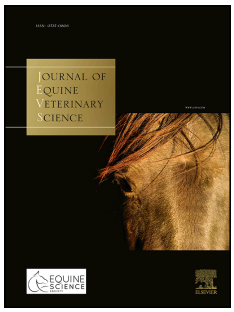

What do we know about clodronate now? A medical and veterinary perspective

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What do we know about clodronate now? A medical and veterinary perspective

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Abstract: There has recently been some controversy over the use of bisphosphonates in horses and some confusion regarding the different classes of bisphosphonate and the differences between the mechanism of actions and effects of each class. This review article explores the different bisphosphonate classes and their different effects and mechanisms of action based on research from both the human and equine veterinary fields. This collaborative review between Veterinary Surgeons and Medical Doctors describes the latest use of bisphosphonates in humans and horses, including safety aspects, and allows comparisons to be drawn between the two fields. Potential future uses of bisphosphonates are also discussed.

Keywords: *bisphosphonate, horse, clodronate, navicular syndrome, osteoarthritis, lameness*

1. Introduction

Clodronate (clodronic acid) is a non-nitrogenous bisphosphonate medication. Bisphosphonates (BPs) are a drug class developed in the late 1960's and are still the most commonly used drugs in the treatment of postmenopausal osteoporosis and other metabolic bone disease in human medicine [1]. Although their most important effect remains the reduction of bone remodeling by inhibition of osteoclastic activity there is growing evidence of additional effects which may expand the use of these therapeutic agents in clinical practice [2,3,4].
There are significant differences between BP classes due to chemical structure and indeed differences between individual BP molecules in respect to these additional effects (immune modulatory, anti-inflammatory and analgesic effects). These effects are independent of the absolute anti-bone resorptive potency [5,6,7,8,9,10,11,12,13].

Clodronate is commonly used in human medicine to treat a variety of bone related diseases including osteoporosis, osteopaenia, osteolytic lesions, hypercalcaemia and bone pain associated with skeletal metastases in patients with breast cancer and multiple myeloma [1,4,14]. Clodronate has also been shown to reduce fracture risk in high-risk patient groups [15,16]. More recently clodronate has been investigated as part of the treatment for musculoskeletal conditions such as osteoarthritis, bone oedema syndrome and for orthopaedic surgery [1,2,17,18,19,20,21,22,23].

Clodronate (Osphos®) has been approved for use in horses in the US and in Europe since 2014 and subsequently also in Canada, Australia, New Zealand and Mexico. Osphos is licensed to treat lameness caused by navicular bone disease in horses.

Confusion exists between different classes and different effects of BPs. Literature reviews have been undertaken to try to evaluate the evidence supporting use of BPs in horses [24] but large-scale, high-quality clinical trials in horses are limited so evidence from in-vitro studies and human clinical trials is used to supplement veterinary evidence.
This review highlights the effects and mechanisms of action of the different BP classes and aims to summarise relevant human medical clodronate literature particularly for those conditions where there is a comparable disease in equine patients.

2. Different mechanisms of action between bisphosphonate classes

Bisphosphonates regulate bone metabolism through inhibition of bone resorption and other mechanisms [7,14,25]. Recently published studies from both human and veterinary medicine have improved our understanding of the specific mechanisms of action of each of the BP classes and the differences between how each BP class exerts its effect. This work has emphasised the differences between each of the individual bisphosphonate medications. Despite not being one of the most potent in terms of anti bone-resorptive effect, analysis of human literature demonstrates that clodronate in particular is a molecule which has effects beyond the expected anti bone-resorptive action. These include anti-inflammatory effects, [9,10,11,12,26,27] potential analgesic effects [8,28,29] and anabolic effects on subchondral bone and cartilage [5,17,29]. These findings, along with low toxicity and a good safety profile [2,6,11,29] have renewed scientific and clinical interest in the clodronate molecule.

It is very important when using bisphosphonates in equine medicine to understand that there are two different, distinct classes of BP and each class exerts its effects via a different mechanism of action due to its physical structure.

1) Non-nitrogenous bisphosphonates (non-NBPs)
2) Nitrogenous bisphosphonates (NBPs)

The classes are named according to whether a nitrogen atom is part of their molecular structure or not and this structural difference affects the mechanism of action, effect and thus, potential indications [1,4,7,14].

