

# Clodronate disodium for treatment of clinical signs of navicular disease – a double-blinded placebo-controlled clinical trial

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**Summary:** Navicular disease (also referred to as palmar foot syndrome or navicular syndrome, although not completely the same) causes lameness in many horses of all activity groups. The objective of this study was to compare clodronate efficacy at improving lameness grade in horses with clinical signs of navicular disease to that of horses given placebo. A randomized, multi-center, double-blinded clinical field study was conducted on 146 horses with forelimb lameness due to navicular disease. Navicular disease was confirmed by lameness examination (with diagnostic nerve blocks) and radiography of the affected distal forelimb(s). At study initiation, treated horses received 1.4 mg/kg clodronate disodium IM, and control (placebo) horses received a corresponding IM volume of 0.9% saline. Primary effectiveness was evaluated at Day 56 using a lameness grading scale, with positive response defined as improvement by at least 1 grade in the limb with the most apparent lameness, with no worsening of the lameness grade (compared to baseline) in the contralateral forelimb. Positive response rates at Days 28 and 180 were also recorded. On Day 56, 75% of horses in the clodronate group were considered showing a positive response, versus only 3% in the placebo group ( $p = 0.003$ ). Of the 86 horses treated with clodronate, 8 horses had an improvement of 3 lameness grades, 45 horses improved by 2 lameness grades and 16 horses improved by one lameness grade. The clodronate positive response rate remained high through Day 180 (65%). Adverse events (primarily abdominal pain) were generally few, mild, and transient. In this study, clodronate disodium was demonstrated to be safe and effective in reducing the clinical signs of lameness associated with navicular syndrome.

**Keywords:** bisphosphonate, clodronate, clodronic acid, navicular syndrome, navicular disease, horse

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## Introduction

Navicular disease (also referred to as palmar foot syndrome or navicular syndrome, although not completely the same) causes lameness in many horses of all activity groups. It usually presents as a chronic forelimb lameness, although cases with acute onset may also be seen. Navicular disease is a degenerative condition of the distal sesamoid (navicular) bone, and/or adjacent soft tissues structures such as the deep digital flexor tendon, the collateral sesamoidean ligaments, the distal sesamoidean impar ligament and the navicular bursa (Dyson et al. 2011). The exact cause and pathogenesis are unknown, but vascular compromise, abnormal biomechanical function and a genetic component may be implicated with a multifactorial etiology most likely (Pool et al. 1989, Dik et al. 2001, Dyson et al. 2011). Changes associated with bone remodeling have been well described, including bone resorption seen as radiolucent areas on radiographs, sclerosis of the flexor compact bone and spongiosa and enthesioid new bone on the proximal border of the navicular bone (Dyson 2008, Verschooten et al. 1989, Pool et al. 1989). More recently, studies using MRI have further added

to the understanding of the different changes that occur in the navicular bone as well as the surrounding soft tissues (Murray et al. 2006, Dyson et al. 2011). Navicular disease cannot be readily cured, and treatment has focused on supportive therapies including rest, corrective shoeing, and anti-inflammatory medications (Schoonover et al. 2005, Belknap 2010). However, the association with bone loss has suggested that anti-resorptive agents such as bisphosphonates may be of benefit for the treatment of the osseous component of the disease (Denoix et al. 2003, Kamm et al. 2008). Bisphosphonates, as shown in experimental animal and human studies, act at several key places to inhibit osteoclast recruitment, adhesion, differentiation and resorptive activity; and to induce osteoclast apoptosis (Fleisch 2000, Russell 2007). Although circulatory half-lives are short, bisphosphonates accumulate rapidly in bone and remain there for long periods subject to skeletal turn over, so that active drug is liberated at the site of bone remodeling (Fleisch 2000).

Bisphosphonates have been used for decades to treat a variety of resorptive conditions in people (Scriba 2000). This class

of drugs has also been evaluated as therapy for a variety of equine orthopedic problems, and the non-nitrogenous bisphosphonate tiludronate has been approved in several countries for treatment of navicular syndrome or bone spavin (Kamm et al. 2008).

Clodronate disodium, like tiludronate, is a non-nitrogenous bisphosphonate. It inhibits bone resorption by impeding the dissolution of hydroxyapatite crystals, as well as by direct cellular effects on osteoclasts (Fleisch 1991, Plosker and Goa 1994). Bisphosphonates have also been described to have anti-inflammatory and analgesic effects as shown in experimental animal model research and human in-vitro studies Plosker and Goa (1994) and Soto and Barbara (2014).

The purpose of the current study was to evaluate the efficacy and safety of clodronate for treatment of clinical signs associated with navicular disease in horses. This information was used to aid regulatory approval for an application as veterinary drug, so as to provide a new treatment option for horses suffering from this condition.

