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Inhibition of cathepsin K reduces cartilage degeneration in the anterior cruciate ligament transection rabbit and murine models of osteoarthritis

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ABSTRACT

Objective: To investigate the disease modifying effects of cathepsin K (CatK) inhibitor L-006235 compared to alendronate (ALN) in two preclinical models of osteoarthritis (OA).
Methods: Skeletally mature rabbits underwent sham or anterior cruciate ligament transection (ACLT)-surgery and were treated with L-006235 (L-235, 10 mg/kg or 50 mg/kg, PO, daily) or ALN (0.6 mg/kg, s.c., weekly) for 8-weeks. ACLT joint instability was also induced in CatK$^{-/-}$ versus wild type (*wt*) mice and treated for 16-weeks. Changes in cartilage degeneration, subchondral bone volume and osteophyte area were determined by histology and μ-CT. Collagen type I helical peptide (HP-I), a bone resorption marker and collagen type II C-telopeptide (CTX-II), a cartilage degradation marker were measured.
Results: L-235 (50 mg/kg) and ALN treatment resulted in significant chondroprotective effects, reducing CTX-II by 60% and the histological Mankin score for cartilage damage by 46% in the ACLT-rabbits. Both doses of L-235 were more potent than ALN in protecting against focal subchondral bone loss, and reducing HP-I by 70% compared to vehicle. L-235 (50 mg/kg) and ALN significantly reduced osteophyte formation in histomorphometric analysis by 55%. The Mankin score in ACLT-CatK$^{-/-}$ mice was ~2.5-fold lower than the ACLT-*wt* mice and was not different from sham-CatK$^{-/-}$. Osteophyte development was not different among the groups.
Conclusion: Inhibition of CatK provides significant benefits in ACLT-model of OA, including: 1) protection of subchondral bone integrity, 2) protection against cartilage degradation and 3) reduced osteophytosis. Preclinical evidence supports the role of CatK as a potential therapeutic target for the treatment of OA.

Introduction

Osteoarthritis (OA) is the most common type of musculoskeletal disorder and the main cause of aged-related disability. In the United States, the prevalence of OA increases with age and more than 80% of individuals older than 65 years are affected by OA [1]. OA is normally characterized by the loss of articular cartilage, subchondral bone sclerosis and osteophyte formation. This disease has been primarily known as a cartilage disorder associated with focal articular cartilage degradation [2]. Proteolytic enzymes produced by chondrocytes, including a number of metalloproteinases (MMP-1, 8 and 13) and aggrecanases (ADAMTS-4 and -5), break down both collagen and proteoglycans. Loss of cartilage worsens progressively over time, leading to reduced joint space and exposure of the underlying bone [2]. However, this disease also involves well-defined synovitis, changes in ligaments and menisci, early increases in the subchondral bone remodeling and subsequent osteophyte formation [2,3].

Cathepsin K (CatK) is highly expressed in actively resorbing osteoclasts and has been demonstrated to be rate limiting in mediating the degradation of matrix proteins, including type I collagen, the major structural protein in bone [4]. Systematic analyses revealed lower levels of CatK mRNA in other cell types, which include synovial fibroblasts, skin fibroblasts, macrophages, dendritic cells, chondrocytes, and epithelial cells of various human tissues [5]. Targeted gene deletion of CatK leads to a high bone mass phenotype in mice due to a significant decrease of osteoclastic bone resorption [6,7]. Potent CatK inhibitors have been demonstrated to effectively prevent estrogen-deficiency induced bone loss in rabbits and non-human primates [8–10]. Moreover, the selective CatK inhibitor odanacatib is currently being developed as treatment for postmenopausal osteoporosis [11].

