Cathepsin K inhibition renders equine bone marrow nucleated cells hypo-responsive to LPS and unmethylated CpG stimulation in vitro

Hayam Hussein\textsuperscript{a,}* , Prosper Boyaka\textsuperscript{b,}* , Jennifer Dulin\textsuperscript{a,}* , Alicia Bertone\textsuperscript{a,b,}\textsuperscript{*}

\textsuperscript{a} Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, United States
\textsuperscript{b} Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, United States

A R T I C L E   I N F O

Article history:
Received 4 August 2015
Received in revised form 15 February 2016
Accepted 22 February 2016

Keywords:
Immune
Cytokine
Inflammation
Toll like receptor
VEL-0230

A B S T R A C T

Cathepsin K (CatK) is an important enzyme regulating bone degradation and has been shown to contribute to the immune response [1]. It is the most abundant cysteine protease expressed in osteoclasts and is believed to be instrumental in collagen and other extra-cellular matrix degradation that is necessary for bone and cartilage turnover [2]. Low expression of CatK has also been detected in other normal tissue types [3–5]. While elevated CatK expression has been reported in psoriatic skin lesions, and in synovial fluid and lining tissue of joints with Rheumatoid arthritis [6]. The involvement of CatK in Toll like receptors (TLRs) signaling has recently been established as another function for CatK in addition to it’s known role in extracellular matrix degradation [3].

The TLRs are a class of pattern recognition molecules with key functions in the innate and the acquired immune systems. The cellular localization of these receptors can vary with some localized at the cell surface and others localized within the endolysosomal compartments [7]. The TLRs interact with a variety of extracellular and intracellular ligands and influence the activity of a wide range of tissues and cell processes [8]. Moreover, they have been found to be major regulators of multiple immune-mediated diseases [9]. The proteases responsible for the cleavage of the TLRs have not been definitively identified, in part owing to disagreement among several reports. Most notably, inhibitors of CatK have revealed a crucial role for CatK in the function of TLR4 and/or TLR9 [3,10]. However, other reports denied this role in either TLR4 or TLR9 signaling pathway [9].

Multiple synthetic inhibitors of CatK, CatKIs, have been developed over recent years with few of them proceeding to clinical trials in animal models and humans [11–13]. Our study aimed to investigate the effect of CatK inhibition on TLR4 and TLR9 signaling pathways in equine whole bone marrow nucleated cells (BMNCs). This cellular fraction was previously characterized [14] and was chosen as our cellular model to include both the lymphoid and non-lymphoid cells (myeloid progenitors, mesenchymal stem and other progenitor cells). The reason behind this choice of including all bone marrow cellular subsets is to determine their inflammatory response to TLR-induced inflammation in the presence of potential signaling crosstalk between these cellular subsets, which will facilitate the pilot investigations of CatK potential contribution to the TLR4 and TLR9 signaling pathways. Moreover, some equine immune (myeloid and lymphoid) and non-immune cells, such as chondrocytes and synovial fibroblast-like cells showed significant inflammatory response when stimulated with Lipopolysaccharides (LPS) in vitro [15–17].
Infectious and inflammatory diseases are major equine health problems, particularly due to the inherent sensitivity of horses to microbial molecules, specifically LPS. Unlike rodents, horses are extremely sensitive to LPS showing the most comparable sensitivity to that of people [18]. Among currently available CatK inhibitors, VEL-0230 (alternatively named NC-2300) is a highly selective and potent inhibitor of CatK that has shown efficacy in inhibiting bone resorption biomarkers in exercising horses and has been proposed as a therapeutic for equine osteo-inflammatory conditions [11].

While the expression of the cluster of differentiation (CD) 90 had previously shown to be restricted to some non-immune cells, including endothelial cells [19,20], it has been identified as an activation-associated cell adhesion molecule that can interact with a corresponding ligand on monocytes and polymorphonuclear cells leading to adhesion of these leukocytes to activated CD90-expressing endothelial cells [21]. Meanwhile, the CD11b molecule is expressed on the surface of many myeloid cells and mediates inflammation by regulating leukocyte adhesion and migration [22]. The CD2 is another adhesion molecule mainly found on the surface of T cells and natural killer (NK) cells, while CD4 is mainly found on the surface of T helper cells, monocytes, macrophages, and dendritic cells. Whereas CD5 is a molecule that primarily found on a subset of IgM-secreting B cells and on T cells [23]. Among different major histocompatibility complex (MHC) classes identified, class II is of major importance due to their critical roles in antigen presentation processes within the adaptive immune response [24].