## Materials and Methods

This multi-center, double blinded, placebo-controlled study was conducted under clinical conditions using client-owned horses examined by equine veterinarians in either the U.S. (5 investigation sites with 1 investigator each) or Germany (one investigator site with 1 investigator) in 2010-2011. No site was included in the statistical evaluation of effectiveness unless it had at least three evaluable clodronate treated horses and two evaluable placebo-treated controls.

Eligible horses were randomized to treatment groups of clodronate disodium [OSPHOS® Solution for Injection, Dechra Ltd., Skipton, North Yorkshire, United Kingdom] or saline control (placebo). Those in the treatment group received 1.4 mg/kg clodronate disodium (60 mg/mL), equivalent to 1.19 mg/kg clodronic acid. Placebo-treated horses received a corresponding volume of 0.9% saline (2.33 mL/100 kg). Injections were administered IM in three sites on Day 0. To ensure blinding of treatment group assignment, vials of clodronate or saline were coded with randomly assigned letter(s) (e.g. A to ZZZ) and were administered by a site treatment administrator, who was not the same person as the investigator.

To facilitate enrollment in this placebo-controlled trial, owners were offered the option of a compassionate use treatment of known clodronate at Day 56 even if deemed a treatment success. At the end of the study (Day 180), all remaining horses received a compassionate use treatment with clodronate.

### Study horses

All were client-owned horses that were housed, fed and exercised according to individual owner preference. Lameness of any breed were eligible for inclusion if they were cooperative with lameness examinations and otherwise clinically healthy. Medical records were evaluated and owners were interviewed for clinical histories. Horses were not eligible if there had been a change in shoe pattern within two weeks prior to

the study, if lameness was not attributable to navicular disease or was potentially attributable to another condition, if there was evidence (scarring) or history of previous neurectomy, or if hematology or serum chemistries were abnormal.

Horses were excluded if they were pregnant, < 4 years of age or had clinical signs of general illness (e.g. impaired general health, active systemic disease, loss of appetite, fever, etc.). Horses with hind limb lameness or evidence/history of musculoskeletal conditions that could have confounded the lameness exams were excluded. Horses were excluded if they had received extracorporeal shockwave therapy within 5 days of study initiation, NSAIDs during the 7 days prior to study initiation, short-acting corticosteroids 10 days prior to the study, or any long-acting corticosteroids, hyaluronic acid, isoxsuprine, pentoxifylline, or a systemically administered chondroprotective agent (i.e. PSGAGs) in the 30-day period before Day 0. Oral chondroprotectives were allowed if treatment was initiated prior to Day 0. Radiographic exclusion criteria included signs indicative of lameness due to primary soft tissue injury, osteoarthritis, fractures or any condition with bony changes other than those related to navicular disease.

Diagnosis of navicular disease was based on a finding of unilateral or bilateral forelimb lameness (AAEP Lameness Scale Grade  $\geq 2$ ), with clear reduction of lameness severity following anesthesia of the distal palmar digital nerves (at which time lameness may have shifted to the other limb). In bilateral lameness, the effectiveness evaluation was focused on the limb with the most apparent lameness. Enrolled horses were also required to have radiographic changes of the navicular bone, including at least one of the following degenerative changes: (1) >7 lucent zones (i.e. synovial invaginations) that were normal in shape, (2) at least one abnormally shaped lucent zone (traditionally known as lollipop-shaped areas) along the distal-horizontal or sloping borders of the navicular bone (i.e. Hallmark sign), (3) poor definition between the palmar cortex and the medulla due to subcortical sclerosis, (4) a crescent-shaped lucent zone in the central eminence of the flexor cortex of the navicular bone, (5) enthesiophyte formation on the proximal border of the navicular bone, (6) asymmetrically shaped navicular bones, or (7) proximal or distal extension of the flexor border of the navicular bone.

Ongoing/scheduled therapies (e.g. anthelmintics or vaccines) were continued except where these might interfere with study evaluation. Prohibited for 72 hours after the Day 0 treatment were medications such as aminoglycosides and tetracyclines that impact or are impacted by serum calcium levels. Administration of long-acting corticosteroids, systemically administered chondroprotective agents, or hyaluronic acid products was prohibited. NSAIDs or short-acting steroidal drugs were allowed only if deemed medically necessary, but all NSAIDs or short-acting steroids were to be withdrawn 7–10 days (respectively) before the scheduled effectiveness evaluations on Days 28, 56, and 180. Concurrent NSAID treatment did not influence the study results as described in the results section.

The pattern of shoeing and foot trimming for the front feet could not be changed during the period from two weeks prior to Day 0 until study termination. At the first visit, investigators recorded the shoeing pattern and confirmed the presence of heel wedges and/or rolled toes. At subsequent visits, investi-

gator determined whether the shoeing pattern had changed and documented the date that the horse was last shod.

