Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.12682

Editorials

Science-in-brief: Bisphosphonate use in the racehorse: Safe or unsafe?

Bone remodelling is of great importance to several orthopaedic diseases of the Thoroughbred racehorse, and bisphosphonates, with their reported effects on bone turnover, may have the potential to alter the remodelling response. The USA recently approved 2 non-nitrogenous members of the bisphosphonate drug family, clodronate and tiludronate, for treatment of navicular disease in the horse. Tiludronate, having already been licensed for similar use in several European countries, was previously available to veterinarians in the USA through Food and Drug Administration approved importation on a case-by-case basis. Currently, commercial advertisements for these recently licensed non-nitrogenous bisphosphonates appear in veterinary specific and, in the USA, general horsemanship publications. Nitrogenous bisphosphonates are not yet licensed in the horse but there are anecdotal reports of their off-label use in the racehorse.

With the increasing awareness of bisphosphonates amongst racehorse trainers, owners and veterinarians, it is prudent to examine the existing evidence to determine if this class of drugs has a place in the off-label treatment of orthopaedic disorders of young and/or racing Thoroughbreds.

In bone, the osteoclast-related antiresorptive effect of bisphosphonates is well documented [1]. The simple assumption that "by inhibiting osteoclasts we stop bone resorption, which means bones are stronger" is a gross oversimplification of bone physiology and drug pharmacology. Bisphosphonates have additional anti-inflammatory and pain relieving effects, in addition to effects in nonosseous tissue such as cartilage. Practitioners must have answers to the following questions prior to embarking in off-label bisphosphonate treatment of the young or racing Thoroughbred: What is the current evidence for use in horses? What conditions of racehorses have anecdotally been suggested for off-label treatment with these drugs and does the evidence support such use? Are there known or theoretical longer term risks to treatment? Finally, can bisphosphonates be safely used in immature horses?

What is the current evidence for use in horses?

The most well reported equine studies involved administration of tiludronate for the treatment of chronic back soreness [2], lower hock osteoarthritis [3] and navicular disease [4]. These studies all yielded favourable results from blinded analysis but, clinical signs were the main determinant of success. All horses were older and none were racing Thoroughbreds so extrapolation of these results to that population may be inappropriate. At least some of the positive changes seen may have been due to pain-relieving or anti-inflammatory effects of tiludronate, rather than a direct effect on bone density, which was not assessed [5]. In another experimental study, unilateral cast immobilisation was used to induce bone resorption and assess the protective effect of tiludronate [6]. From this equine research, it is clear that serum biomarkers (CTX-1) of bone resorption are significantly reduced following treatment with tiludronate. Several other studies have been undertaken, and other members of the family, such as the nitrogenous bisphosphonates pamidronate [7] and zoledronate [8] have been investigated for short term safety and usefulness in the horse. In summary, equine specific investigations of bisphosphonates are sparse compared with human studies.

Many questions related to bisphosphonate use in the horse have, therefore, not yet been answered by scientific evaluation in this species. In other species, many members of the bisphosphonate class have been studied and the pharmacokinetics and physiological effect of each drug may be different. Additionally, interspecies differences may exist and there is no population directly comparable to the racehorse. For these reasons, until racehorse-specific research is undertaken, extrapolation from experimental and clinical research in other species must be undertaken with caution.

What conditions of racehorses have been suggested for off-label treatment with these drugs and does the evidence support such use?

Based on reported success in other sport horse disciplines and extrapolation from human research, some veterinarians have suggested that bisphosphonates may be useful in the young and racing Thoroughbred for the treatment or prevention of disease. Specifically: reduction in stress fracture risk; treatment of palmar/plantar osteochondral disease (POD); sesamoiditis/suspensory branch insertional enthesis; osteoarthritis (OA); and subchondral bone cysts. Rather than list the specific modes of action of bisphosphonates, it is probably of more benefit to report the evidence of how bisphosphonates may influence each disease in an attempt to explain why bisphosphonates may, or may not, be an appropriate treatment option for each disease.

