, GlaxoSK reported jointly with Human Genome Science the advancement of relacatib into Phase I trials (2007) for the treatment of patients with bone metastases and also of postmenopausal osteoporosis (GlaxoSK study ID: SB-462795/008 - www.gsk-clinicalstudyregister.com). There are currently no published data on the clinical trials. Several compounds have been extensively published by GlaxoSK, exploring the substrate/inhibitor binding sites of cathepsin K and the efficacy of selected inhibitors in preclinical studies [55,79-87]. As a result, ketones and azepanones have been revealed as promising cathepsin K inhibitors.

4.4. Odanacatib

Black and colleagues have developed the nitrile-based cathepsin K inhibitor, odanacatib (formerly MK-0822 from Merck & Co.), which displays high potency for cathepsin K ($IC_{50} = 0.2$ nM) and increased selectivity versus cathepsins B, L and S when compared to balicatib and relacatib [88]. Potency and selectivity was due to the presence of the 4-fluoroleucine side chain at P2 position interacting within the S2 pocket. For the first time, IC_{50} values have been determined for human cathepsins F, V (> 1,000-fold) as well as cathepsins C, H, and Z (> 50,000-fold). Interestingly, a similar selectivity was observed in whole cell assays. Despite the relatively low selectivity against cathepsin S measured *in vitro* ($IC_{50} = 60$ nM), odanacatib poorly inhibited antigen presentation (a feature of cathepsin S activity, [89,90]) in cellular assays. In preclinical studies, this molecule presented good pharmacokinetic parameters such as minimal *in vitro* metabolism and long half lives, and oral bioavailability [88]. In ovariectomized monkeys its effects were promising since it suppressed bone resorption without negatively affecting bone formation (bone mineral density was increased in hip and spine) [91]. The compound has also been evaluated in humans for safety and efficacy. Odanacatib, compared with placebo, demonstrated a dose-dependent reduction in bone resorption markers (serum C-telo and urine N-telopeptides of type 1 collagen, biochemical markers of the rate of bone degradation) and an increase in bone mineral density (BMD) at the total hip, lumbar spine, and femoral neck in postmenopausal women with low BMD when given at doses of 10, 25, or 50 mg per week. BMD increased for 24 months of the 2-year study. Comparison of the odanacatib effects on bone formation markers with others anti-cathepsin K inhibitors is described elsewhere [92]. Odanacatib is well tolerated and no major side effects have been observed (for review: [92,88]). It has been reported that this cathepsin K inhibitor has been moved into phase III trials [92].

4.5. Other Developments

Several specific cathepsin K inhibitors MV061194, MV061748, MV061940, MV061645 and MSX-081 are currently under development by Medivir AB (for review: [65]). MV061194 is a selective, potent reversible cathepsin K inhibitor ($K_i = 2.5$ nM) [46]. High selectivity has been measured versus cathepsins L and S ($K_i > 100$ μ M) and cathepsins B and H ($K_i > 10$ μ M). Preclinical studies have shown a selected drug candidate to be active in reducing bone degradation. Furthermore, this compound also augments bone formation

probably by preventing cathepsin K activity, not only restricting collagen degradation but also the degradation of other crucial matrix-embedded growth factors such as osteocalcin, IGF-1, and bone morphogenetic protein, BMP-2 [46]. Medivir AB announced the initiation of its first human trial (phase I) on healthy volunteers with a new small molecule inhibitor, an oral form of MIV-701, in 2007. Studies with 300-mg daily dose on post-menopausal women (during 2 weeks) indicated of 50% lower bone degradation, with mild and transient side effects (headache, gastrointestinal disturbance) (www.medivir.com, press releases issued 11/22/2007). More recently, Medivir AB announced the designation of MIV-710a as a drug candidate for osteoporosis and osteoarthritis (www.medivir.com, press releases issued 02/09/2009). The chemical structures of the selected inhibitors remain unknown to the public and no pharmacokinetic data about the compounds have been disclosed.