Horses continued their accustomed exercise regimen, as determined by the owner and based on the comfort level of the horse. Owners were interviewed at the scheduled evaluations to determine whether the exercise program had been consistent and if the horse had been exercised appropriately. Horses with changes to the level of exercise (decrease/increase or type) were not terminated from the study solely due to this change in exercise. Several horses had both increases and decreases in exercise throughout the study. No horse was considered non-evaluable nor was terminated for the reason of a change (decrease/increase) in exercise.

### Assessments

Complete physical examinations were performed on Days 0, 28, 56, and 180 (as appropriate). Examinations included assessment of body weight (via girth tape), temperature, respiration rate, heart rate, appearance, attitude, activity, and appetite. Hematology and serum chemistry samples were obtained on Days 0, 28 and 56. For individual horses, lameness was assessed over time by the same blinded investigator using the 0–5 scale developed by the American Association of Equine Practitioners (AAEP) prior to treatment (Day 0) and at Days 28, 56, and 180 [American Association of Equine Practitioners website 2015]. The lameness evaluation included examination at the walk and trot while traveling down a straight line and circling to both right and left. Horses were also lunged if this did not cause undue discomfort. Other causes of lower foot pain (e.g. hoof bruises, hoof cracks, laminitis) were excluded based on clinical examination and use of hoof testers.

The same blinded investigator who performed the initial lameness evaluation confirmed the diagnosis by reduction in lameness following anesthesia of the distal palmar digital nerves in the limb with the most apparent lameness. Level of lameness in both forelimbs was graded prior to the nerve block. In bilaterally lame horses with identical grades in both legs, the limb with most lameness was selected at the discretion of the investigator. If desired, nerve block of the other forelimb was permitted with reassessment and grading following this block.

The foot with the most apparent lameness was subjected to a navicular series of digital radiographs on Days 0 and 180. Lateromedial, dorsoproximal-palmarodistal oblique (Oxspring's projection), palmaroproximal-dorsodistal oblique (skyline projection), and horizontal dorsopalmar views were obtained for each horse. The foot was cleaned and packed with a moldable packing product to eliminate artifacts; removal of shoes was at the discretion of each investigator. All radiographs were reviewed by a single board-certified radiologist (R.C. Cole), with Day 0 films assessed for study eligibility, and Day 180 films categorized as worsened, unchanged, or improved over baseline. Day 56 radiographs were not obtained as it was deemed to be too short of a period to document any major changes in bony structures.

Following injection, horses were observed for approximately 30 minutes for signs of immediate adverse events, then for an

additional 1.5 hours. Owners were asked to report any abnormalities observed over the course of the study. Owners or primary caregivers were asked to maintain a diary and were interviewed on Days 28, 56, 180, and 240 (at Day 240 if needed because of compassionate use clodronate treatment at Day 180).

Horses developing hind-limb lameness during the study were evaluated on a case-by-case basis, but no horses were terminated for this reason. All horses that received compassionate use treatment with known clodronate after the Day 56 efficacy evaluation were excluded from the Day 180 efficacy evaluation.

### Statistical analyses

The individual horse served as the experimental unit. Clinically valid evaluable cases were included in the efficacy assessment, and all horses that had been administered clodronate or placebo were included in the safety analyses. All data were analyzed with a computer software program (SAS<sup>®</sup>, Cary, NC, USA). Statistical significance was evaluated at  $\alpha = 0.05$  (two sided tests) unless otherwise indicated.

The results from a pilot study in 23 horses with diagnosed navicular disease (12 horses in the clodronate group, 11 horses in the placebo group), showed that the positive reponse rate in the clodronate group was around 90% while success in the placebo group was around 10%. Given this expected effect size, an enrollment ratio of 3:1 was chosen using a minimum of 100 clodronate treated horses and a minimum of 33 placebo horses.

Percent positive response (with 95% confidence interval [CI]) at the Day 56 evaluation served as the primary effectiveness endpoint. Positive response was defined as improvement by at least 1 grade in the limb with the most apparent lameness, with no worsening of the lameness grade (compared to baseline) in the contralateral forelimb. Differences in positive response rates between the groups were assessed using a generalized linear mixed model appropriate for binary data [SAS/STAT 9.2 User's guide 2008]. Treatment group was included in the model as a fixed effect, with site and site-by-treatment interaction included as random effects. Because of the likelihood of high failure rates in the placebo group, the "residual" term was included as an additional random effect so as to guard against over-dispersion. Positive response rates were also calculated for Days 28 and 180, but statistical tests were not performed as the primary effectiveness endpoint was success rate at Day 56 and no control group was available at Day 180.

For safety data analysis, categorical outcomes and continuous outcomes with less than five discrete levels were evaluated using a generalized linear mixed model appropriate for repeated-measures ordinal data, assuming a multinomial distribution and a cumulative logit link [SAS/STAT 9.2 User's guide 2008]. Continuous outcomes and those measured multiple times during the study (i.e. hematology, serum chemistry, and physical exam) were evaluated by repeated-measures analysis of covariance. Treatment, visit, and treatment-by-visit interaction were included in the model as fixed effects,

with site, site-by-treatment interaction, and site-by-treatment-by-visit interaction included as random effects. Pre-treatment values were included as covariates when available. Blood results from the US and German laboratories were analyzed separately. Safety outcomes were considered statistically significant at  $\alpha = 0.10$ .