Several diseases in the racehorse arise from an inappropriate bone remodelling response and can be considered under the heading 'stress remodelling'. Examples include: stress fractures of the tibia, humerus, scapula or pelvis; sclerosis of the third carpal bone; and palmar/plantar osteochondral disease. Inappropriate remodelling can affect almost any bone as a result of the failure of functional adaptation to high strains or strain rates placed upon it [9]. There are 2 parts to the theoretical use of bisphosphonates in this type of injury: prevention and repair. The theoretical reasoning for 'prophylactic stress remodelling/fracture treatment' has arisen from a lack of understanding of bone physiology amongst some practitioners. Because stress fractures are a propagation of stress remodelling and the first stage of remodelling is bone resorption, some practitioners suggest that "if bone resorption is halted, the bone will not become weakened and therefore stress fractures will not occur." It is true that, in man, higher serum levels of biomarkers of bone resorption have been identified prior to subsequent stress fracture development [9], thus it is conceivable that halting this pathway may be desirable. This theory, however, is not borne out by what is known about bone physiology in other species. Beagles given high doses of risedronate and alendronate demonstrated microdamage accumulation and the bone was weaker with 19% less energy required to break a rib [10]. In a rat ulna stress-fracture model, the nitrogenous risedronate failed to prevent stress fractures and actually delayed healing as evidenced histologically at 10 weeks' post fracture [11]. In human army recruits, prophylactic risedronate administration did not decrease the risk of subsequent stress fractures [12]. Although not yet evaluated in the horse, these studies suggest that prophylactic treatment of bones under high strain rates, such as in the training Thoroughbred, may actually weaken bone and predispose to stress fracture: the opposite of the intended effect.

Microcracks occur in bone due to normal (or training induced) stresses and strains [1]. In contrast to complete fractures, the only way that bone can heal these microcracks is to initiate resorption as the first step in the healing process across the crack [11]. Osteoblasts then form new, osteonal bone across the fracture line and the bone matures as healed, fully functioning bone. Interestingly, as the osteoblast 'follows' the osteoclast

across the fracture, inhibition of the osteoclast actually resulted in decreased bone formation up to 44% in one stress-fracture-repair study using alendronate [13]. In other words, if the osteoclast cannot initiate the healing of these microcracks, they will ultimately propagate. Bisphosphonates may additionally inhibit the healing of microcracks by a 'protective' effect in specific cells that do not internalise the drug such as the osteocyte [13]. This is important because the role of the osteocyte in bone homeostasis is to sense changes in its environment and, in areas of impending stress fracture, osteocytes undergo apoptosis which initiates the cascade of osteoclast development and recruitment. The osteocyte can, therefore, be considered the orchestrator of stress fracture repair. It is believed that bisphosphonates, by having an antiapoptotic effect on the osteocyte, inhibit osteoclast recruitment and decrease the ability to repair microcracks. With osteoclast inhibition, the bone collagen becomes older, more mineralised, and contains less water [9,14]. As a result, the bone mineral density and stiffness increase but the toughness (the bone's ability to yield without failure) decreases and this may predispose to propagation of microcracks. Bisphosphonate treated bone, for want of a better description, has the material properties of a concrete block: very strong but very easy to fracture [14]. By interfering with the homeostasis of bone mineral density, there is the risk that we may actually create long bones that are predisposed to fracture. The dose, frequency, and individual drug at which these theoretical risks may be encountered has not been evaluated in the horse. It has been postulated that the distribution of bisphosphonate is not uniform but is higher in actively remodelling bone and higher in cancellous than cortical bone, which may mitigate some of the risk regarding long bone stress fractures [15]. However, all bones in the young or racing Thoroughbred are undergoing active remodelling as a consequence of their training regimes, thus uptake may be more uniform than is seen in man. Until racehorse-specific research is undertaken, the uptake pattern of bisphosphonates in this demographic will remain unknown.