Amura Pharmaceuticals has developed a novel series of potent 5,5-bicyclic ketone inhibitors of cathepsin K (K_i in low nanomolar range) with high selectivity (greater than a hundred fold against closely related cathepsins S and L). They were reported to display potent anti-osteoclast activity (i.e., decreased CTX levels) coupled with improved bone formation (i.e., increased levels of osteocalcin) in osteoporosis rat models and human cells [93-95]. The orally active bicyclic cathepsin K compounds revealed good pharmacokinetics and dynamics *in vitro* (with a good ADMET i.e. absorption, distribution, metabolism, excretion and toxicology). Presently, a potent candidate is being selected for a phase I trial as a potential treatment for osteoporosis (www.amura.co.uk). Again, the structure(s) of the drug candidate(s) have not been disclosed.

5. Potential side and off-target effects of cathepsin K inhibitors

In contrast to the currently used bisphosphonates, which affect the quantity and functionality of the whole osteoclast population, cathepsin K inhibitors are significantly more selective by exclusively turning off the arguably single most important protease in bone resorption. As discussed above, all current inhibitor development strategies are directed to specifically inhibit cathepsin K by blocking the active site of the protease. This strategy was driven by the early assumption that cathepsin K is an osteoclast specific target with little or no non-osteoclastic functions and therefore the inhibition of the hydrolytic activity of cathepsin K should not result in non-bone related side effects. However, cathepsin K expression has been demonstrated in various other cell types, which include synovial fibroblasts, skin fibroblasts, macrophages, dendritic cells, chondrocytes, epithelial cells of various tissues, and melanocytes [33-36,96-98]. Whereas some of the non-osteoclast cell types exhibiting cathepsin K expression open a window of opportunity to consider cathepsin K as a novel target for other diseases (Figure 2) (chondrocytes: osteoarthritis; synovial fibroblasts: rheumatoid arthritis; macrophages/giant multinucleated cells: atherosclerosis), other sites of expression may raise concern. It cannot be excluded that the adverse skin phenotype [73] which led to the termination of the balicatib inhibitor trial may have been related to cathepsin K inhibition in skin and not to off-target side effects. The histological analysis of human pycnodysostosis (human cathepsin K deficiency) tissue specimens revealed the accumulation of collagen fibrils in osteoclasts as well as various types of fibroblasts, thus indicating that fibroblasts also exploit cathepsin K as significant collagenolytic protease [44]. Moreover, cathepsin K-deficient mice exhibited characteristics of lung fibrosis in a bleomycin disease model [72]. Both of these observations indicate that cathepsin K inhibitors would affect the turnover of type I collagen not only in bone but in skin and lungs as well. However, the degradation of collagen by fibroblasts appears to be a predominantly lysosomal event [35] whereas osteoclast-mediated collagen degradation is likely to happen extensively in the sub-osteoclastic resorption lacuna [37]. This may explain the advantage of the non-lysosomotropic odanacatib compound over balicatib. In contrast to balicatib, the odanacatib clinical trials reported neither skin nor lung related adverse side effects [92,99].

Of potential concern are also recent reports of cathepsin K activities related to non-collagenous substrates. Brix and co-workers have shown that cathepsin K is expressed in the thyroid epithelium and that the protease is likely involved in thyroid-globulin processing as revealed in studies using cathepsin K deficient mice [100,34]. Most interestingly, the same group reported preliminary results about severe learning/memory impairments in cathepsin K-deficient mice [101]. The mechanism is not known but one speculation is that thyroxin T3 whose release from thyroglobulin is potentially mediated by cathepsin K is responsible for the learning impairment [102]. Of course, a cathepsin K inhibitor-mediated memory defect in the elderly target population for osteoporosis treatment would be of great concern. Along this line, cathepsin K has been implicated in the pathogenesis of schizophrenia [103].