## Results

Of the 211 horses evaluated, 146 met inclusion criteria and received treatment with either clodronate or placebo. Enrolled horses represented a wide variety of breeds, ranged from 4 to 22 years of age, and weighed 367 to 601 kg. There were 101 geldings, 41 mares and 4 stallions enrolled. Breeds included: 72 American Quarter Horses, 12 Rheinländer, 8

American Paint Horses, 5 each of Belgian Warmblood, Dutch Warmblood, Hanoverian, Sachsen Anhaltiner, Thoroughbred and Westfale, 4 Holsteiner, 3 each of Oldenburg and Selle Français and 1 each of American Quarterhorse/Morgan mix, Appaloosa, Warmblood, Belgian Sporthorse, Hessian Warmblood, Irish Horse, Latvian Warmblood, Swedish Warmblood, Bavarian Warmblood, Selle Luxembourgeois and Württemberger. Breed was unknown for three horses.

AAEP lameness grades were similar between groups (Grade 2, 66% and 71%, and Grade 3, 34% and 29%, respectively for clodronate and placebo (Table 1) and radiographic findings between groups also were similar (Table 2). The pre-enrollment duration of the forelimb lameness was highly variable in both groups. Average lameness duration was 445 days (SD  $\pm$  309 days) in the clodronate group and 321 days

**Table 1** Number and percentage of horses with AAEP lameness grades 0 - 3 at Day 0, Day 28, Day 56 and Day 180

	Grade 0	Grade 1	Grade 2	Grade 3
Day 0				
Clodronate (n=111)			73 (66%)	38 (34%)
Placebo (n=35)			25 (71%)	10 (29%)
Day 28				
Clodronate (n=89)	19 (21%)	40 (45%)	25 (28%)	5 (6%)
Placebo (n=29)	1 (3%)	8 (28%)	13 (45%)	7 (24%)
Day 56				
Clodronate (n=86)	41 (48%)	25 (29%)	16 (19%)	4 (5%)
Placebo (n=28)			20 (71%)	8 (29%)
Day 180				
Clodronate (n=61)	25 (41%)	23 (38%)	12 (20%)	1 (1%)
Placebo (n= NA <sup>a</sup> )				

<sup>a</sup> Not applicable - no evaluable controls due to compassionate use treatment after Day 56

**Table 2** Radiographic findings at enrollment by treatment group and lameness score

Group		More than 7 lucent zones (synovial invaginations) on distal horizontal/sloping border	At least 1 abnormally shaped synovial invagination on distal horizontal/sloping border	Poor definition between the palmar cortex and medulla due to subcortical sclerosis	Crescent-shaped lucent zone in the central eminence of the flexor cortex	Enthesiophyte formation on the proximal border	Asymmetry of the navicular bone	Proximal or distal extension of the flexor border
Clodronate Grade 2 <sup>a</sup>	n	7	72	33	17	18	2	0
	%	6.3 %	64.9 %	29.7 %	15.3 %	16.2 %	1.8 %	
Clodronate Grade 3 <sup>a</sup>	n	3	37	24	12	13	0	0
	%	2.7 %	33.3 %	21.6 %	10.8 %	11.7 %		
Clodronate Total <sup>a</sup>	n	10	109	57	29	31	2	0
	%	9 %	98 %	51 %	26 %	28 %	2 %	0 %
Placebo Grade 2 <sup>b</sup>	n	3	25	14	4	8	0	1
	%	8.6 %	71.4 %	40 %	11.4 %	22.9 %		2.9 %
Placebo Grade 3 <sup>b</sup>	n	1	10	7	3	2	1	1
	%	2.9 %	28.6 %	20 %	8.6 %	5.7 %	2.9 %	2.9 %
Placebo Total <sup>b</sup>	n	4	35	21	7	10	1	2
	%	11 %	100 %	60 %	20 %	29 %	3 %	6 %

<sup>a</sup> Clodronate group n (total) = 111 <sup>b</sup> Placebo group n (total) = 35

(SD  $\pm$  266 days) in the placebo group, with no significant difference between groups ( $p > 0.05$ ). Prevalence of radiographic findings by baseline lameness score is shown in Table 2.

By the time of the primary-efficacy analysis (Day 56), 6 horses were excluded for the following reasons: injury (3), owner withdrawal (1), sold (1), failed to return (1). An additional 26 horses were excluded when two sites were removed from the statistical analysis due to insufficient number of placebo control horses. Due to compassionate use treatment with known clodronate after the Day 56 evaluation, there were no evaluable placebo-treated controls available at Day 180. Twenty-five placebo horses and 17 clodronate-treated horses received compassionate use treatment after the Day 56 evaluation. Of these, one placebo-treated horse and two clodronate treated horses had been graded as a "positive response". The owners of these horses opted for compassionate use treatment with hopes of observing additional improvement.