In man, it has been suggested by some that bisphosphonates aid the healing of stress fractures [16], although the use of bisphosphonates to treat human stress fractures in athletes is controversial, with the general recommendation being that until well-designed safety studies are undertaken it is prudent to limit the use of bisphosphonates in the treatment of stress fractures [9]. Bisphosphonates decrease bone remodelling and decrease the rate of stress fracture repair as assessed histologically [13]. Callus formation or periosteal reaction is largely unaffected by bisphosphonate use [11]. One case report identified that 4 (of 5) human athletes treated post-injury with pamidronate resumed training in a pain-free manner within 72 h of scintigraphic confirmation of tibial stress fractures and that all patients resumed training within a 3-week period [16]. However, the strain forces on the tibia of a training racehorse are not comparable to those of a human runner and attempting to train a racehorse with a long bone stress fracture so soon after diagnosis is a recipe for disaster.

The resolution of pain demonstrated so quickly after bisphosphonate administration in human athletes has potential implications for the use of bisphosphonates in all competition horses, not just the Thoroughbred racehorse. In man and horses, it has been postulated that active bone resorption may be painful and that inhibition of this process may alleviate pain. By reducing the development of an acidic environment at the ruffled border of the osteoclast, acid sensitive ion channels within free nerve ending are not activated, thus pain is reduced [14]. That is probably part of the mechanism of analgesia but there are multiple experimental reports of the pain-relieving effects of bisphosphonates independent of osteoclast inhibition, and these analgesic effects may occur at lower than typical therapeutic doses. Tiludronate reduces the release of the inflammatory mediator nitric oxide and other inflammatory cytokines from macrophages [17]. Bisphosphonates may also have direct inhibitory effects in developing glial nerve cells [18] and, in dogs, spinal levels of substance P were reduced in an experimental OA model [19]. Although the nitrogenous bisphosphonates may have more potent antiresorptive effects, the non-nitrogenous tiludronate and clodronate may have more potent anti-inflammatory, analgesic effects [20]. Clodronate has been shown to decrease levels of several matrix metalloproteinase (MMP) enzymes in vitro and even demonstrated an analgesic effect when injected directly into the cerebral ventricles of mice perhaps through direct interaction with neurons [20]. Relating these potential analgesic effects to the small human case study [16] and the existing equine clinical studies [2–4], suggests that extreme caution should be exercised when deciding upon treatment of racing Thoroughbreds with stress fractures. If clinical signs alone are being used to assess improvement, a situation could arise where a horse 'appears' free from lameness and, therefore 'healed'. It is possible, however, that the horse may be benefiting from the analgesic effects of bisphosphonate whilst actually having a delayed bone healing response due to bisphosphonate. Furthermore, this concern is not unique to stress fractures but equally applies to other musculoskeletal diseases.

Bisphosphonate–analgesia represents a significant ethical dilemma for the industry, magnified by the fact that these drugs have extremely short serum and urine half-lives and are difficult to extract from bone for analysis. Horses may be trained, raced or sold whilst bisphosphonates are still active within the animal and the only way to identify them is by accessing the medical record of the attending veterinarian. Bisphosphonates remain active within the bone for extremely long periods relative to their plasma half-lives. The drug stays 'buried' within the bone matrix until it is recruited in an area of active bone turnover. Tiludronate has been identified in the bones of treated horses as late as 6 months' post-treatment [5] and, in man, several of the newer generation nitrogenous bisphosphonates have bone durations of up to 10 years [9]. This concern should be considered prior to initiating off-label treatment in the racehorse.

Like stress fractures, POD is also the result of a failure of bone to adapt to training loads. The palmar/plantar subchondral bone attempts to adapt by becoming denser and eventually more brittle leading to potentially irreversible collapse of the subchondral bone and clinical signs of joint effusion, pain and lameness. There is limited evidence to support the use of bisphosphates in POD and a recent Havemeyer meeting report recommended that bisphosphonates had no place in the treatment of this disease based on current evidence [21]. As POD is a result of increased bone density it seems counterintuitive to use drugs that are known to increase bone mineral density further. In fact, in man, overuse of pamidronate resulted in 'marble bone disease' (osteopetrosis) in a child [22]: an extreme case of these drugs resulting in dense, brittle bones. Additionally, tiludronate has anti-vascular endothelial growth factor properties that may impede vascularisation [23] and, at least in theory, may compound the disease because it has recently been cited that impaired blood supply may be a factor in its development [24]. For these reasons, bisphosphonates, which increase bone density, slow the rate of bone turnover, and have the potential to impair blood flow are probably contraindicated in POD.