We hypothesized that CatK inhibition in horses will affect BMNCs other than mature osteoclasts, altering their pro-inflammatory cytokines secretion and the expression of surface markers’ and the MHC II molecule, rendering these cells hypo-responsive to the TLR-induced inflammation. Thus, careful considerations to the side effects of cathepsin K inhibitors on the immune system during its therapeutic application in bone resorption disorders should be taken, whereas they may have dual benefits in the treatment of osteo-inflammatory disorders. We have studied two different inflammatory models in horses’ BMNCs: The LPS (TLR4 ligand) and the unmethylated CpG (TLR9) stimulation with the objectives of: 1. determine whether CatK inhibition will alter the cytokine secretion by stimulated BMNCs; specifically interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α), and 2. determine the changes in the BMNCs surface markers’ expression (CD11b, CD2, CD4, CD5 (ly-1), and CD90 (Thy-1)) and MHC II molecule under CatK inhibition.

2. Materials and methods

All experimental procedures were approved by the “Institutional Animal Care And Use Committee” at The Ohio State University.

2.1. Bone marrow aspiration and nucleated cell isolation

Six horses were sedated with 0.01 mg/kg body weight (b.w.) detomidine hydrochloride (Domitor, Pfizer Animal Health, Exton, PA) and 0.02 mg/kg b.w. butorphanol (Torbugesic, Fort Dodge Animal Health, Fort Dodge, Iowa) intravenously. An area of 5 cm × 20 cm over the sternum was clipped, scrubbed, aseptically prepared and the intersternebral spaces were identified by palpation or ultrasonography. Local infiltration of 2 ml anesthetic solution, mepivacaine hydrochloride 2% (Carbocaine, Cooke-Waite Laboratories, Inc., NY), was performed subcutaneously over the midpoint in the sagittal plane of two adjacent sternebrae. A stab incision using a No. 11 scalpel blade was then made through the skin and a Jamshidi biopsy needle (11 gauge, 10 cm, Ranfac Corp, Avon, Mass) pre-rinsed with heparin (Multiparin 5000 iu/ml) was introduced for approximately 4–6 cm until it contacted the sternbra. The needle was then further pushed 3 or 4 cm into the sternbra and 60 cm³ of bone marrow was aspirated into two 30 ml syringes, preloaded with 3 ml heparin each. The two 30 cm³ syringes were then loaded into one Marrowstim® concentration system (BIOMET Biologics, Warsaw, IN, USA). Bone marrow nucleated cells were immediately isolated according to the manufacturer’s instructions and the method described by Ishihara et al. [14], and then transferred into sterile 10 ml syringes. The syringes were then placed on ice for immediate transfer to the laboratory.

2.2. Cell culture and experimental treatments

Horse BMNCs were collected and pelleted (all centrifugation steps were performed at 300 × g at 6°C for 5 min). The cells were washed twice with 1X DPBS (Invitrogen, Carlsbad, CA) and cultured in Dulbecco’s Modified Eagle’s medium (DMEM; GIBCO, Grand Island, NY, USA) supplemented with 10% heat inactivated fetal bovine serum (GIBCO). For application of the different experimental conditions, cells were diluted to 10⁶ cells/ml and dispensed to 12-well plates. Cells were then seeded as per well alone or with either one of two CatKI (VEL-0230) concentrations; 1 μM or 10 μM. The cells were seeded non-stimulated or stimulated with either LPS (Sigma–Aldrich, MO, USA) at concentration of 1 μg/ml or unmethylated CpG motif (InvivoGen, CA, USA) at concentration of 5 μg/ml with each CatKI concentration in the media. The plates were incubated for 48 h in 5% CO₂ at 37°C. The CatKI concentrations were chosen based on the effective inhibitory concentrations on previous research and our pilot experimental investigations (see supplementary data), while LPS concentration and CpG sequence and concentration were chosen on the basis of pilot experiments using horses peripheral blood mononuclear cell (Comparative Orthopedic Research Laboratory, The Ohio State University, Unpublished data).