The first BPs to be developed and used clinically did not contain nitrogen (non-NBPs). Nitrogenous bisphosphonates (NBPs) are the most recently developed BP class and have higher potency in terms of anti-bone resorptive effect. This makes them more suitable to treat specific diseases of bone metabolism in humans but serious side effects are more commonly reported with this BP class [2,5,6,28] and, due the their mechanism of action, they do not have any of the additional effects reported with non-NBPs. There are isolated case reports describing the use of the NBP zoledronic acid in a small number of healthy horses and in horses suffering from a rare bone-fragility disorder [30,31] but efficacy and safety have not been established in horses with commonly encountered orthopaedic diseases or in sufficient numbers of horses treated over a sustained period of time.

There are no veterinary licensed NBPs. Some of the human licensed products include: alendronate (Fosomax), ibandronate (Boniva), zoledronate (Reclast/Zometa) and risedronate (Actonl).

The veterinary licensed BPs (clodronate and tiludronate) are from the original generation of 'simple' BPs and have a molecular structure which does not contain nitrogen, non-NBPs. The therapeutic benefits of these medicines and their safety profiles have been successfully demonstrated for several bone diseases in horses.

It is important to note that non-NBPs are considered as therapeutic medications by the FEI (and other local jurisdictions) and are categorized as *controlled medications,* which can be safely used to treat competition horses as long as an appropriate withdrawal period is observed. In contrast, NBPs are not considered as therapeutic agents and are listed by the FEI as *banned substances* which are prohibited from use in

competition horses at any time and should never be administered to a horse intended for competition. No detection time has been established for any of the banned NBPs. The half-life of the NBPs is significantly longer than that of non-NBPs. The terminal half-life of the NBP alendronate is estimated to be at least 10 years in humans [32] which could have an impact on medication control if horses were to be treated with one of these molecules.

In general, non-NBPs are synthetic, non-hydrolysable analogues of pyrophosphate that contain P-C-P rather than P-O-P bonds. They are potent calcium chelators that rapidly target exposed metabolically active bone mineral surfaces in vivo, having an effect on bone resorbing osteoclasts and ultimately cause osteoclast inhibition and apoptosis. Bone resorbing osteoclasts and their intracellular mitochondria are the primary target of the non-NBP. The molecule is internalised within the osteoclast through endocytosis and disrupts the formation of ATP within the mitochondria, generating a cytotoxic analogue which causes cellular apoptosis [14]. This mechanism explains how additional anti-inflammatory effects can be seen with certain non-NBPs, including clodronate [9,11] and why they are not seen with NBPs [5].

NBPs have a different mechanism of action, these BP's do not utilise the ATP-ase energy depleting pathway, rather they inhibit the mevalonate pathway by inhibiting farnesyl pyrophosphate synthase. The inhibition is due to the docking of the nitrogenous bisphosphonates into the pyrophosphate $Mg2^{+}$ molecule, which leads to a decrease of the formation of isoprenoid lipids such as farnesyl and geranyl-pyrophosphates. These proteins are responsible for cellular functions including cytoskeleton assembly and intracellular signaling [4,7,14]. This unique mechanism also explains why some side effects (a rise in body temperature and flu-like symptoms), resembling a typical acute phase response [33], are seen with NBP therapy but do not occur following non-NBP treatment [2]. As the acute phase reaction is a specific feature unique to NBP treatment, it has been suggested that this reaction is mediated via γδ T cell activation due to an accumulation of metabolic intermediates caused specifically by inhibition of the mevalonate pathway [33]. Dramatic and serious side effects, including osteonecrosis of the jaw, atypical femoral fractures have been reported in human patients treated with NBPs. These are seen far less frequently following use of non-NBPs [6].

Currently there is very little safety, efficacy or toxicity data regarding the use on NBPs in the horse. Nieto et.al. (2013) [30], investigated the pharmacokinetics and pharmacodynamics of one dose of zoledronic acid in 8 healthy horses. They found CTX-1 remained below baseline after the one year study period. They did not follow the horses past the end of the study period so cannot quantify how long CTX-1 remained reduced in these horses or if there were any long term adverse effects of the medication. The type of equine diseases commonly treated with BPs do not necessarily benefit from the relatively increased potency of bone resorptive effect seen with this class of BP and NBPs do not provide any of the potential additional anti-inflammatory benefits described with non-NBPs.