### Efficacy

On Day 28, 67% of horses in the clodronate group were considered having a positive response versus 21% of those in the placebo group (Table 3). By the time of the primary-efficacy assessment (Day 56), the modelled positive response rate in the clodronate group had risen to 75%, which was significantly higher ( $p = 0.003$ ) than that in the placebo group (3%). These Day-56 model rates are very similar to the crude values of 80% (69/86) for clodronate and 4% (1/28) for placebo. Among clodronate treated horses, Day-56 lameness grades had improved by one (16 horses), two (45 horses), or three (8 horses) grades as compared to the control group in which only one horse had an improved grade (Table 4).

Of the 68 clodronate treated horses that were deemed having a positive response on Day 56, 61 were evaluable at Day 180. Of these 61 horses, 52 remained showing a positive response at Day 180 based on improvement in lameness grade as compared to Day 0. However, 21 of these 61 evaluable horses demonstrated an increase in lameness grade at Day 180 as compared to their Day 56 evaluation. Including the 18 treatment failures at Day 56, the calculated overall positive response rate for clodronate solution at Day 180 is 65.8% (52/79). The modelled positive response rate in the clodronate group for Day 180 was 84% (Table 3).

A total of 59 clodronate-treated horses were assessed radiographically on Day 180. Of these, three (5%) showed evidence of improvement, with the remainder unchanged over baseline.

The majority of the NSAIDs were administered after the Day 56 efficacy evaluation; there were only 4 instances of phenylbutazone and 1 instance of firocoxib administration from Days 0–56. The results from these 5 horses were excluded from the efficacy analysis, thus NSAID administration did not complicate the study results at Day 56. Of the 9 horses that received NSAIDs after the Day 56 visit, only one was evaluable at Day 180, and the NSAID (phenylbutazone) had been discontinued for greater than 7 days prior to the Day 180 evaluation.

### Safety

All 146 enrolled horses were included in the safety assessment. None were terminated from the study as a result of adverse events related to treatment.

**Table 3** Positive response (95% confidence interval) during the study<sup>a</sup>

	Number of horses (n)		Positive response (%) (95% CI)	
	Clodronate	Placebo	Clodronate	Placebo
Day 28	89	29	67% (58%-77%)	21% (6%-35%)
Day 56	86	28	75% <sup>b</sup> (35%-94%)	3% (0.3%-26%)
Day 180	61	NA <sup>c</sup>	84% (74%-93%)	NA <sup>c</sup>

<sup>a</sup> least-square means and 95% CI from the generalized linear mixed model. <sup>b</sup> significantly different ( $p = 0.003$ ) from placebo

<sup>c</sup> Not applicable - no evaluable controls due to compassionate use treatment after Day 56

**Table 4** Change in AAEP lameness grade at Day 28, 56 and 180 / Veränderung der Lahmheitsgrade nach AAEP am Tag 28, 56 und 180

	Day 28				Day 56				Day 180			
	Clodronate		Placebo		Clodronate		Placebo		Clodronate		Placebo	
	n	%	n	%	n	%	n	%	n	%	n	%
Improvement by 3 grades	4	4.5	0	0	8	9.3	0	0	5	8.2	0	0
Improvement by 2 grades	26	29.2	2	6.9	45	52.3	0	0	28	45.9	0	0
Improvement by 1 grade	34	38.2	8	27.5	16	18.6	1	3.6	20	32.8	0	0
No change	25	28.1	18	62.0	16	18.6	26	92.9	8	13.1	0	0
Worsened by 1 grade	0	0	1	3.4	1	1.2	1	3.6	0	0	0	0
Total horses	89		29		86		28		61		0	

Between Days 0 and 56, adverse events were reported for 30 horses (27%) in the clodronate group and 7 horses (20%) receiving placebo. Those adverse events considered to have probable, possible, or unknown relationships to clodronate (definition as used in the Guidelines on Pharmacovigilance for Medicinal Products for Veterinary Use by the European Union (Union 2011)) included pawing and/or rolling (2), colic (2), head bobbing (2), injection-site swelling (2), hives (1), lameness (6), lip licking (3), nervousness (7), and yawning (3). All were considered to be mild and transient, which was also the case for the adverse events in the control group. Between Days 56 and 240, adverse events were recorded for 34 horses receiving clodronate, but none were considered related to treatment. There were no apparent or observed interactions between clodronate and concurrent medications administered during the course of the study.