Moreover, cathepsin K might be critically involved in the metabolism of other important regulatory peptides such as kinins. Bradykinin (BK) and related kinins are vasoactive peptide hormones generally released from the kallikrein-kininogen system, but there may exist alternative pathways as well (for review: [104]). The pharmacological effects of kinins are mediated by bradykinin receptors (B1- and B2-type). Kinins are short-term mediators involved in various physiological and pathophysiological events (i.e. vasodilation and constriction of smooth muscle, cardiac homeostasis, regulation of blood pressure, proinflammatory properties in lung). Kinins are subsequently cleaved by kininases. Whilst the kininase activity of ACE (angiotensin-converting enzyme) and NEP (neutral endopeptidase) is well established, their relative contribution to kinin degradation is controversial (for review: [105]). We demonstrated in previous studies that cathepsins L and K might be involved in kinin metabolism [106-108]. While cathepsin L is a kininogenase *in vivo* [107], cathepsin K is the only known mammalian cysteine cathepsin that exhibits a potent kininase activity *in vitro*. Since bradykinin induces bronchial constriction and hyperresponsiveness in asthmatic patients, cathepsin K may have a positive effect during an asthma crisis by inactivating pro-inflammatory kinins. We have recently demonstrated the biological relevance of his hypothesis in rats showing that cathepsin K modulates the BK-dependent contraction of isolated bronchial smooth muscles and impairs BK-induced transient falls in systemic blood pressure [109]. Furthermore critical active site residues involved in the kininase activity of cathepsin K were identified [110].

In summary, while many of the non-osteoclast related functions of cathepsin K are still in an early stage of characterization, they nevertheless need to be seriously considered in the design of future cathepsin K inhibitor trials.

6. Cathepsin K as potential therapeutic target for diseases other than osteoporosis

6.1. Cathepsin K and arthritis

The common feature of various arthritides is the progressive loss of articular cartilage and the damage of the underlying bone structure. In particular, rheumatoid arthritis is characterized by subchondral bone loss and the mechanism of bone loss is similar or equal to that of osteoporosis. Bone loss can even be accelerated due to the use of glucocorticoid treatment regimes in rheumatoid arthritis and is treated with antiresorptives such as bisphosphonates [111]. It seems conceivable that cathepsin K inhibitors should be beneficial for the inhibition of osteoclast-mediated bone resorption in arthritis as well. The role of cathepsin K in rheumatoid arthritis models is highlighted by the findings that overexpression of cathepsin K leads to spontaneous synovitis and cartilage erosion [112] and cathepsin K null mice in the collagen-induced arthritis model revealed decreased bone and articular cartilage resorption (manuscript submitted, DB). It has been demonstrated that cathepsin K

is also expressed in cell types that have been directly implicated in cartilage destruction such as synovial fibroblasts [113,35] and chondrocytes [114,96]. The role of synovial fibroblasts in collagen turnover was demonstrated by the finding that fibroblasts treated with cathepsin K inhibitors (Fig.1B) and cathepsin K-deficient human fibroblasts reveal the intracellular accumulation of undegraded collagen fibrils [35,44]. Thus, the inhibition of cathepsin K in rheumatoid arthritis may require a targeted delivery of inhibitors to the joints as a systemic inhibition of cathepsin K may lead to fibrosis in lung and skin. Strategies to achieve this have been recently discussed [93]. We have demonstrated that some of the classical anti-rheumatoid arthritis drugs such as gold derivatives and quinines may directly or indirectly inhibit the activity of rheumatoid arthritis-associated cysteine proteases such as cathepsins K and S [115]. It should be noted that the central role of cathepsin K in arthritic joint destruction has been recently questioned by the finding that cathepsin K deficiency only partially inhibits but does not prevent bone destruction in a TNF- α overexpressing mouse model [116]. It was demonstrated that in this aggressive arthritis model matrix metallo-proteinases are significantly up-regulated and that they may substitute for the cathepsin K activity. Furthermore, it has been shown that various stimulations including that by TNF- α lead to an up-regulation of cathepsin L in mouse models [117]. It is important to note that the contribution of mouse cathepsin K to extracellular matrix degradation is less pronounced when compared with that of human cathepsin K. Whereas human cathepsin K deficiency reveals the accumulation of intracellular collagen fibrils in osteoclasts as well as fibroblasts [44], this feature is not seen in cathepsin K-deficient mouse cells [118,44]. However, the application of a broad-based cathepsin inhibitor leads to a similar accumulation of collagen fibrils suggesting that cathepsins other than cathepsin K contribute to type I and II collagen degradation in mice. Potential candidates are cathepsins L and B which have been implicated in collagen turnover in the past [22].