3. Clodronate in human medicine

As one of the first generation, non-NBP medications clodronate has been used in human medicine for several decades. It is available as oral, intramuscular and intravenous preparations and is indicated for the treatment of hypercalcaemia and/or osteolysis of malignancy, multiple myeloma and for the prevention and treatment of post-menopausal osteoporosis.
It seems that clodronate has a distinct mechanism of action even within this BP class [3,10,2] with activities in addition to the well documented anti bone-resorptive activity [3,7,23]. Clodronate demonstrates anti-inflammatory effects, reducing release of inflammatory cytokines IL-1b, IL-6, and TNF-α, [2,10,11,13] nitric oxide [26] and PGE_2 [27]. The anti-inflammatory effect is likely to be mediated by the specific clodronate metabolite AppCCl2p [10]. Clodronate also inhibits MMP-1 [29] and, potentially, has both central and peripheral antinociceptive activity [6,8,28]. Studies have also demonstrated a positive chondromodulatory effect of clodronate increasing cartilage extra cellular matrix accumulation [5].
This unique mechanism of action has led to investigation of clodronate in humans for the treatment of bone pain, bone oedema syndromes including chronic regional pain syndrome and several forms of osteoarthritis [1,2,17,19,20,21,23,34,35]. Clodronate has been studied as a potential treatment for neuropathic and inflammatory pain in humans. Due to its mechanism of action, clodronate inhibits vesicular nucleotide transporter (VNUT), a key molecule carrying ATP as part of pathological nociception. Of all of the BPs, clodronate is by far the strongest inhibitor of VNUT, even when compared to other non-NBPs such as

tiludronate. Results are promising as clodronate has been found to be more effective and have comparatively fewer side effects than existing drugs [36].
In humans clodronate has also demonstrated a significant, protective, anti-fracture effect both in patients with pre-existing skeletal fragility [15] and also in a double-blind, placebo controlled, randomised study where patients were not selected for osteoporosis [16].

3.1. Clodronate research: Osteoarthritis

The complete pathogenesis of osteoarthritis (OA) is still not entirely clear but recent work suggests that the subchondral bone plays a crucial role in development of the disease [18]. The role of BPs in treatment and prevention of OA in people is still not fully defined and several meta-analyses have investigated this question [18,21]. Davis, et.al (2013) [21] found that conclusive evidence that BPs in general are effective for treatment of OA pain is limited. However, it is interesting that whilst only two studies specifically investigating clodronate were included in the meta-analysis, both of these studies found beneficial, positive effects of clodronate treatment [20,34].
The more recent meta-analysis [18] found that BP therapy was effective in relieving pain and stiffness and improved function, osteophyte score was also significantly reduced. Differences in study protocols and the variation in types of BP used for these studies makes comparisons difficult and further trials are indicated.
The ER.O.D.E study (2017)[17] is a blinded, randomised study investigating the effect of intramuscular clodronate for the treatment of erosive osteoarthritis of the hand in people. The treatment group (24 patients) initially received clodronate (200mg) administered intramuscularly (IM) in addition to their usual NSAIDs or other analgesic drugs. The control group (16 patients) received only their usual analgesic and NSAID drugs without the addition of clodronate. A serum biomarker of OA, COMP, was measured before treatment and after 6 months in order to try to monitor disease progression. Serum COMP levels are correlated to the presence of synovitis, OA severity, alterations in subchondral bone turnover and radiographic progression. The patients who received clodronate experienced significantly less pain, consumed significantly less anti-inflammatory and analgesic drugs, had fewer tender and swollen joints, had greater hand strength and lower patient and physician-reported disease activity scores than their counterparts who did not receive clodronate. At 6 months, the clodronate treated group also had significantly decreased serum COMP levels compared to the control group, who had no significant decrease. Participants in the study experienced no significant adverse effects of clodronate treatment and the authors concluded that clodronate is an effective symptomatic treatment of painful erosive OA of the hand and may play a role as a disease modifying OA medication.

This finding is supported by Rosa, et.al. (2014)[5] who found that in-vitro clodronate exerts an anabolic effect on articular chondrocytes and has a positive chondro-modulatory effect. In this study, the anabolic effect induced by clodronate on chondrocyte cultures was mediated by the export of a specific intra-cellular ATP-analogue and its interactions with the purinergic pathway. This stimulation upregulated short-term matrix synthesis by a relatively small degree (13-14%) but there was a sustained effect of an 80-90% increase in proteoglycan and collagen content (ECM accumulation) observed over the long term, which may support the evidence for use of clodronate to ameliorate progression of cartilage degeneration and improve OA management.

It has been demonstrated that use of clodronate as part of a therapeutic treatment strategy for OA causes upregulation of SOX9 gene expression [37]. This suggests that clodronate stimulates mesenchymal stem cell differentiation preferentially to the chondrogenic lineage, counteracting cartilage degeneration and demonstrating the potential use of clodronate as a therapeutic and disease modifying treatment for OA.