Shortly after clodronate administration on Day 0, 10 horses appeared uncomfortable, nervous, or otherwise experiencing pain, agitation, or potential signs of mild colic. Eight of these 10 horses were hand-walked until clinical signs resolved (approximately 15 minutes), one resolved after receiving an anti-inflammatory for hives, and signs resolved in one without treatment. Four additional horses experienced licking, yawning, head shaking, and/or rolling following administration of clodronate, but these signs quickly resolved spontaneously in three cases, and were considered normal behavior for the fourth horse.

There was no significant ( $p < 0.10$ ) change in body weight, temperature, respiratory rate, or heart rate associated with clodronate treatment. Clodronate treatment was not associated with any clinically significant change in hematology or serum chemistry at any of the time points measured in this study.

## Discussion

Horses treated with clodronate had a significantly higher positive response rate than did horses receiving placebo. Improvement was apparent by Day 28, which is approximately one month sooner than reported for cases of navicular syndrome treated with IV tiludronate (Denoix et al. 2003). Two thirds of treated horses showed at least 6 months of improvement from a one-time IM clodronate dose. This long lasting effect is in accordance with the pharmacokinetic and pharmacodynamic characteristics of clodronate, which include a relatively rapid clearance from the blood and a long lasting incorporation into bone tissue of several months (Plosker and Goa 1994).

Traditional treatments for navicular syndrome revolve around therapies such as corrective shoeing and treatment with anti-inflammatories and other drugs (Schoonover et al. 2005, Belknap 2010). In the current study, the improvement produced by clodronate was in the absence of any ancillary treatment that may have confounded the results. Long-acting anti-inflammatory drugs were prohibited, as were shorter-acting agents administered near the time of study initiation. Shoeing was kept unchanged from two weeks before the initial assessment until study termination.

Diagnosis in the current study was achieved using blinded lameness evaluation, radiographs, and nerve blocks, rather

than imaging techniques such as MRI or CT scan, or means of objective lameness evaluation. Objective lameness evaluation such as force-plate assessment or body-mounted inertial sensor based system could have increased the reliability of lameness evaluation, although in the current study individual horses were evaluated by the same investigator over time, thereby excluding inter-observer variation (Schoonover et al. 2005, Keegan et al. 2010, Keegan et al. 2013). The chosen diagnostics were considered to be both sufficient and cost effective, but may have increased the risk of including horses with soft tissue injury of the heel, and of tendon or ligament injury within the foot. However, any introduced misclassification would have been equal for treatment and control group (non-differential) given the random nature of the design (randomized based on order of presentation), thereby skewing results toward the null and reducing the power to detect a significant effect (Rothman and Greenland 2008). The clear and significant improvement noted in the current study suggests that such misclassification was slight and/or that the benefits of clodronate treatment may have been underestimated.

There were relatively few adverse events potentially related to clodronate treatment, and these were considered to be mild and transient. Most common were clinical signs related to abdominal discomfort or mild nervous system effects, including nervousness, yawning, lip-licking, head-bobbing, and pawing/rolling. The vast majority resolved quickly with no intervention or after hand walking. Swelling at the site of administration occurred in only two (0.8%) of the 248 clodronate injections (experimental and compassionate use). Over the course of the study, treatment had no significant effect on parameters commonly associated with pain such as heart or respiratory rate. There were no signs of elevated temperature or demineralized fractures, as has been observed with some bisphosphonates (Fleisch 1991, Plosker and Goa 1994). These findings are consistent with the excellent safety record of clodronate use in people (Plosker and Goa 1994).

The current study results support IM clodronate as a well-tolerated and effective treatment for horses diagnosed with a forelimb lameness, a positive reaction to palmar digital nerve block and radiological changes in the navicular bone. However, the fact that the effectiveness evaluation in this study was only carried out by means of a subjective lameness examination at three points in time should be considered. Further studies are necessary, using objective techniques for the evaluation of both lameness and pain causing inflammatory processes, to evaluate the effectiveness of clodronate in lameness caused by navicular syndrome.

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## Animal Welfare Statement

In support of worldwide regulatory approvals, the study was designed and conducted according to Good Clinical Practi-

ce, Guidance GL9, International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products. All study related procedures were conducted under best veterinary practices. Approvals to conduct the clinical trial were received from the applicable regulatory agencies in the U.S. and Germany.

### Informed consent statement

Informed consent was obtained from each owner or authorized representative.

### Conflict of interest statement

Financial support for this study was provided by Dechra Ltd, Skipton, North Yorkshire, United Kingdom. The information from this study was used to aid regulatory approval for an application as veterinary drug. The authors Poole and Longhofer were employed by Dechra at the time of the study.