CatK has been implicated in the pathogenesis of OA [12]. CatK expression was found to be highly elevated in the synovium of rheumatoid arthritis and OA patients [13]. Increased CatK protein could be localized to synovial fibroblasts, stromal multinucleated giant cells and chondrocytes at the site of cartilage erosion [13]. A recent study revealed that in addition to osteoclasts in subchondral bone in OA, detectable CatK expression was found in human chondrocytes repairing

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fibrocartilage in OA [14]. Compelling evidence to support the direct involvement of CatK in OA came from studies demonstrating CatK degrades key cartilage components including type II collagen and aggrecan [12,13]. In naturally occurring equine OA, CatK and its degradation product C-telopeptide from collagen type II (CTX-II) were significantly increased in OA cartilage [15,16]. Up-regulation of CatK expression was demonstrated in articular chondrocytes in a Del1 transgenic mouse model of OA [17,18]. To further support the role for this enzyme in the pathogenesis of OA, transgenic mice overexpressing CatK under its own promoter became susceptible to progressive synovitis, which, upon aging, resulted in synovial hyperplasia and fibrosis and subsequent destruction of articular cartilage and bone [17].

Collective evidence suggests that pharmacological inhibition of CatK potentially leads to direct disease-modifying effects by reducing cartilage degradation or indirectly by modifying subchondral bone remodeling. We previously established the disease modifying action of alendronate (ALN), a potent bisphosphonate in the joint instability induced OA model in rats [19]. Treatment with a CatK inhibitor, SB-553484, resulted in mild to moderate chondroprotective effects and reduced degradation markers of bone (CTX-I) and cartilage (CTX-II) in the canine partial medial meniscectomy model of OA [20]. More recently, prolonged treatment of the Dunkin-Hartley guinea pig model of spontaneous OA with another CatK inhibitor, AZ12606133, decreased cartilage turnover as determined by urinary CTX-II levels, and reduced mechano-sensitivity in response to joint movement [44]. However, in contrary to other studies, Takahashi et al. demonstrated that down regulation of CatK expression in the synovium using small interference RNA was reported to accelerate the progression of OA in the anterior cruciate ligament transection (ACLT) model of OA in rabbits [21].

In the present study, we evaluate the role of CatK in the disease progression using two different preclinical models of OA. The disease-modifying effects of a potent CatK inhibitor L-006235 (L-235) are evaluated in skeletally mature ACLT-rabbits. In this model, L-235 displays significant chondroprotective effects, reduces the cartilage degradation marker, urinary CTX-II, and the histological Mankin score for cartilage damage. Moreover, L-235 dose-dependently reduced osteophyte formation. The effects of CatK deficiency on OA progression are further assessed in the ACLT-induced joint instability of CatK knock-out mice as compared to the aged matched wild type (*wt*) mice. In these mice cartilage degeneration as determined by Mankin score in ACLT-Cat $K^{-/-}$ mice was significantly lower than that in ACLT-*wt* mice.

Materials and methods

Anterior cruciate ligament transection model of osteoarthritis

In rabbits

All procedures were approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories. Thirty-five adult (9–13 months old, 3.3–4.6 kg) male New Zealand white rabbits (Taconic, NJ) were randomized based on body weights at baseline. Rabbits were anesthetized with ketamine/xylazine, followed by intubation and maintenance on isoflurane. The right knee joint was exposed through a medial parapatellar approach. The patella was dislocated laterally and the knee was placed in full flexion; the anterior cruciate ligament (ACL) was transected as previously described [19,22]. Lachman test was performed in the ACLT-rabbits to confirm complete ACL-transection, while subluxation of patella and flushing with saline were performed in the sham operated animals. Buprenorphine hydrochloride (0.1 mg/kg) (Reckitt & Colman Products Ltd., Hull, England) was given as an analgesic. Rabbits were allowed to move freely in soft bedding plastic cages.