In humans, intra-articular clodronate has also been studied as part of the management of knee OA, a common degenerative condition which can be difficult and costly to manage. All three published studies investigating clodronate treatment for knee OA have demonstrated positive effects. Cocco, et.al. (1999) [23] found a significant reduction in spontaneous pain and pain on active movement correlated with reductions in synovial fluid concentration of inflammatory mediators (PGE_2, LTB_4 and $TBXb_{2)}$ when clodronate was administered. Subsequently, Rossini, et.al. (2009) [20] found that weekly intra articular clodronate injections were at least as effective as hyaluronic acid in controlling pain and improving functional movement of the knee. The most recent study [19] compared IA clodronate to placebo and noted a significant reduction in pain, improvement in patient and physician global assessment significant reduction in acetaminophen consumption in the

clodronate group compared to the placebo treated group five weeks after final injection. These findings could impact how we treat OA in horses in the future and certainly appear to warrant further investigation.

3.2. Clodronate research: Bone oedema syndromes

In man, bone marrow oedema (BME) is defined as a clinico-radiological diagnosis. It is an aspecific response of bone to several different clinical pictures with inflammatory (rheumatoid arthritis, spondyloarthritis) or degenerative causes or it can be caused by overload (osteoarthritis, osteonecrosis), infections, trauma, surgery, transient osteoporosis of the hip, regional migratory osteoporosis and complex regional pain syndrome [38].
Bone oedema is commonly diagnosed following MR imaging of horses with joint pain/lameness, particularly involving the distal limb.
Complex regional pain syndrome (CRPS) is part of the BME syndrome in people, when it is not associated with nerve injury it is defined as type 1-CRPS. Other potential imaging findings for CRPS are localised or diffuse osteoporosis in the painful limb and increased radiopharmaceutical uptake on nuclear scintigraphy [39]. Causes are unknown but this condition causes pain, oedema, swelling and vasomotor disorders and is challenging to treat.
Despite no evidence of increased osteoclastic activity in CRPS, BPs seem to show a good anti-inflammatory/analgesic effect in these cases, particularly in the early stages of the disease [40]. It is postulated that the anti-inflammatory/analgesic effect of BPs are due to their ability to decrease production of TNF-alpha and other pro-inflammatory cytokines which decreases activity of mononuclear cells [35], they could also reduce synthesis of prostaglandins and reduce proton concentration in the local environment, a process involved in sensitisation of peripheral nociceptors [22].
Varenna et.al. (2000) [35] compared clodronate treatment with placebo treatment for 32 patients with CRPS-1. They found that, forty days after treatment, the clodronate group showed significantly improved pain scores and that this improvement was sustained after 180 days from start of treatment. When the study participants who initially received placebo subsequently received clodronate treatment, there was a significant positive difference on their pain scores at day 40 compared with day 40 following placebo treatment. This study in humans recommended that clodronate is administered early in the course of BME disease to maximise efficacy.

4. Clodronate in equine medicine

For horses, clodronate is available as a licensed, intramuscular preparation (Osphos). Interest in the clodronate molecule for use in equine medicine has increased following the positive reports from the human medical literature and research in other veterinary species.

It has been demonstrated that clodronate is effective in reducing lameness in horses suffering from navicular disease. Frevel, et.al. (2017) [42] found that 75% horses treated with clodronate improved by at least 1 lameness grade 2 months after treatment and that 65% of the horses maintained this improvement 6 months after treatment. At day 56, 61% of the horses showed an improvement of 2 or 3 grades in lameness and 48% of the Osphos-treated horses had become sound compared to none of the placebo-treated horses.
Mitchell, et.al. (2018) [43] agreed with the previous findings that clodronate significantly improved lameness score in horses with navicular disease. They noticed an improvement in lameness score after one week with a performance improvement remaining after eight weeks. Interestingly, this study also measured a bone turnover marker, CTX-1, which is considered one of the most relevant bone turnover markers when monitoring BP effect in humans. They found that there was no change in CTX-1 following clodronate treatment despite the improvement in lameness. It must however be noted that the first measurement of CTX-1 in this study was only taken 1 week post-treatment and therefore a short-lived decrease in CTX-1 during the initial days following treatment may have been missed. The improvement in lameness could be due to reduction in inflammatory cytokines and macrophages, due to chondro-modulatory effects or other mechanisms discussed previously in this paper. The authors of this study suggest that that a single dose of clodronate will improve lameness in horses without impacting bone morphology or increasing fracture risk.