Cathepsin K has also been implicated in the pathogenesis of osteoarthritis [119]. Morko et al. observed an upregulation of cathepsin K in articular chondrocytes in a transgenic mouse model of osteoarthritis. Cathepsin K was found near the sites of matrix destruction whereas control mice revealed little expression of this cysteine protease [120]. The strongest support for a direct involvement of cathepsin K in osteoarthritis has been provided in studies using a neopeptide antibody which recognizes a specific cathepsin K-catalyzed cleavage site in type II collagen. In naturally occurring equine osteoarthritis, cathepsin K as well as cathepsin K-mediated type II collagen degradation was significantly increased in osteoarthritic cartilage [97].

6.2. Cathepsin K and atherosclerosis

Atherosclerosis is characterized by the formation of blood flow obstructing plaques which also contribute to the erosion of the underlying tunica media in the artery walls. Moreover, ruptures of plaques are the main causes of infarcts and strokes as they lead to blood vessel thrombus formation. It has been demonstrated that cathepsin K contributes both to blood vessel erosion as well as plaque destabilization [121,122]. Cathepsin K-deficient mice on atherosclerotic-prone ApoE^{-/-} background showed more than 70% less buried fibrous caps than their cathepsin K-expressing litter mates. Buried fibrous caps are linked to the occurrence of past plaque ruptures [123]. Moreover, the number of elastic lamina breaks was also significantly reduced in cathepsin-deficient mice. These studies underline the importance of cathepsin K in the progression of atherosclerosis and that cathepsin K inhibitors might be beneficial in controlling potentially fatal plaque ruptures. The role of cathepsins in cardio-vascular diseases has been recently reviewed [124,125].

6.3. Cathepsin K and metabolic function

In conjunction with their role in vascular remodeling and atherosclerosis, several reports linked cysteine cathepsins with metabolic functions. A strong correlation has been described between obese subject's body mass index and the overexpression of cathepsin S (mRNA and protein levels) (for review: [126]). Given the involvement of cathepsin S in the development of atherosclerotic processes, cathepsin S is probably a plausible candidate to link obesity and cardiovascular diseases [127,128] but the mechanisms whereby enlarged adipose tissue affects vascular function remain poorly defined. Like the closely related cathepsin S, the production of cathepsin K is also enhanced in the white adipose tissue of overweight/obese patients and in animal models. Cathepsin K has been proposed to be a novel marker of adipogenesis, and it is thought that the enzyme might be involved in the pathogenesis of obesity by promoting adipocyte differentiation via the cleavage of osteonectin or other SPARC-related (Secreted Protein Acidic and Rich in Cysteine) components of the extracellular matrix, which are critical for cell adhesion, differentiation, and angiogenesis [129-131]. Recently, cathepsin K null mice showed reduced adiposity under high fat diet treatment in younger mice [132]. All data combined provide new experimental evidence for the involvement of cathepsin K in adipose tissue. Cathepsin K inhibitors are valuable tools to further dissect the involvement of cathepsin K in obesity and may represent a future treatment potential.

6.4. Cathepsin K and cancer

Findings demonstrating the involvement of cysteine cathepsins in various cancers associated with malignant progression have been presented in several papers (for review: [133-135]). On the basis of their up-regulation, their extracellular secretion into tumor associated acidic pericellular micro-environments, and their potent extracellular matrix-degrading activity in many human tumors, including breast, lung, brain, bone, prostate, and melanoma, cathepsins may represent potential diagnostic and prognostic markers [136,137]. Indeed, cathepsins have been considered ideal therapeutic targets in cancer and therefore cathepsin inhibitors have been proposed as promising anticancer agents, particularly in the light of the failure of clinical trials using MMP inhibitors in the late 1990s (for review: [60,135]). A significant extracellular role of cathepsins in tumor progression and metastasis would support the use of non-lysosomotropic inhibitors which could have potent effects by targeting cell-surface or secreted cathepsins in several types of tumors, thus limiting the inhibition of intracellular cathepsins in normal cells. It has been proposed that cathepsin K inhibitors could be useful for the treatment and prevention of bone metastases [65]. Besides its expression in breast, lung, melanoma and thyroid cancers, cathepsin K has been also implicated in prostate tumors associated with increased invasive potential [138-143,98].