In mice

Characterization of high bone mass phenotype of Cat $K^{-/-}$ mice has been previously described [7]. ACLT was performed on the right knee joints of 21–23 weeks old male (n = 8) and female (n = 10) $CatK^{-/-}$ and aged matched male (n = 10) and female (n = 13) wild-type C57BL/6 littermates as previously described [23]. Briefly, all surgical procedures were performed using a surgical loupe. ACL was transected with micro-scissors. Complete transection was confirmed with the anterior drawer test. Following surgery, animal care was as described above. Animals were randomized based on body weight and with matched numbers of male and female mice: normal wild type (*wt*), sham-*wt*, ACLT-*wt*, sham-Cat $K^{-/-}$, and ACLT-$CatK^{-/-}$. Sham (n = 4–5/group) and ACLT (n = 7–9/group) mice were necropsied at 10- and 16-weeks post-surgery.

Treatments

L-235 is a potent and reversible inhibitor of human and rabbit CatK, effectively inhibiting bone resorption by rabbit osteoclasts (IC_{50} = 5nM). In vitro potency and selectivity profile of L-235 was previously reported [31]. The equivalent osteoporosis dose of L-235 at 10 mg/kg (PO, daily) or ALN (200 μg/kg, sc, weekly) was arbitrarily defined based upon the dose required for full protection against estrogen deficiency induced bone loss in rabbits [9]. The effective dose of ALN in the ACLT-rats was previously demonstrated to require at least 3-fold the doses employed in the OVX-model of osteoporosis [19]. Thus, in the ACLT-rabbits, the doses of L-235 used were 10 mg/kg and 5-fold of this dose, as compared to ALN dosed at approximately 3-fold of its equivalent dose selected from OVX-rabbits.

Efficacy of the CatK inhibitor L-006235 (L-235) in ovariectomized rabbits was previously described [9]. Sham-animals (n = 7) were dosed P.O. daily with 0.5% Methocel (w/v) vehicle. ACLT-rabbits (n = 7/group) were dosed with either vehicle (ACLT + Veh), ACLT + ALN (200 μg/kg, 3× per week), ACLT + L-235 (10 mg/kg), or ACLT + L-235 (50 mg/kg). A suspension of L-235 in 0.5% Methocel was dosed P.O. daily. All treatments were initiated one week post-surgery. Blood and urinary samples were collected at 3, 5, and 7 weeks post-surgery. At 8-weeks post-surgery, animals were necropsied and joint tissues were collected for histopathological analyses.

Gross morphology and tissue preparation

In studies with ACLT-rabbits, the right joints were disarticulated, cleaned and fixed in 4% paraformaldehyde (Fisher Scientific, NJ) for 24 h. Gross appearance of the rabbit distal femur was recorded by a digital camera (DIX, Nikon, Japan) with a 1:4 Nikon lens (Nikon, Japan) to evaluate osteophyte formation. Osteophytes were scored 0–3: 0; no osteophyte, 1; mild (single small), 2; moderate (small multiple or single large), 3; severe (large multiple). Tibiae were then cut in half at the center of the articular surface along with the medial collateral ligament in frontal section with a band saw (EXAKT Tech. Inc, Norderstedt, Germany). Anterior parts were fixed in 4% paraformaldehyde for another 24 h and decalcified in 0.5 M EDTA prior to paraffin embedding. Paraffin embedded tissues were sectioned and stained with toluidine blue-O (0.2% TB-O/0.1 M sodium acetate buffer, pH 4.0) for Mankin scoring. Other sections were stained for tartrate resistant acid phosphatase (TRAP) activity [24]. Immunohistochemical analysis was performed as previously described [19,25]. Posterior parts were stored in 70% ethanol until embedded in methylmethacrylate. Plastic sections (5 μm thick) were stained with Masson's trichrome for bone histomorphometry analysis. Details on tissue processing for histology and histomorphometry were performed as previously described [19,22]. For *wt* and $CatK^{-/-}$ mice, the right knee joints were fixed in 4% paraformaldehyde for 48 h then switched to 70% ethanol. Hindlimbs were scanned by μ-CT then decalcified in 0.5 M EDTA prior to paraffin embedding. The paraffin embedded tissues were frontal sectioned and stained with toluidine blue-O for Mankin scoring.