Cathepsin K – A New Molecular Target for Osteoporosis

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Abstract

Cathepsin K (Cat K) is a cysteine protease of the papain family, now considered to be the major enzyme responsible for degradation of the organic bone matrix. It is highly and selectively expressed in osteoclasts and, under acidic conditions, has the unique ability to degrade type I collagen helical regions. Complete deficiency of Cat K activity leads to pycnodysostosis, a severe and rare autosomal recessive bone sclerotic disorder, and Cat K null-mice are osteopetrotic. Thus this protease is an attractive target for inhibition of bone resorption. Cat K inhibitors have been reviewed recently. Therefore, the following Perspective will focus on inhibitors that have been extensively characterized in animal models of bone loss, particularly the inhibitors that have advanced to clinical trials for the treatment of osteoporosis. Other disease targets such as osteoarthritis and bone metastasis will also be discussed.

Introduction - Structure, Function, Localization and Genetics of Cathepsin K

Osteoclasts resorb bone by removing both the inorganic (hydroxyapatite) and the organic components (90% is fibrillar type I collagen) of mineralized bone. Following tight attachment to the bone surface, osteoclasts secrete protons into a sealed extracellular compartment. The acidic pH (about 4-5) removes the bone mineral and exposes the underlying matrix (Figure 1). About forty years ago, based on a model of parathyroid hormone-stimulated, resorbing bone explants, Vaes proposed that lysosomal acid hydrolases were the proteases degrading the organic matrix of bone (1) and demonstrated that E-64 and leupeptin, inhibitors of lysosomal cysteine proteases, blocked bone resorption in vitro and in vivo (2). Although the identity of the enzyme(s) was not known, several cathepsins including B and L were thought to be involved in bone resorption. In 1994, cathepsin K (Cat K) was discovered using differential display of osteoclast and rabbit macrophage cDNA libraries in rabbits (3). Subsequently, the human and murine enzymes were also cloned (4,5).

Cat K is a member of the CA1 family of lysosomal cysteine proteases. In humans, there are eleven functionally diverse members of this family (6), with each cathepsin having an active site comprised of cysteine, aspartagine and histidine residues. Cat K and other cysteine proteases are synthesized as inactive pre-pro enzymes from which the propeptide must be removed for activation. In vitro, this is an autocatalytic process at pH 4 (7), making this enzyme an ideal candidate for the acidic conditions of the resorption lacunae. Unlike the other cathepsins, Cat K not only degrades type I collagen in the telopeptide regions, but is capable of cleaving the triple helical domains at multiple sites (8). Cat K is abundantly expressed in osteoclasts along the bone and cartilage...
Figure 1: Schematic representation of a resorbing osteoclast. The osteoclast acidifies the resorption lacunae by secreting H\(^+\) and Cl\(^-\) ions for demineralization, and lysosomal cathepsin K for degradation of type I collagen.

resorption lacunae, near the ruffled border (9), and in intracellular lysosomes and transcytotic vesicles (10). Cat K expression in other cells is much lower than that observed in osteoclasts either at the level of mRNA or protein (embryonic lung, and neonatal dermal fibroblasts (11;12)), but is higher in synovial fibroblasts and macrophages of rheumatoid arthritic joints (13) and in breast and prostate tumors (14; 15).

The proof of concept for Cat K being the major collagenase responsible for degradation of the bone matrix was provided by the discovery that deficiency of this enzyme causes a rare autosomal recessive bone sclerosing disorder called pycnodysostosis (16) triggered by mutations in the Cat K gene. Affected individuals are typically short in stature and, in spite of dense bones, suffer from increased non-traumatic fractures and were shown to accumulate undigested collagen fibrils in osteoclasts (17). While targeted disruption of the Cat K (CSTK) gene in mice results in osteopetrosis without an effect on bone quality (18-20), in one study Cat K knockout mice backcrossed into a C57BL/6J background developed bone fragility (21). Furthermore, transgenic mice that overexpress Cat K have reduced trabecular bone volume as a result of accelerated bone turnover (22).

Based on its localization, selectivity of expression, biochemical activity, and (mouse and human) genetics, Cat K has become a pharmaceutical target for the treatment of osteoporosis and potentially for other diseases associated with high bone turnover such as osteoarthritis, and metastatic bone disease. Cat K inhibitors designed to diminish bone resorption in vitro and in vivo have been recently reviewed (6; 23;24), therefore, the following Perspective is intended to provide an update on inhibitors that have been extensively
characterized in animal models of bone loss, particularly focusing on those that have advanced to clinical trials for the treatment of osteoporosis.