Insertional injuries of the suspensory ligament are well documented in the racehorse [25]. Pain arises due to inflammation between the sesamoid bone and the suspensory branch or at the proximal origin of the suspensory ligament. This inflammatory cycle leads to lysis of bone with intermittent pain, local swelling, and radiographic change such as enlarged vascular channels on the sesamoids [26] or lysis/sclerosis on the palmar surface of third metacarpal/metatarsal bones. In 2001, the European Agency of Medicinal Products cited insertional sesamoid/suspensory branch injury as a condition which could be treated with tiludronate [27]. By reducing osteoclastic resorption, it was proposed that pain would be relieved, insertional Sharpey fibres preserved, and the cyclical inflammatory nature of the disease arrested, although there is no published evidence to support this theory. The risk in the racehorse is that it is not known whether the abatement of clinical signs is due to analgesic effects of bisphosphonate or a true resolution of the disease process. Again, it is possible that treatment reduces clinical signs without resolving the condition: potentially predisposing to a future failure of the suspensory apparatus. These risks remain theoretical concerns but may be especially important as some practitioners use bisphosphonates to treat sesamoiditis in yearling Thoroughbreds based on the anecdotal assertion that the radiographic appearance of sesamoiditis appears to resolve. The ethical implications of the potential long term risk of such off-label use should be considered and, again, bisphosphonate use for sesamoiditis and suspensory enthesis in racehorses must be discouraged until more critical research has been undertaken.

Nonracehorse studies have demonstrated a positive effect of bisphosphonate on clinical signs related to OA of the lower hock joints

[3,7]. In man, intra-articular (i.a.) clodronate was as effective a pain reliever as i.a. hyaluronic acid for knee OA [28]. There are many mechanisms of therapeutic action in OA [29,30]: bisphosphonates may stabilise the subchondral bone or even protect chondrocytes from undergoing apoptosis. They chelate the zinc required for proinflammatory MMP function and reduce inflammatory cytokines concentrations within the joint. In both man and horses, it is generally accepted that bisphosphonates can result in amelioration of clinical signs dues to OA [3,7,31]. There is a dose dependent response in equine chondrocyte explants: low doses decrease apoptosis and sulfated glycosaminoglycan release, whereas higher doses result in increased sulfated glycosaminoglycan release and MMP concentrations [30]. At this time it is considered that i.a. use of tiludronate may lead to concentrations that are potentially damaging to cartilage, although very low doses may be protective. Again, care must, however, be taken in the extrapolation of these experimental data to clinical cases. Tiludronate is also known to induce synovial inflammation when administered i.a. and the drug will enter the systemic circulation from this route [32]. Systemic or low dose i.v. regional perfusions may be considered safe to chondrocytes, but high dose regional perfusions may be toxic to chondrocytes and care should be employed when calculating which dose to use and which route to administer [33]. Further investigation in larger numbers of horses is warranted and, until then, i.a. use should be considered experimental.

Developmental subchondral bones cysts secrete proinflammatory prostaglandin E2, nitric oxide and MMPs, which result in pain and recruitment of osteoclasts, resulting in cyst expansion [34]. There is a theoretical rationale for use of bisphosphonates here because they are antiresorptive and anti-inflammatory, and have anti-MMP effects [15]. However, in addition to potentially adverse systemic effects as outlined earlier, there are potential negative effects to healthy chondrocytes.

Are bisphosphonates safe in the juvenile horse?