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Erweiterte Zusammenfassung

### Dinatriumclodronat (Clodronsäure) für die Behandlung klinischer Beschwerden bei der Strahlbeinerkrankung – eine klinische placebokontrollierte Doppelblindstudie

Die Strahlbeinerkrankung tritt bei Pferden aller Nutzungsarten auf. Es handelt sich dabei um eine degenerative Erkrankung des distalen Sesambeins (Strahlbein). Als Ursache kommen Überlastung oder Verletzung, Durchblutungsstörungen oder erbliche Veranlagung in Frage. Die Verknüpfung zum Knochenabbau lässt vermuten, dass ein antidegenerativer Wirkstoff wie Bisphosphonat zur Verbesserung des Krankheitsbildes beiträgt. Bisphosphonate wirken an einigen Schlüsselstellen, wo sie die Osteoklastenmobilisierung, -adhäsion und -differenzierung sowie resorptive Vorgänge hemmen; außerdem induzieren sie die Apoptose der Osteoklasten. Ziel dieser Studie war es, die Wirksamkeit von Clodronsäure (als Dinatriumclodronat) bei Pferden mit Strahlbeinerkrankung anhand der Verbesserung des Lahmheitsgrades gegenüber Placebo-behandelten Pferden zu vergleichen. Clodronsäure ist wie Tiludronsäure ein nicht-stickstoffhaltiges Bisphosphonat. Sie hemmt die Knochenresorption, indem sie sowohl die Auflösung von Hydroxylapatit-Kristallen behindert als auch direkte zelluläre Effekte auf die Osteoklasten ausübt.

Die randomisierte, multizentrische, klinische Doppelblindstudie wurde mit 146 Pferden mit einer Vorderhandlahmheit aufgrund der Strahlbeinerkrankung durchgeführt. Die Diagnose wurde durch eine Lahmheitsuntersuchung (einschließlich diagnostischer Anästhesie) und Röntgenuntersuchung der betroffenen Vordergliedmaßen bestätigt. Bei Studienbeginn erhielten Pferde der Behandlungsgruppe 1,4 mg/kg Dinatriumclodronat i.m., die Pferde der Kontrollgruppe (Placebo) erhielten ein entsprechendes Volumen einer 0,9%igen Kochsalzlösung i.m.. Die Injektionen wurden am Tag 0 an drei verschiedenen Stellen verabreicht. Alle studienbezogenen Verfahren wurden

unter den Bedingungen der „besten tierärztlichen Praxis“ durchgeführt, um eventuelle Schmerzen, Leiden oder Schäden zu minimieren. Alle Pferde, die an der Studie teilnahmen, wurden individuell nach Besitzerwünschen gefüttert, gehalten und bewegt. Es kamen lahme Pferde aller Rassen in Betracht, solange sie für die Lahmheitsuntersuchung kooperativ und ansonsten klinisch gesund waren. Medizinische Vorberichte wurden ausgewertet und die Besitzer wurden befragt. Pferde, bei denen der Hufbeschlag 2 Wochen vor Studienbeginn geändert wurde, kamen nicht in die Auswahl. Des Weiteren wurden Tiere ohne Strahlbeinerkrankung, nachweislicher Neurektomie oder verändertem klinischen Blutbild von der Studie ausgeschlossen. Trächtige Tiere, Tiere jünger als 4 Jahre oder Tiere mit klinischen Anzeichen einer generalisierten Erkrankung (z.B. Appetitlosigkeit, Fieber, etc.) wurden von der Studie ausgeschlossen. Pferde mit einer Hinterhandlahmheit oder muskuloskeletalen Veränderungen, die die Lahmheitsuntersuchung beeinflussen könnten, wurden ebenfalls ausgeschlossen. Ausgeschlossen wurden auch solche Pferde, die bis 7 Tage vor Beginn der Studie mit NSAIDs, bis 10 Tage davor mit kurzwirksamen Kortikosteroiden oder 30 Tage vor Studienbeginn mit langwirksamen Kortikosteroiden behandelt wurden. Ein Studienausschluss erfolgte ebenfalls, wenn 10 Tage vor dem Tag 0 eine Behandlung mit Hyaluronsäure, Isoxsuprin, Pentoxifyllin oder mit einem systemisch wirksamen chondroprotektiven Medikament durchgeführt wurde. Orale Chondroprotektiva (Ergänzungsfutter) waren erlaubt, wenn die Verabreichung vor Tag 0 erfolgte. Radiologische Ausschlusskriterien waren Anzeichen für eine Lahmheit aufgrund einer Weichteilverletzung, Arthrosen, Frakturen oder andere Knochenveränderungen, die nicht zur Strahlbeinerkrankung gehören.