Considering the critical role of cathepsin K in bone remodeling, cathepsin K inhibitors ought to be beneficial in the treatment of cancers characterized by extracellular matrix degradation. Clinical trials with odanacatib support this assumption. 43 women with breast cancer and metastatic bone disease received 5 mg of drug daily over 4 weeks and showed urine NTX level reductions comparable to 4 mg of intravenous zoledronic acid. Surprisingly, as one of the adverse effects skin rushes and pruritis were listed for two patients although the symptoms disappeared after one week without discontinuation of the medication (www.merck.com/newsroom/press_releases/research_and_development/2008_0527.html). Obviously, this will require strict observation in future studies as it might be reminiscent to the problems reported for balicatib. The implication of cathepsin K in other diseases and metabolic pathways is summarized in Figure 2.

7. Expert opinion

Bisphosphonates and estrogen replacement therapy are still the main therapeutic staples for current treatment regimes of osteoporosis. However, various risk-benefit considerations as well as expiring patent protections push for the development of novel anti-resorptive drugs. Cathepsin K inhibitors are taking center stage with regard to the understanding of the drug target and the stage of drug development. In contrast to the rather general target, estrogen, which is involved in a multitude of regulatory functions or the approach to eliminate a whole cell type such as osteoclasts by the bisphosphonate approach, the selective inhibition of a single and pathology causing enzyme such as cathepsin K appears to be advantageous over classical anti-resorptive approaches (Figure 3).

However, the non-osteoclastic functions of cathepsin K must be strongly considered. Preliminary findings that cathepsin K appears to be involved in blood pressure regulation, certain brain functions, and skin and lung homeostasis are of particular concern for the future application of chronically administered anti-osteoporotic cathepsin K inhibitors. Other findings regarding the role of cathepsin K in arthritis, atherosclerosis, and certain cancers may widen the therapeutic window of cathepsin K inhibitors.

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Figure 1.

A) Schematic representation of the degradation of triple helical collagens by cathepsin K (random cleavage sites) and matrix metallo proteinases (one cleavage site). B) Electron microscopy images (osmium staining) of human RA-synovial fibroblasts cultivated on bovine cartilage discs (original magnification $\times 10,000$). The left panel shows the inhibition of fibrillar collagen degradation in a synovial fibroblast by the potent cathepsin K inhibitor LHVS (accumulation of undigested collagen fibrils in lysosomal/endosomal vesicles; see arrows). The right panel shows the lack of inhibition of collagen degradation in the presence of the potent cathepsins S, L and B but ineffective cathepsin K inhibitor, Mu-hPhe-Np-VSNp (no intracellular accumulation of striated collagen fibrils) [145,35].

Figure 2. Physiological and pathophysiological implications of cathepsin K (expressed or not) in mammals. Cancers or related diseases are in italic. Potential consequences of a treatment with a cathepsin K inhibitor are indicated (left panel: off-target; right panel: therapeutic target for osteoporosis and other diseases).

Figure 3.

Drug development strategies for osteoporosis therapy: A) systemic interference (Hormone replacement therapy), B) osteoclast cell targeting (apoptosis of osteoclasts by bisphosphonates), C) single molecular function targeting (prevention of bone collagen matrix degradation by cathepsin K inhibition).

Table 1

Novel inhibitors of cathepsin K in pre/clinical development

Cat K inhibitors	Structure description	Activity	Ref.
Novartis balicatib (AAE581) Phase II (discontinued)		IC_{50} (nM) cat K = 1.4 cat B = 4,800 cat L = 503 cat S = 65,000	[62]
GlaxoSK relacatib (SB-462795) Phase I		$K_{i,app}$ (nM) cat K = 0.041 cat B = 13 cat L = 0.068 cat S = 1.6 cat V = 0.063	[78]
Merck/Celera odanacatib (MK-0822) Phase III		IC_{50} (nM) cat K = 0.2 cat B = 1,034 cat L = 2,995 cat S = 60 cat V = 762 cat F = 795 cat C, H, Z > 10,000	[88]
Medivir AB MV061194 MIV-701 Phase I (completed)	<p>Structure unknown</p>	IC_{50} (nM) cat K = 2.5 cat B, H > 10,000 cat L, S > 100,000	[46]
Amura bicyclic ketone cat K inhibitor Preclinical studies	<p>Structure unknown</p>	IC_{50} (nM) cat K = 10 cat B > 10,000 cat L > 3,500 cat S > 4,500	[144]