2.3. Cell count and measurements of cell viability

Cell total count and viability were performed using a hemocytometer and trypan blue staining (InvivoGen). Further assessment of apoptosis and viability was performed using Annexin V/PE Apoptosis detection kit (BD Biosciences, Bedford, MA) and subsequent flow cytometric analysis.

2.4. Cytokines assay

The concentrations of IL-1β, IL-6, and TNF-α in culture media were measured using equine-specific ELISA kits (Genorise Scientific, Inc., Glen Mills, PA, USA) according to the manufacturers’ protocols. All measurements were performed in duplicates, optical density was obtained using a standard colorimetric method and averaged concentrations were calculated for each duplicate.

2.5. Immunostaining and analysis of BMNCs lineages and MHC II molecule

Single-cell preparations (5 × 10⁵ cells) were stained for 30 min in the dark at 4°C with one of the following fluorochrome-labeled monoclonal antibodies (Abs): anti-human CD90, mouse anti-equine CD11b, mouse anti-equine CD2, mouse anti-equine CD4, and mouse anti-equine CD5 (BD Biosciences, eBiosciences, San Diego, CA, and hermo Fischer Scientific Inc., Rockford, IL). For MHCI immuno-staining, cells were stained with anti-equine MHCIi monoclonal Ab (Clone CVS20, AbD Serotec, Raleigh, NC) followed by anti-mouse secondary antibody (FITC rat anti-mouse IgG1, clone AB5-1, BD Biosciences, San Jose, CA, USA). After incubation, cell
washed twice in FACS buffer and re-suspended in 200 µl FACS buffer for flow cytometry analysis (Accuri Cytometers, San Jose, CA). A gating procedure was generated by generating a cytogram of SSC versus forward scatter light and gated to exclude cell debris by including only cells with relatively high SSC and forward scatter light values (Fig. 1). Quadrant cursors were set by using an appropriate negative control; either unstained control cell preparation or fluorochrome-labeled secondary antibody control preparation. Quadrants were set so that in negative controls, 99% of the cells were localized in the left lower quadrant. The percentage and absolute number of cells labeled by each antibody as well as the mean fluorescence intensity (MFI) were determined for each experimental condition per each animal.

2.6. Data and statistical analyses

Data for all outcomes were statistically analyzed as raw values and percent change from control values (normalized to non-stimulated control, LPS-stimulated cells, or CpG-stimulated cell with 0 µM CatKI). Normalized data was then graphed as mean with standard error of the mean (SEM). Statistical analysis was performed using SPSS v.18.0 (IBM Corp., Armonk, NY, USA). The normal distribution within the different variables was investigated using Shapiro–Wilks test. Comparison between different experimental conditions was performed using the one-way analysis of variance with repeated measures for normally distributed data followed by Duncan’s Multiple Range Test, and Friedman test for skewed data. Significance was determined when $P < 0.05$ and trend differences were discussed when $0.10 > P > 0.050$.

3. Results

3.1. Cathepsin K inhibition promoted BMNCs viability and reduced cell apoptosis

Treatment of non-stimulated, LPS-stimulated, and CpG-stimulated cells with the CatKI (VEL-0230) significantly increased cell viability and total cell count in all experimental conditions as measured by trypan blue staining. The data were compared for the non-stimulated, LPS-stimulated, and CpG-stimulated cells in Fig. 2A. This was further confirmed by Annexin-7AAD fluorescent staining which revealed a significant reduction in the percent of cells undergoing apoptosis with CatKI treatment in all experimental conditions (Fig. 2B). The decrease in the percent apoptotic cells was CatKI-concentration dependent.

3.2. Cathepsin K inhibition significantly decreased cytokine production of either naïve or stimulated BMNCs

Different cytokine protein analyses revealed significant reduction in IL-1 β, IL-6, and TNFα concentrations in culture supernatants of non-stimulated cells, or cells stimulated with LPS and CpG. This reduction in cytokine secretion was evident with either one or two CatKI molar concentrations. The cytokine secretions of the non-stimulated cells showed either a trend or significant decrease with the greater CatKI concentration (10 µM). Horse was a significant different variable in all analyzed parameters of all experimental conditions ($P < 0.001$). Table 1 is showing mean ± SEM cytokine concentrations (pg/ml of culture supernatant) of BMNCs in different experimental conditions. Table 1 shows the mean ± SEM cytokine concentrations (pg/ml of culture supernatant) of BMNCs in different experimental conditions.