Licensed bisphosphonates are only labelled for use in horses older than 4 years and the US Food and Drug Administration has issued warnings against the use in younger horses. Similarly, in the UK, no racehorse is permitted to receive bisphosphonate prior to reaching age 36 months. This generalisation derives from experience with this drug class in man. Even though bisphosphonates have been extensively studied in children since the 1960s, there few age-specific safety and efficacy data with use restricted to specific medical conditions such as osteogenesis imperfecta, compassionate ameliorative use or research [14,35]. A growing rabbit study demonstrated a transient effect on physeal cell morphology and a 3% decrease in long bone length but this has not been borne out in man [36]. Equine-specific research has demonstrated that healthy, trained juvenile horses have higher levels of bone remodelling biomarkers than those that are exercise-restricted [37]. Indeed, increased bone remodelling is a prerequisite for a healthy, athletic skeleton. Those same biomarkers are also increased in racehorses in training compared to nontraining controls. Of importance, administration of tiludronate decreases the serum concentrations of these biomarkers [6]. One can infer from these studies that a certain level of bone remodelling is necessary in the juvenile horse and that tiludronate, by reducing this turnover, may have a negative influence on appropriate skeletal development in the young athlete. Use of bisphosphonates is generally discouraged in adolescent human athletes [9]: the group most comparable to juvenile racehorses. Additionally, human females of potential childbearing age are discouraged from using bisphosphonates due to the potential for fetal accumulation of the drug, leading to fetal skeletal abnormalities. This potential risk is often cited and should be considered by equine veterinarians when treating female patients.

Conclusion

Bisphosphonates may be useful in the treatment of specific orthopaedic conditions in the horse and several products have been evaluated, considered safe in the short term with disease-specific licenses for equine use. No study, however, has evaluated bisphosphonate use in the training or racing Thoroughbred. Therefore, off-label use of bisphosphonate in the racing and juvenile Thoroughbred should not be undertaken without careful consideration. Current evidence, albeit from other species, suggests that bisphosphonates may be contraindicated in many orthopaedic conditions of the racehorse, especially in younger cases. Insufficient evidence for efficacy or long-term safety currently exists to support bisphosphonate use in this population. Racehorses in training are exposed to tremendous skeletal strains that necessitate bone modelling and remodelling. Attempting to 'control' bone metabolism in such an empirical way, particularly with an extremely long-lasting drug, may create more harm than good in this population and, until greater racehorse-specific research on the effects of bisphosphonates is undertaken, this drug class should be used with extreme caution, if at all.

The ethical dilemma of using an 'off-label' drug that has analgesic effects, is difficult to detect with conventional forensic methods, yet remains bound to, and potentially active in, skeletal tissues for extended periods must be considered. This is especially important to the Thoroughbred industry, where horses may change ownership frequently.

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References

1. Ott, S.M. (2005) Long-term safety of bisphosphonates. *J. Clin. Endocrinol. Metab.* **90**, 1897-1899.
2. Coudry, V., Thibaud, D., Riccio, B., Audigié, F., Didierlaurent, D. and Denoix, J.M. (2007) Efficacy of tiludronate in the treatment of horses with signs of pain associated with osteoarthritic lesions of the thoracolumbar vertebral column. *Am. J. Vet. Res.* **68**, 329-337.
3. Gough, M.R., Thibaud, D. and Smith, R.K.W. (2010) Tiludronate infusion in the treatment of bone spavin: a double blind placebo-controlled trial. *Equine Vet. J.* **42**, 381-387.
4. Denoix, J.M., Thibaud, D. and Riccio, B. (2003) Tiludronate as a new therapeutic agent in the treatment of navicular disease: a double-blind placebo-controlled clinical trial. *Equine Vet. J.* **35**, 407-413.
5. Kamm, L., McIlwraith, W. and Kawcak, C. (2008) A review of the efficacy of tiludronate in the horse. *J. Equine. Vet. Sci.* **28**, 209-214.
6. Delguste, C., Amory, H., Doucet, M., Piccot-Crézollet, C., Thibaud, D., Garnero, P., Detilleux, J. and Lepage, O.M. (2007) Pharmacological effects of tiludronate in horses after long-term immobilization. *Bone* **41**, 414-421.
7. De Simone, E.A., Perrone, G., Caggiano, N., Lastra, Y., Rubatino, F., Díaz, J., Ferretto, A., Montes de Oca, C., Roldán, E. and Chiappe Barbará, M.A. (2015) Levels of cytokines and matrix metalloproteinases 2 and 9 in the synovial fluid of osteoarthritic horses treated with pamidronate. *J. Equine. Vet. Sci.* **35**, 577-583.
8. Nieto, J.E., Maher, O., Standley, S.D., Knych, H.K. and Snyder, J.R. (2013) Pharmacokinetics, pharmacodynamics, and safety of zoledronic acid in horses. *Am. J. Vet. Res.* **74**, 550-556.
9. Shima, Y., Engebretsen, L., Iwasa, J., Kitaoka, K. and Tomita, K. (2009) Use of bisphosphonates for the treatment of stress fractures in athletes. *Knee Surg. Sports Traumatol. Arthrosc.* **17**, 542-550.
10. Mashiba, T., Turner, C.H., Hirano, T., Forwood, M.R., Johnston, C.C. and Burr, D.B. (2001) Effects of suppressed bone turnover by bisphosphonates on microdamage accumulation and biomechanical properties in clinically relevant skeletal sites in beagles. *Bone* **28**, 524-531.
11. Kidd, L.J., Cowling, N.R., Wu, A.C., Kelly, W.L. and Forwood, M.R. (2001) Bisphosphonate treatment delays stress fracture remodeling in the rat ulna. *J. Orthop. Res.* **29**, 1827-1833.
12. Milgrom, C., Finestone, A., Novack, V., Pereg, D., Goldich, Y., Kreiss, Y., Zimlichman, E., Kaufman, S., Liebergall, M. and Burr, D. (2004) The effect of prophylactic treatment with risedronate on stress fracture incidence among infantry recruits. *Bone* **35**, 418-424.
13. Sloan, A.V., Martin, J.R., Li, S. and Li, J. (2010) Parathyroid hormone and bisphosphonate have opposite effects on stress fracture repair. *Bone* **47**, 235-240.
14. Marini, J.C. (2003) Do bisphosphonates make children's bones better or brittle? *N. Engl. J. Med.* **349**, 423-426.