**Cat K Inhibitors for Suppression of Bone Loss - *In Vitro and In Vivo* Pharmacology**

Briefly, cysteine protease inhibitors are low molecular weight compounds carrying an electrophilic moiety, referred to as a warhead, which targets a nucleophilic cysteine in the active site of this enzyme. Ideally, effective and safe Cat K inhibitors should bind reversibly, have good bioavailability and potency and be selective for Cat K with limited inhibition of other cysteine protease family members. For characterization of potency and selectivity, the Cat K inhibitors are evaluated in enzyme assays using purified recombinant human Cat K and other related cathepsins L, B and S. In addition, detailed interactions of Cat K and its inhibitors can be viewed as high resolution X-ray crystallographic structures (25). For assessing functional efficacy *in vitro*, some inhibitors were tested in isolated rabbit osteoclast-mediated bone resorption assays, since rabbit Cat K is 94% homologous to human Cat K. A covalent and reversible inhibitor with a nitrile warhead, L-006235, was thus identified with the following properties: (i) forms a reversible thioimidate bond with the active site cysteine of cathepsin K; (ii) inhibits human Cat K with a Ki of 0.25 nM; and (iii) bone resorption by rabbit osteoclasts (IC$_{50}$ = 5 nM); and (iv) is >4000- fold selective against cathepsins L, B and S (26).

The traditional rat and mouse models of bone loss are not appropriate for testing *in vivo* efficacy because of the differences between the rat/mouse and human Cat K enzymes (87-88% homology). Therefore, the *in vivo* efficacy of this compound was tested in ovariectomized (OVX) rabbits. When given orally, once daily for 27 weeks to adult newly-OVX rabbits, L-006235 partially (2 mg/kg/d) or fully (10 mg/kg/d) blocked bone loss due to estrogen deficiency with efficacy similar to alendronate, which was used as a control. However, unlike alendronate, L-006235 had no effect on cancellous and endocortical mineralizing surface (MS/BS) or on Haversian bone formation (27). These results were surprising given that in adult bone turnover, formation is tightly coupled to bone resorption. Furthermore, SB-553484, which inhibits mouse Cat K with an IC$_{50}$ of 26 nM, prevented bone loss in OVX mice while increasing cortical bone volume and cortical thickness (28). There is some precedence for these data in Cat K knockout mice, where histomorphometric analysis of cancellous bone revealed that bone formation rates were increased (18-22;29).

In non-human primates, as in other animal models of bone loss, serum and urinary biomarkers of bone resorption and formation are increased as a result of OVX. In addition, rhesus and cynomolgus monkeys have identical amino acid sequences to human Cat K (30-31). Proof of concept for L-006235 as an anti-resorptive agent was therefore further tested in OVX rhesus monkeys treated for 11 days, and was found to dose-dependentbly suppress the increases in collagen degradation products (uNTx) (26). Other Cat K inhibitors, balicatib (AAE581 from Novartis), relacatib (SB-462795 from GlaxoSmithKline) and odanacatib (MK-0822 from Merck), when tested for longer periods (9 to 18 months) in OVX cynomolgus (balicatib and relacatib) and in rhesus (odanacatib) monkeys, all significantly reduced bone resorption markers. However, balicatib and relacatib also increased bone formation markers relative to vehicle-treated animals. In one of these studies, alendronate was used as a control and was demonstrated to suppress both bone resorption and formation markers as expected.

The effects of the Cat K inhibitors on OVX monkey bones can be summarized as follows: (i) balicatib prevented the loss of vertebral and femoral BMD, increased bone strength, and suppressed cancellous bone formation rate (MAR), while increasing periosteal bone formation and cortical thickness (32); (ii) relacatib also reduced bone formation at cancellous sites while increasing femur periosteal bone (33) possibly due to transiently increased PTH.
plasma levels (34); (iii) odanacatib increased BMD of the spine and hip, and reduced bone turnover in trabecular and endocortical surfaces in rib and ilium biopsies (35). In conclusion, Cat K inhibitors, in mouse, rabbit and in some monkey bone loss models, suppressed bone resorption without affecting bone formation.

Recently, there has been great interest in the development of drugs for osteoporosis with these properties, i.e., with uncoupling of bone formation and resorption, possibly due to secretion of anabolic factors either from non-resorbing osteoclasts (36-38) or released from bone matrix by resorbing cells (39). Although patients with pycnodysostosis have reduced bone turnover with no suggestion of uncoupling of resorption and formation (40), these studies were done in only two patients, a child and a young adult. More studies are clearly needed, but given the rarity of patients with this condition, may be slow to emerge.