The IL-1 β, IL-6, and TNFα normalized data are shown in Fig. 3A–C. The non-stimulated cells (Fig. 3A) showed a significant decrease in IL-1β secretion with both CatKI molar concentrations,
Table 1
Mean ± SEM cytokine concentrations (pg/ml of culture supernatant) of BMNCs in different experimental conditions.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Un-stimulated cells</th>
<th>LPS-stimulated cells</th>
<th>CpG-stimulated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CatKI (0 μM)</td>
<td>CatKI (1 μM)</td>
<td>CatKI (10 μM)</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>2014.8 ± 611.6</td>
<td>1731.3 ± 603.8</td>
<td>1682.3 ± 597.2**</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>38.8 ± 3.3</td>
<td>38.8 ± 3.3</td>
<td>30.8 ± 3.2**</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>5.3 ± 2.1</td>
<td>2.8 ± 2.8</td>
<td>1.0 ± 0.7*</td>
</tr>
</tbody>
</table>

* 0.10 > P > 0.05 compared to CatKI (0 μM) within the same experimental group.

1 P < 0.05 compared to CatKI (0 μM) within the same experimental group.

** P < 0.01 compared to CatKI (0 μM) within the same experimental group.

Fig. 3. Cathepsin K inhibition significantly decrease cytokine production of either naive or stimulated BMNCs. The mean ± SEM % change from control in IL-1 β, IL-6, and TNFα production of BMNCs treated with 0, 1, or 10 μM of a CatKI are shown for (A) Non-stimulated BMNCs. (B) LPS-stimulated BMNCs. (C) CpG-stimulated BMNCs. Statistical comparison results are denoted; (*) when group is significant compared to the CatKI (0 μM), P < 0.05. (**) When group is highly significant compared to the CatKI (0 μM), P < 0.01. (***) When group has a trend change compared to the CatKI (0 μM), 0.10 > P > 0.05.

A significant decrease in IL-6 and a trend reduction in TNFα secretion with the greater CatKI concentration only. The LPS-stimulated cells (Fig. 3B) showed a significant decrease in all cytokine secretion with both CatKI concentrations with the exception of TNFα secretion showing a trend reduction at the greater CatKI concentration only. The CpG-stimulated cells (Fig. 3C) showed a significant reduction in the secretion of all analyzed cytokines with both CatKI concentrations.

3.3. Cathepsin K inhibition altered different equine BMNCs surface markers and MHC II molecule expression

One or two concentrations of the CatKI (VEL-0230) treatments significantly altered cell surface markers and MHC II molecule expression as shown in Table 2. The horse was a significant different variable in all analyzed parameters of all experimental conditions (P < 0.001). The normalized data for the non-stimulated cells are shown in Fig. 4A for % positive cells and in Fig. 4B for MFI. There were significant reductions in the % of cells positive for CD90, and CD11b with one or both CatKI concentrations. A trend decrease in the % of CD4 positive cells was found at the greater CatKI concentration. Meanwhile, significant reductions in the MFI of CD2 with one or both CatKI concentrations were observed. A significant increase in the MFI of MHCII was detected at the greater CatKI concentration only.

The normalized data for the LPS-stimulated cells is shown in Fig. 5A for % positive cells and in Fig. 5B for MFI. There were significant reductions in the % of cells positive for CD90, CD11b, CD4, and MHCII with one or both CatKI concentrations. Meanwhile, significant reductions in the MFI of CD90, CD11b, CD2, CD4, and CD5 with one or both CatKI concentrations were observed. A significant increase in the MFI of MHCII was detected at the lower CatKI concentration.

