THE CATHEPSIN K INHIBITOR L-006235 DEMONSTRATES BOTH DISEASE MODIFICATION AND ATTENUATION OF PAIN BEHAVIOUR IN THE IN THE MIA MODEL OF OSTEOARTHRITIS

James J. Burston[1,2], Luting. Xu[1,2], Paul I. Mapp[1], Urszula Grabowska[3], Karin Tunblad[3], Erik Lindström[3], Victoria Chapman[1,2]

[1]Arthritis Research UK Pain Centre, University of Nottingham, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.[2]School of Life Sciences, University of Nottingham, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom. [3]Medivir AB, Huddinge, Sweden

BACKGROUND

Cathepsin K (CatK) is a cysteine protease predominantly expressed in the osteoclast and involved in bone resorption (1). CatK expression is up-regulated in human osteoarthritis (OA) synovium (1) and CatK mRNA expression is increased in human OA bone (1). CatK over-expression in mice leads to spontaneous synovitis and cartilage degeneration (2). Inhibitors of CatK attenuate lesion severity and biomarkers of collagen degradation in a canine model of OA (3), and have significant effects on subchondral bone integrity, cartilage degradation and osteophytosis in a rabbit model of OA (4). CatK inhibition also reduces mechanosensitivity of knee afferent nerve activity in a guinea pig model of spontaneous OA, thus suggesting a role for CatK in joint nociception during disease progression (5).

AIM

To investigate the effects of a selective CatK inhibitor, L-006235 (6), on both pain behaviour and joint pathology in a model of OA in the rat.

METHODS

Male Sprague Dawley rats received intra-articular injection of saline or monosodium iodoacetate (MIA; 1 mg) into the left knee joint. One day before injection, rats were dosed with either vehicle or 30 mg/kg L-006235 (p.o. twice daily) and then every day for 28 days. Pain behaviour (weight bearing on the hind limbs and changes in distal hind paw withdrawal thresholds) was quantified before and after intra-articular injection of saline or MIA and at several time-points during the 28 day treatment period. Rats were then euthanized and tissues taken for *ex-vivo* analysis. Tibiofemoral joints were removed and post-fixed in neutral buffered formalin (4% formaldehyde), decalcified in EDTA, processed and scored (7). Haematoxylin and eosin staining was conducted. Cartilage surface integrity was scored from 0 (normal) to 5 (full-thickness degeneration), and a total joint damage score (range 0–15) was calculated as cartilage surface integrity × length of cartilage involved in thirds. Inflammation was graded on a scale from 0 (lining cell layer 1–2 cells thick) to 3 (lining cell layer >9 cells thick and/or severe increase in cellularity). Osteophyte scores ranged from 0 (no osteophyte) to 3 (osteophyte >160 µm). Sections from the posterior half of the knee joints were dewaxed and re-calcified with calcium chloride and magnesium chloride, before tartrate-resistant acid phosphatase (TRAP5b) staining was conducted using a commercially available kit (F386A, Sigma-Aldrich, Dorset, UK). TRAP positive osteoclasts were quantified as previously described (7), under 40 times magnification from one end of the growth plate to the other end using the following criteria; 1) displayed purplish to dark red cytosol, 2) number of nuclei >3/osteoclast, 3) located within the subchondral bone area, comprising the area between the cartilage/bone junction and the growth plate. All behaviour and histology experiments were conducted in a blinded fashion.

All data are presented as Mean ± SEM.

RESULTS

L-006235 prevented the development of MIA-induced weight bearing asymmetry and lowering of ipsilateral hindpaw withdrawal thresholds. Effects of L-006235 were not immediate and only significant from day 14 post model induction, indicating that L-006235 does not alter early inflammatory pain associated with the MIA model but rather prevents the development of OA-like pain behaviour. At the end of the study, intra-articular injection of MIA was associated with a significant increase in cartilage damage score, which was significantly reduced by L-006235. Furthermore, MIA-induced increase in osteophyte score was significantly reduced by L-006235, and there was a non-significant decrease in synovitis. L-006235 did not alter the number of TRAP5b positive multinucleated osteoclasts in MIA rats.

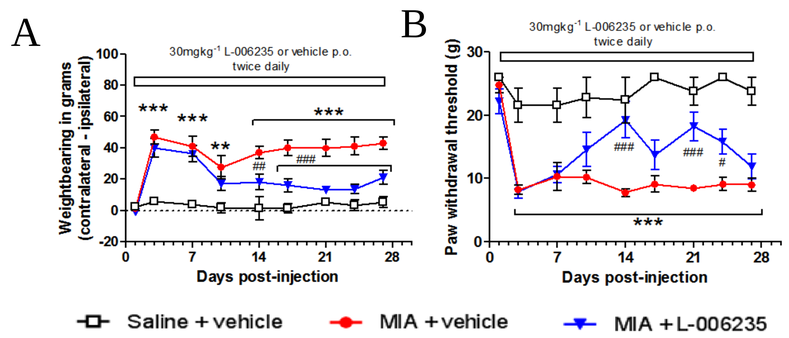

Figure 1: Oral administration of the CatK inhibitor L-006235 (30mg/kg) significantly attenuated the development of MIA-induced weight bearing asymmetry (**A**) and MIA-induced lowering of ipsilateral paw withdrawal thresholds (**B**). Statistical analysis ANOVA or Kruskal Wallis with Bonferroni or Dunn's post-hoc test respectively, **p<0.01, ***p<0.001 vs saline control or #p<0.05, ##p<0.01, ###p<0.001 vs MIA + vehicle group (n= 6-10 rats per group).