**Clinical Trials and Safety of Cat K Inhibitors**

Relacatib, balicatib and odanacatib have all been evaluated in humans for safety and efficacy. Relacatib is an equipotent inhibitor of Cat K, L and V, while exhibiting some selectivity against Cat S and B (41). There are currently no published data on relacatib in clinical trials as it was reportedly discontinued following Phase I studies. In a one-year dose ranging study (5, 10, 25 and 50 mg/day) in postmenopausal women, balicatib at 25 and 50 mg reduced serum CTx by 61% and urinary NTx by 55%. However, serum bone formation markers (osteocalcin, bone specific alkaline phosphatase and N-terminal propeptide of type I collagen (P1NP)) were not reduced by the 10, 25 and 50 mg doses, and were increased in the 5 mg dose group. Although there were dose-dependent BMD increases in lumbar spine and hip of 4.5 and 2.2%, respectively, the development of balicatib has been discontinued, reportedly due to skin adverse events including scleroderma-like skin thickening and rashes (42). Several Cat K inhibitors, including balicatib and L-006235, are basic, nitrogen-containing compounds that demonstrate lysosomotropic properties, i.e., increased accumulation in acidic lysosomes. In whole cell assays, both compounds accumulated in lysosomes and displayed increased potency (~100-fold) against off-target cathepsins L, B and S (43). In a recent in vivo study, balicatib and L-006235 were found to have activities against Cat L, B and S in rats and mice (44). It is therefore possible that the skin toxicities in human trials were caused by increased accumulation of balicatib in lysosomes of human skin fibroblasts, in which cathepsins B and L are highly expressed and localized along with Cat K.

These findings led to the synthesis and evaluation of non-basic inhibitors, including odanacatib, which do not accumulate in lysosomes, therefore maintaining selectivity in whole cell assays (45). Odanacatib inhibits human cathepsin K with an IC$_{50}$ of 0.2 nM, and rabbit osteoclast-induced bone resorption in vitro with an IC$_{50}$ of 23 nM. In addition, odanacatib exhibited long half-lives in several preclinical species, including 6 h in the rat, 57 h in the dog and 18 h in the monkey. In Phase I clinical trials, odanacatib was shown to have an apparent half-life of 45-50 hours (46) in humans, making it possible to evaluate the safety and tolerability of weekly doses in clinical trials (47). In a 12-month, randomized, double blind, placebo-controlled study in postmenopausal women, odanacatib at the highest dose tested (50 mg/week) increased lumbar spine BMD by 3.4%, femoral neck BMD by 2.5%, and reduced uNTx/Cr by 58% and produced less reduction of serum bone formation markers compared to that seen historically with bisphosphonates. Odanacatib was generally safe and well-tolerated. Patients in this study continue to be evaluated for 24 months and the results will be presented in 2008 (47).

**Other Indications**

Metastatic bone disease (MBD) is characterized by very high levels of bone turnover in proximity to tumors (48;49). Bone resorption inhibitors such as bisphosphonates represent the current
standard of care for the treatment of bone metastases primarily due to breast, prostate or multiple myeloma; yet, this mode of treatment is palliative. It has been proposed that other strong anti-resorptives such as Cat K inhibitors could be useful in the treatment and prevention of bone metastases. Cat K is expressed in breast and prostate cancers (14;15). Evidence for a treatment effect of cathepsin K inhibitors has been presented in the form of preclinical MBD models in which human breast cancer cells are implanted in nude mice or rats. Cat K inhibitors, either dosed immediately after tumor implantation or following detectable osteolytic lesions at the tumor injected site, were demonstrated to prevent or reduce breast cancer-induced osteolysis and skeletal tumor burden (50;51). When prostate cancer cells were injected into the tibia of SCID mice, treatment with a Cat K inhibitor either in prevention or in treatment mode, was also reported to effectively block the progression of skeletal lesions and cancer growth in bone (52).

Cat K inhibitors may be beneficial for the treatment of osteoarthritis. Cat K expression is increased in tissues isolated from synovia from joints of human OA patients and is specifically found in synovial fibroblasts and articular chondrocytes (recently reviewed in (53)). Cat K not only efficiently degrades triple helical type II collagen, one of the two major extracellular components of cartilage, but it also degrades aggrecan at acidic pH (6). This enzyme is thus suggested to play a direct role, along with metalloproteinases, in articular cartilage degradation. Cat K inhibitors, through antiresorptive activity, may also reduce subchondral bone turnover, thus producing an indirect benefit, contributing to modifying disease progression in osteoarthritic joints (54).

**Summary**

Cat K inhibitors have been shown to inhibit bone resorption in several animal models and in clinical trials. In some animal models of bone loss, Cat K inhibitors are effective without inhibiting bone formation. In clinical trials after 12 months of treatment, balicatib and odanacatib increased both lumbar spine and hip BMD, and reportedly showed limited suppression of bone formation markers, as compared to the known reduction of these markers by bisphosphonates. These results suggest an apparent decoupling of bone formation and resorption. A phase III fracture prevention trial with odanacatib is underway. Further human studies of these new agents should determine bone safety, and possible neutral or stimulatory effects on bone formation.

**Conflict of Interest:** Dr. Rodan reports that she is a consultant to Merck & Co. and owns stock in the company. Dr. Duong reports that she is an employee of Merck & Co. and owns stock in the company.

**References**


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