The normalized data for the CpG-stimulated cells is shown in Fig. 6A for % positive cells and in Fig. 6B for MFI. There were significant reductions in the % of cells positive for CD2, CD4, CD5 and MHC II with one or both CatKI concentrations. A biphasic change in the % of CD11b positive cells were found with a significant increase observed at the lower CatKI concentration and a significant decrease observed at the greater CatKI concentration. Meanwhile, significant reductions in the MFI of CD11b, CD2, CD4, and MHC II with one or both CatKI concentrations were observed. A significant increase in the MFI of CD5 was detected at the greater CatKI concentration.
### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-stimulated cells</th>
<th>CatK(0 μM)</th>
<th>CatK(1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD90</td>
<td>% positive cells</td>
<td>51.3 ± 0.3</td>
<td>46.6 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>231 ± 3.8</td>
<td>231 ± 0.1</td>
<td>219 ± 0.0</td>
</tr>
<tr>
<td>CD11b</td>
<td>% positive cells</td>
<td>55.7 ± 0.5</td>
<td>44.7 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>223 ± 3.8</td>
<td>223 ± 0.1</td>
<td>223 ± 0.0</td>
</tr>
<tr>
<td>CD2</td>
<td>% positive cells</td>
<td>29.2 ± 2.4</td>
<td>24.5 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>273 ± 1.4</td>
<td>273 ± 0.1</td>
<td>273 ± 0.0</td>
</tr>
<tr>
<td>CD4</td>
<td>% positive cells</td>
<td>6.5 ± 2.4</td>
<td>5.2 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>450 ± 1.4</td>
<td>450 ± 0.1</td>
<td>450 ± 0.0</td>
</tr>
<tr>
<td>CD5</td>
<td>% positive cells</td>
<td>2.9 ± 2.4</td>
<td>2.2 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>290 ± 1.4</td>
<td>290 ± 0.1</td>
<td>290 ± 0.0</td>
</tr>
<tr>
<td>MHCII</td>
<td>% positive cells</td>
<td>14.0 ± 2.9</td>
<td>12.7 ± 0.0</td>
</tr>
<tr>
<td>MFI</td>
<td>175 ± 1.4</td>
<td>175 ± 0.1</td>
<td>175 ± 0.0</td>
</tr>
</tbody>
</table>

* P < 0.05 compared to non-stimulated cells within the same experimental group.

** P < 0.01 compared to non-stimulated cells within the same experimental group.

### 4. Discussion

The results of this study indicated that CatK inhibition had a potent immuno-modulatory effect on horse’s BMNCs in vitro. This effect was evident in non-stimulated cells exposed to the culture environmental conditions in vitro, and in the two inflammatory models; the LPS stimulation (TLR4 ligand) and the unmethylated CpG stimulation (TLR9 ligand).

In this study, CatK inhibition in horses’ BMNCs, via blockage of the active site of the enzyme CatK, markedly decreased pro-inflammatory cytokine secretion, expression of certain cell surface markers, and expression of MHC II molecule in response to TLR4 and TLR9 agonists. However, both non-stimulated and stimulated BMNCs exhibited greater viability and total cell count with lower apoptotic activity when CatK functions were impaired.

Both TLR4 and TLR9 signaling pathway share the myeloid differentiation factor (MyD88)-dependent pathway. Thus, a proteolytic function for Catk at one or more aspects of this pathway is plausible in horses as revealed by the CatK inhibition effect on BMNCs. Although TLR4 can additionally signal independently of MyD88 through TIR-domain-containing adapter-inducing interferon-β (TRIF), which results in 75% of the TLR4-induced inflammatory response in most animal species and human [25,26], this pathway (the MyD88- independent) is of a negligible function in horses and does not contribute to their relatively higher sensitivity to LPS stimulation [18]. Surprisingly, CatK inhibition modulated horses’ BMNCs inflammatory response to both TLR4 and TLR9, unlike mice.
dendritic cells, which found to be hypo-responsive to TLR9 but not TLR4 when a similar CatK functions were impaired [3].

The production of pro-inflammatory cytokines is an important consequence of TLRs-induced cell activation that mediates tolerance to microbial infection [27] and many of the pathophysiological effects including an increase in bone resorption activities [28]. The major cytokines produced by the MyD88 pathway are IL-1β, IL-6, TNF-α, and IL-10. In this study, the availability of equine-specific cytokine analyses provided an accurate quantification for three of these major cytokines in a highly sensitive manner. We have detected a statistically significant decrease in the production of IL-1β, IL-6, and TNFα in the presence of a CatK, which was CatK concentration-dependent in both LPS and CpG inflammatory models. Theses results do confirm the proposed role of CatK in the MyD88 signaling pathway and/or a possible role for CatK in the proteolytic activities required for extracellular secretion of these cytokines, including IL-1β by which secretion is an inflammasome-dependent. Although TLR stimulation and downstream signaling alone are not sufficient to trigger the secretion of IL-1β, they are considered critical mediators of IL-1β precursor synthesis, which, in turn, is maturated and secreted as a result of a second signal that is inflammasome-dependent. Moreover, stimulation with TLR ligands was found to directly activate the nucleotide-binding oligomerization domain-like receptors (NIRs) in human monocytes inducing IL-1β secretion [29]. A similar inhibition in pro-inflammatory cytokines secretions (IL-12 and IL-23) was observed when rat dendritic cells cultured in vitro with multiple concentration of VEL-0230. This inhibition was observed with CpG but not LPS stimulation [3]. Interestingly, our result came in agreement with a recent study that reported a significant decrease in the pro-inflammatory mRNA (IL-23, IL-6, and TNFα) and corresponding cytokines expression in response to both TLR4 and TLR9 stimulation in a mouse model of periapical disease using another CatKI, odanacatib [10].