15. Barbara, A.C. and Soto, S.A. (2014) Bisphosphonates: pharmacology and clinical approach to their use in equine osteoarticular diseases. *J. Equine. Vet. Sci.* **34**, 727-737.
16. Stewart, G.W., Brunet, M.E., Manning, M.R. and Davis, F.A. (2005) Treatment of stress fractures in athletes with intravenous pamidronate. *Clin. J. Sports Med.* **15**, 92-94.
17. Monkonnen, J., Simila, J. and Roger, M.J. (1998) Effects of tiludronate and ibandronate on the secretion of proinflammatory cytokines and nitric oxide from macrophages *in vitro*. *Life Sci.* **62**, 95-102.
18. Dehghani, F., Conrad, A., Kohl, A., Korf, H.W. and Hailer, N.P. (2004) Clodronate inhibits the secretion of proinflammatory cytokines and NO by isolated microglial cells and reduces the number of proliferating glial cells in excitotoxically injured organotypic hippocampal slice cultures. *Exp. Neurol.* **189**, 241-251.
19. Rialland, P., Colombe, O., Moreau, M., Pelletier, J.P., Martel-Pelletier, J., Beaudry, F., del Castillo, J.R.E., Bertaim, T. and Gauvin, D. (2014) Association between sensitisation and pain-related behaviours in an experimental canine model of osteoarthritis. *Pain* **155**, 2071-2079.
20. Kim, S., Seiryu, M., Okada, S., Kuroishi, T., Takano-Yamamoto, T., Sugawara, S. and Endo, Y. (2013) Analgesic effects of the non-nitrogen-containing bisphosphonates etidronate and clodronate, independent of anti-resorptive effects on bone. *Eur. J. Pharmacol.* **699**, 14-22.
21. Smith, M.R.W., Kawcak, C.E. and McIlwraith, C.W. (2016) Science in brief: report on the Havemeyer Foundation workshop on subchondral bone problems in the equine athlete. *Equine Vet. J.* **48**, 6-8.
22. Whyte, M.P., Wenkert, D., Clements, K.L., McAlister, W.H. and Mumm, S. (2003) Bisphosphonate-induced osteopetrosis. *N. Engl. J. Med.* **349**, 457-463.
23. Yoshida, M., Tokuda, H., Ishisaki, A., Kanno, Y., Harada, A., Shimuzu, K. and Kozawa, O. (2005) Tiludronate inhibits prostaglandin F 2α-induced vascular endothelial growth factor synthesis in osteoblasts. *Mol. Cell. Endocrinol.* **236**, 59-66.
24. Alber, M.T., Brown, M.P., Merritt, K.A. and Trumble, T.N. (2014) Vascular perfusion of the palmar condyles of the equine third metacarpal bone: a possible contributing factor to palmar osteochondral disease? *Osteoarthr. Cartil.* **22**, S128-S129.
25. Plevin, S. and McLellan, J. (2014) The effect of insertional suspensory branch desmitis on racing performance in juvenile Thoroughbred racehorses. *Equine Vet. J.* **46**, 451-457.
26. McLellan, J. and Plevin, S. (2014) Do radiographic signs of sesamoiditis in yearling Thoroughbreds predispose the development of suspensory ligament branch injury?. *Equine Vet. J.* **46**, 446-450.
