Effect Of Repeated Oral Administration Of A Cathepsin k Inhibitor On Bone Turnover And Bone Quality In Healthy Adult Exercising Horses

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Introduction: Cathepsin K (CTSK) is a lysosomal protease highly expressed in osteoclasts and is involved in degradation of bone matrices, mainly type I collagen. Cathepsin K remained a potential therapeutic target for bone diseases in human and several animal disease models in which osteoclast activity is increased, such as osteoporosis and autoimmune arthritis. Several studies have also investigated the effect of both CTSK as well as its inhibition on bone resorption in horses. Our laboratory has access to VEL-0230, alternatively named NC-2300, a highly specific irreversible inhibitor of bone CTSK. The drug suppressed osteoclast-mediated bone resorption in vitro and in vivo in rat models. The drug binds to intracellular and extracellular CTSK and interferes with CTSK-mediated bone resorption by osteoclasts. Multiple reports exist on using other anti-resorptive drugs in equine musculoskeletal disorders which are associated with increased bone resorption activities. Examples included the use of tiludronate for navicular disease and lesions of the thoracolumbar vertebral column, and zoledronate for bone fragility disorders. In addition, the pharmacological effect of some anti-resorptive agents has been studied in healthy adult horses, such as gallium nitrate, tiludronate, and zoledronate. Unlike the bisphosphonate anti-resorptive drugs, VEL-0230 induced a rapid, short-acting inhibitory effect on bone resorption as proven by efficacy studies in humans, non-human primates, dogs and rats. These studies demonstrated that CTSK sits at the nexus of both musculoskeletal system (bone) and immune system. It contributes to both bone resorption and the production of pro-inflammatory cytokines mediated by Toll-like receptor 9 signaling of dendritic cells. Our laboratory has identified an optimal oral dose and dosing interval of the CTSK inhibitor (VEL-0230) in healthy adult horses correlated to a significant decrease in a plasma bone resorption biomarker. Moreover, our in vitro data demonstrated that either one or two selected concentrations of VEL-0230 inhibited immune response and pro-inflammatory cytokines secretion of horses’ bone marrow cells to lipopolysaccharides and unmethylated CpG motifs. These findings suggest that VEL-0230, with its dual anti-resorptive and anti-inflammatory properties, could target osteo-inflammatory disorders in a specific manner. This study aimed to investigate the effect of repeated oral administration of a CTSK inhibitor (VEL-0230) on bone turnover and bone quality in healthy adult exercising horses.

Methods: Twelve mature horses (range 2-5 years), sufficiently sound to be exercised without medication on a treadmill, were aligned in a randomized, controlled, double blinded experimental trial. As shown in Fig.1, Horses were acclimated to a treadmill and exercised 3 times a week to sustain a constant level of conditioning and to mimic a training schedule (warm up at walk (4.3 mph) for 5 minutes, a trot (10 mph) for 5 minutes, a gallop (25 mph) for 5 minutes, then cool down walk for 5 minutes). After 11 days (d) of quarantine, acclimation was done for 1 week (d -14 - Day -7). Horses were then maintained in exercise for the duration of the study. Horses were randomly
assigned to either a CTSK inhibitor (CTSKI) (n=6) or a control (n=6) group in a double blinded manner. At day 0, VEL-0230 and a vehicle were administered orally at a dose of 4mg/kg b.w. once a week for 4 successive weeks in the CTSKI and control group, respectively. Prior to each dose administration, all horses had a muzzle placed for 6 hours and had their mouths rinsed with water by dose syringe immediately before dosing. Blood samples were obtained via jugular vein catheterization at 0, 6, 24 and 96 hours post each dose administration for complete blood picture, serum biochemistry and plasma separation for quantitative analysis of bone biomarkers [plasma carboxy-terminal cross-linking telopeptide of type I collagen (CTX-1) and osteocalcin]. Baseline (d -11 to -3) and post study (d 31-39) radiographs for lateral surface of the third metacarpal bone (MCIII) of both front limb and nuclear scintigraphy of the distal fore limbs were also performed. The thickness of dorsal cortex of the MCIII at mid-diaphysis as well as the thickness of the thickest cortex was measured for each limb on radiographs. In addition, three region of interest (ROI) were analyzed using nuclear scintigraphy and radioactivity (counts/ROI) was measured for each limb. Weekly physical exams with body weight and conditioning scoring (score 1-9) were performed immediately before each dose administration. Bone marrow samples were aspirated from the sternum on d 22 and bone marrow nucleated cells were isolated for quantitative comparison of osteoclastogenesis using specific gene expression analysis for osteoclast progenitors as well as mature osteoclasts. Two calcein doses for bone labeling were administrated intravenously in five days interval on d 23 and 28 for six horses and on d 24 and 29 for the other six horses. Bone biopsies were obtained from Tuber coxae (TC) and first phalanx (P1) three days after the second calcein dose administration (d 31 or 32) and were processed for bone histomorphometric analysis, micro CT scanning and Immunohistochemistry of CTSK.

**Results:** Selected Results: There was no statistical significant difference between control and CTSKI groups in the mean dorsal cortical thickness of the MCIII. However, time (pre to post-study) significantly reduced the dorsal cortical thickness in both groups as seen in Fig. (2). VEL-0230 induced a statistically significant decrease in bone resorption as revealed by plasma CTX-1 concentrations before and after each dose administration (Fig. 5 shows the results of the 4th dose). Data for osteocalcin did not reveal significant changes or inhibition in bone formation within the CTSKI group compared to the control one.

**Discussion:** Based on these selected results, repeated oral administration of a CTSK inhibitor did not interfere with adaptive cortical bone remodeling induced by exercise through the experimental time as revealed by dorsal cortical thickness measurements. In addition, VEL-0230 induced a statistically and biologically significant decrease in bone resorption (≥ 70 %) as revealed by plasma CTX-1 concentration before and after each dose administration with no inhibitory effect on bone formation. We concluded that weekly oral administration of VEL-0230 can inhibit bone resorption in young exercising horses. Coupled with its anti-inflammatory effect, VEL-0230 has the potential to be effective for equine osteo-inflammatory diseases.

**Significance:** This study investigated the efficacy and safety of repeated oral administration of a cathepsin K inhibitor on bone turnover and bone quality in young exercising equine athletes.
Fig. 1 Experimental design of a randomized, controlled, double blinded clinical trial to investigate the effect of VEL-0230 repeated oral administration on healthy exercising horses.
Fig. 2 Mean ± SD Dorsal cortical thickness of MCIII measured on radiographs obtained pre and post-study in control and Cathepsin K inhibitor (CTSKI) groups. Time pre to post study was significant factor regardless of group, P < 0.05.
Fig. 3 Mean ± SEM plasma CTX-1 concentrations pre & 6 hours post 4th dose administration of a vehicle & VEL-0230 in control and treatment group respectively. P value is reported for each statistical comparison and significance * was determined when P < 0.05.