In people, a reduction in CTX-1 is associated with an increased risk of a specific fracture configuration involving the femur, an atypical femoral fracture. This risk is related to the anti-bone resorptive potency of the BP used and is seen much more commonly following treatment with the more potent NBPs. Generally this risk is low

when non NBPs are used due to the different mechanisms of action of the two groups [6]. In the rare human cases of atypical femoral fracture where non NBPs have been used these tend only to occur following long term, high dose therapy for osteoporosis [44]. This fracture configuration has not been reported in horses and safety data from Osphos indicates that no negative effect on bone density or bone strength was found even with repeated overdose of clodronate in horses [45].

Side effects following use of clodronate in horses are generally uncommon, mild and self-limiting and include head bobbling, transient swelling/pain at the injection site, pawing the ground, hives and pruritus. Nervousness, lip licking, yawning and mild colic have been commonly reported but any colic signs usually resolve with walking. Rarely, as with other BPs, episodes of renal insufficiency have been reported and are more frequently observed in horses treated concurrently with potentially nephrotoxic agents such as NSAIDs [41]. It is advisable to avoid use of these agents at the same time as using clodronate in horses and to monitor renal parameters if there is any concern. Clodronate (Osphos) is licensed only for horses aged 4 years or older [41]. Use of clodronate in younger horses was not studied during registration as navicular bone disease more commonly manifests in adult horses.

There are concerns that the use of BPs in horses, particularly NBPs where less evidence exists, could weaken bone, delay bone healing and decrease new bone formation/repair following any stress fracture or microdamage. As previously mentioned, studies using clodronate have indicated that bone morphology (including bone density, strength and structure) and general bone remodelling (assessed by measurement of the bone turnover marker CTX-1) [43,45] are not adversely impacted by clodronate administered at clinical doses. Additionally it has been shown that non-NBPs do not impact bone on a structural or cellular level following standard dosing schedules and that bone healing in an equine biopsy model was only minimally affected by clodronate administration [46].
In contrast, a study investigating the pharmacokinetics, pharmacodynamics and safety of zoledronic acid in horses, found a significant decrease in CTX-1 plasma concentrations during a period of 1 year after administration of the drug. No further information regarding the effects of NBPs on bone morphology, remodelling or fracture risk is currently available in horses.

Mashiba, et.al. (2001) [47] found increased skeletal micro-damage in Beagle dogs but this was following 12 months of daily administration of 6x the human recommended dose of the potent NBPs risedronate and alendronate. Kidd, et.al. (2001) [48] found that daily administration of the NBP risedronate delayed healing of ulna stress fractures in rats but that woven bone formation and periosteal reaction was not affected. This finding was supported by Sloan, et.al. (2010) [49] who examined the effects of parathyroid hormone and the NBP alendronate on stress fractures in rats, they found that whilst alendronate suppressed intracortical bone formation periosteal bone formation is not significantly affected.
It should be remembered that the NBPs used in these studies have a different mechanism of action compared to non-NBPs (where more data exists) including clodronate. Indeed clodronate has been demonstrated to have an anti-fracture risk effect in some human studies [15,16].

When reviewing BP studies and considering potential effect on fracture healing it is important to consider which BP is being evaluated, to differentiate between the two different BP drug classes and also to consider the different ways these medications are utilised in humans compared to in horses, particularly to the difference in duration of treatment.

4.1. *Clodronate in horses: The future*

Although clodronate is already a well-known and extensively utilised molecule, recent research in both human and equine medicine has revealed much more about the unique, additional mechanisms of action of this specific molecule. We now know that clodronate has additional positive biological effects beyond the well-known anti bone-resorptive action. These additional pharmacological properties could open up exciting possibilities to treat other, common musculoskeletal conditions in the equine patient. Meanwhile equine studies have also consistently demonstrated both a good therapeutic effect and a good safety profile for clodronate when used to treat navicular bone disease in horses.

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Animal Welfare Statement

Concerning the following manuscript:

'What do we know about clodronate now? A medical and veterinary perspective'

The authors have not carried out direct experimentation to write this review article. To the best of our knowledge the articles included in the review were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals and we agree that unnecessary cruelty in animal experimentation is entirely unacceptable.

Competing Interest Statement

Concerning the following manuscript:

'What do we know about clodronate now? A medical and veterinary perspective'

Richard Markell has acted as a veterinary consultant to Dechra Veterinary Products EU.

Gianantonio Saviola has acted as a medical consultant (comparative medicine) to Dechra Veterinary Products EU.

Elizabeth Barker and Chris Dujardin are employed by Dechra Veterinary Products EU.

J.D. Conway is employed by Dechra Veterinary Products US.