The recognition of ligands by TLRs results in activation of monocyte-derived dendritic cells, and other antigen-presenting cells, to secrete pro-inflammatory cytokines that promote dendritic cell maturation, antigen uptake and presentation. Reducing the biological activities of these pro-inflammatory cytokines can alleviate the brunt of attack of diseases and subsequent lesions. The biological activities of TNFα include the induction of other inflammatory factors, activation of the inflammatory cells, increasing expression of the adhesion molecules and nitric oxide. Similar biological activities are also induced by IL-1β. Whereas IL-6 is mainly responsible for inducing B cell maturation, synthesis of the acute phase proteins, and fever [30].

The results of this study revealed a significant decrease in the % of cells positive for CD90, CD11b, CD2, CD4, CD5, and MHC II molecules. All of which have important functions in the innate and/or adaptive immune response. In a partial disagreement to Asagiri et al. [3], and in agreement with other previous research [6,31], our data provided evidence for an anti-inflammatory effect...
of CatK inhibition in horses’ BMNCs. While CatK depletion caused a dramatic reduction in the pro-inflammatory cytokines secretion of mice dendritic cells in response to TLR9 stimulation, it did not affect the formation, morphology and antigen presenting ability of these cells [3]. Our data provided an evidence for decreased antigen presenting ability of horses BMNCs exhibited by the decrease in the percentage of MHC II positive cells under the effect of CatK inhibition. Similarly to our results, CatK inhibition revealed an anti-inflammatory role in mouse models for experimental autoimmune encephalomyelitis and psoriasis as CatK-knockout mice showed significant lower encephalomyelitis compared to wild type mice, and VEL-0230 tropical treatment significantly ameliorated psoriatic skin lesions in a mouse model for Psoriasis [3,6]. Moreover, CatK, uniquely among other cysteine proteases, was found to have a kininase activity impairing symptoms of the bradykinin–dependant lung inflammatory diseases [31]. Contrary to the anti-inflammatory role previously reported for CatK inhibition, CatK depletion significantly aggravated the chronic colitis in a mouse model suggesting an antimicrobial role for extracellular CatK within the gut mucosa [5]. However, such antimicrobial activity for CatK was excluded within the immune response to influenza virus vaccine in vitro [32].

Cathepsin K inhibition represents a novel strategy for developing agents to treat osteoporosis, osteoarthritis, and other disorders characterized by increased bone resorption in humans. It has also been proposed for treatment of certain osteo-inflammatory conditions in horses [11]. The anticipated outcome of a CatK inhibitor would be to slow down the resorption of bone during certain biologic stress risers seen in inflammatory bone diseases, such as exercise-induced bone trauma. Such outcome is very likely to arise following the decrease in pro-inflammatory cytokines secretions by inflammatory cells which could possibly lead to attenuation in the induction of T helper 17 cells, which are an osteoclastogenic T cell subset known to play an important role in the inflammatory bone loss [4]. Additionally, it is possible to utilize CatK inhibition to minimize the deleterious effects of TLR activation induced by microbial ligands in horses.

In conclusion, CatK inhibition in horses did affect BMNCs other than mature osteoclasts rendering them hypo-responsive to both TLR4- and TLR9-induced inflammation, and predicting a proteolytic activity for CatK within the MyD88 pathway and/or the following proteolytic events required for extracellular secretion of the produced cytokines (Fig. 7).

Conflict of interest

None to declare.

Acknowledgments

This work was partially supported by FreeStride Therapeutics Inc. The authors thank Eunsoo Kim and Dr. Lauren Smanik for providing generous technical assistance with the study, and Tim Vojt for his medical illustration services.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cimid.2016.02.005.

References