27. Committee for medicinal products: Tiludronic acid, disodium salt summary report. The European Agency for evaluating medicinal products. Jan 2001. Available from www.emea.eu.int/pdfs/vet/mrls/077401en.pdf
28. Rossini, M., Ombretta, V., Ramonda, R., Bianchi, G., Olivieri, I., Lapadula, G. and Amai, S. (2009) Intra-articular clodronate for the treatment of knee osteoarthritis: dose ranging study vs hyaluronic acid. *Rheumatology* **48**, 773-778.
29. Hayami, T., Pickarski, M., Wesolowski, G., McLane, J., Bone, A., Destefano, J., Rodan, G.A. and Duong, L.T. (2004) The role of subchondral bone remodeling in osteoarthritis: reduction in cartilage degeneration and prevention of osteophyte formation by alendronate in the rat anterior cruciate transection model. *Arthritis Rheum.* **50**, 1193-1206.
30. Duesterdieck-Zellmar, K.E., Driscoll, N. and Ou, J.F. (2012) Concentration-dependent effects of tiludronate on equine articular cartilage explants incubated with and without interleukin-1B. *Am. J. Vet. Med.* **73**, 1530-1539.
31. Strassle, B.W., Mark, L. and Leventhal, L. (2010) Inhibition of osteoclasts prevents cartilage loss and pain in a rat model of degenerative joint disease. *Osteoarthr. Cartil.* **18**, 1319-1328.
32. Duesterdieck-Zellmer, K.F., Moneta, L., Ott, J.F., Larson, M.K., Gorman, E.M., Hunter, B., Löhr, C.V., Payton, M.E., Morré, J.T. and Maier, C.S. (2014) Effects of low and high dose intraarticular tiludronate on synovial fluid and clinical variables in healthy horses – a preliminary investigation. *PeerJ.* **2**, e534.
33. Hunter, B.G., Duesterdieck-Zellmer, K.F. and Larson, M.K. (2015) Tiludronate concentrations and cytologic findings in synovial fluid after intravenous regional limb perfusion with tiludronate in horses. *PeerJ.* **3**, e889.
34. Von Rechenberg, B., Guenther, H., McIlwraith, C.W., Leutenegger, C., Frisbie, D.D., Akens, M.K. and Auer, J.A. (2000) Fibrous tissue of subchondral cystic lesions in horses produce local mediators and neutral metalloproteinases and cause bone resorption *in vitro*. *Vet. Surg.* **29**, 420-429.
35. Bachrach, L.K. and Ward, L.M. (2008) Clinical review: bisphosphonate use in childhood osteoporosis. *J. Clin. Endocrinol. Metab.* **94**, 400-409.
36. Smith, E.J., Little, D.G., Briody, J.N., McEvoy, A., Smith, N.C., Eisman, J.A. and Gardiner, E.M. (2005) Transient disturbance in physeal morphology is associated with the long-term effects of nitrogen containing bisphosphonates in growing rabbits. *J. Bone Miner. Res.* **20**, 1731-1741.
37. Billinghurst, R.C., Brama, P.A.J. and van Weeren, P.R. (2003) Significant exercise-related changes in the serum levels of two biomarkers of collagen metabolism in young horses. *Osteoarthr. Cartil.* **11**, 760-769.