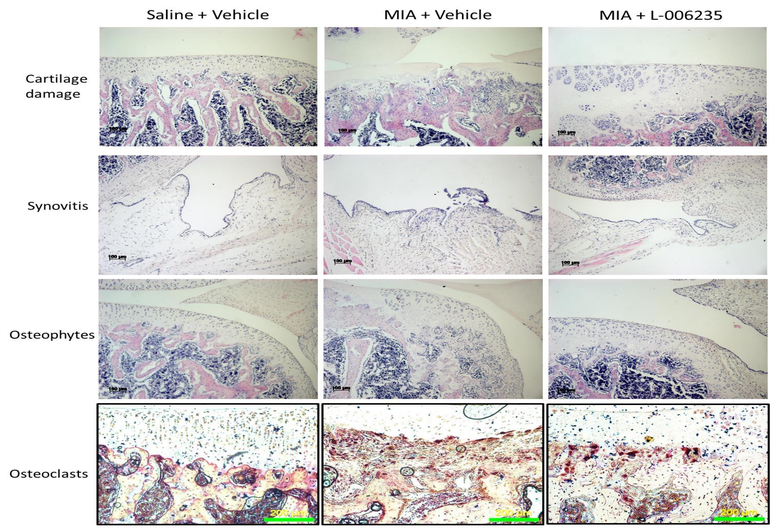

Figure 2: Effect of the CatK inhibitor L-006235 (30mg/kg, twice daily for 28 days) on cartilage damage, synovitis, osteophytosis (scale bar = 100 µm) and osteoclastogenesis (scale bar = 200 µm) at day 27 following intra-articular injection of MIA into the left knee joint.

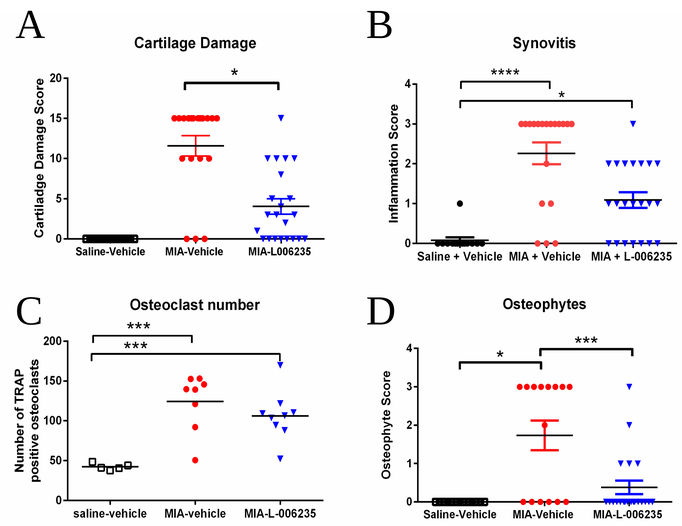

Figure 3: Intra-articular injection of MIA resulted in significant joint pathology at 27 days post model induction, compared to saline injected rats. Oral L-006235 significantly reversed MIA-induced cartilage damage (**A**) and osteophytosis (**D**), but did not alter MIA-induced synovitis (**B**) or osteoclastogenesis (**C**). Data are mean ±S.E.M, analysed with One-way ANOVA with Bonferroni's post-hoc test for parametric data or Kruskal-Wallis with Dunn's post-hoc test for non-parametric data (*p<0.05, ***p<0.001, ****p<0.0001).

CONCLUSIONS

The CatK inhibitor L-006235 reduced MIA-induced development of pain behaviour and associated cartilage damage and osteophytosis. Future studies will evaluate whether L-006235 can reverse established OA-like pain and joint pathology, thereby evaluating the potential of this drug/class of drugs to not only treat OA pain but also to slow the development of OA joint damage and thereby extend the time to total joint replacement.

REFERENCES

1. Logar DB et al., (2007). Expression of bone resorption genes in osteoarthritis and in osteoporosis. J Bone Miner Metab 25(4): 219-225.
2. Morko MJ et al., (2005) Spontaneous development of synovitis and cartilage degeneration in transgenic mice overexpressing cathepsin K. Arthritis Rheum. 52(12):3713-7.
3. Connor JR. et al., (2009). Protective effects of a cathepsin K inhibitor, SB-553484, in the canine partial medial meniscectomy model of osteoarthritis. Osteoarthritis and Cartilage 17(9): 1236-1243.
4. Hayami T et al., (2012). Inhibition of cathepsin K reduces cartilage degeneration in the anterior cruciate ligament transection rabbit and murine models of osteoarthritis. Bone 50(6): 1250-1259.
5. McDougall JJ et al., (2010). Cathepsin K inhibition reduces CTXII levels and joint pain in the guinea pig model of spontaneous osteoarthritis. Osteoarthritis and Cartilage 18(10): 1355-1357.
6. Palmer et al., (2015). Design and synthesis of tri-ring P3 benzamide-containing aminonitriles as potent, selective, orally effective inhibitors of cathepsin K. J Med Chem 48(24):7520-34.
7. Sagar DR et al., (2014). Osteoprotegerin reduces the development of pain behaviour and joint pathology in a model of osteoarthritis. AdAnn Rheum Dis 73(8):1558-65.