Eine vollständige Allgemeinuntersuchung wurde an den Tagen 0, 28, 56 und 180 durchgeführt (soweit erforderlich). Proben für die Blut- und Serumuntersuchungen wurden an den Tagen 0, 28 und 56 entnommen. Die Lahmheit wurde von unabhängigen Untersuchern mittels der Punkteskala (0 - 5) der „American Association of Equine Practitioners“ (AAEP) vor der Behandlung (Tag 0) und an den Tagen 28, 56 und 180 durchgeführt. Die Lahmheitsuntersuchung beinhaltete die Evaluation in Schritt und Trab auf gerader Strecke und auf dem Zirkel sowie auf der rechten und linken Hand. Andere Hufveränderungen (wie z.B. Hufprellung, Huffissur, Hufrehe) wurden mittels klinischer Untersuchung und Hufzangenprobe ausgeschlossen. Der gleiche Untersucher, der die klinische Lahmheitsuntersuchung durchgeführt hatte, evaluierte den Lahmheitsgrad nach diagnostischer Anästhesie des N. digitalis palmaris distalis der Vordergliedmaße mit dem höheren Lahmheitsgrad. Der Fuß mit der deutlichsten Lahmheit wurde an Tag 0 und 180 einer radiologischen Untersuchung hinsichtlich der Strahlbeinerkrankung unterzogen. Digitale Röntgenaufnahmen wurden von jedem Pferd als lateromediale und horizontal dorsopalmar Aufnahme sowie nach Oxspring und als Skyline erstellt. Alle Aufnahmen wurden von einem zertifizierten Radiologen überprüft; an Tag 0 auf Eignung für die Studie und an Tag 180 kategorisiert nach verbessert, verschlechtert oder unverändert. An Tag 56 wurden keine radiologischen Untersuchungen durchgeführt, da dieser Tag als zu früh erachtet wurde, um relevante Veränderungen der Knochenstruktur sehen zu können.

Nach erfolgter Injektion wurden die Pferde für etwa 30 Minuten beobachtet, um sofortige unerwünschte Ereignisse zu erkennen und im Anschluss für weitere 1,5 Stunden. Die Besitzer wurden gebeten, beobachtete Auffälligkeiten während des Studienverlaufs zu dokumentieren. Eigentümer oder Tierpfleger wurden verpflichtet, ein Tagebuch zu führen und wurden an den Tagen 28, 56, 180 und 240 interviewt (an Tag 240 nur wenn an Tag 180 eine „mitfühlende Behandlung“ mit Clodronsäure stattgefunden hatte). Pferde, die während der Studie eine Hinterhandlahmheit entwickelten, wurden von Fall zu Fall evaluiert, aber nicht von der Studie ausgeschlossen.

Die maßgebliche Evaluation der Wirksamkeit wurde an Tag 56 anhand einer Lahmheitsgradskala durchgeführt. Ein Behandlungserfolg wurde als Verbesserung um mindestens 1 Grad an derjenigen Extremität mit der deutlichsten Lahmheit definiert, ohne dass eine Verschlechterung des Lahmheitsgrades (im Vergleich zum Ausgangswert) an der kontralateralen Vordergliedmaße zu beobachten war. Die Erfolgsraten hinsichtlich der Lahmheitsgrade an den Tagen 28 und 180 wurden ebenfalls protokolliert.

Am Tag 56 konnte bei 75 % der Pferde der Behandlungsgruppe ein Behandlungserfolg nachgewiesen werden. Von den 86 mit Clodronsäure behandelten Pferden wurde bei 8 Pferden eine Verbesserung der Lahmheit um drei Lahmheitsgrade, bei 45 Pferden eine Verbesserung um 2 Lahmheitsgrade und bei 16 Pferden eine Verbesserung um einen Lahmheitsgrad bei der Untersuchung an Tag 56 festgestellt. In der Placebogruppe wurde zum selben Untersuchungszeitpunkt bei 3 % der Pferde eine Besserung der Lahmheit dokumentiert. Die an Tag 180 durchgeführte Lahmheitsuntersuchung ergab, dass in der mit Clodronsäure behandelten Gruppe 65 % weiterhin eine Verbesserung der Lahmheit zeigten im Vergleich zu Tag 0.

Die Ergebnisse der vorliegenden Studie konnten die zuvor aufgestellte Hypothese bestätigen, dass Pferde mit der Diagnose der Strahlbeinerkrankung, nach einer Behandlung mit Clodronsäure eine Besserung ihrer Lahmheit zeigen. Zusätzlich konnte gezeigt werden, dass Clodronsäure sich auch langfristig positiv auf die Knochenresorption auswirkt. Klinisch konnte dieser Effekt durch eine langfristige Besserung des Lahmheitsgrades nachgewiesen werden. Die Hypothese, dass Clodronsäure ein in der Anwendung sicheres Arzneimittel darstellt, konnte ebenfalls bestätigt werden, da die aufgetretenen unerwünschten Wirkungen als vorübergehend und mild beschrieben wurden.

In der vorliegenden Studie wurde die Evaluierung der Veränderung des Lahmheitsgrades und der strukturelle Veränderungen ausschließlich durch visuelle Beurteilung bzw. mittels Röntgenuntersuchung einer Gliedmaße vorgenommen. Weitere Belege der Wirksamkeit von Clodronsäure in Zusammenhang mit dem Hufrollen-Syndrom erfordern Studien unter Einbeziehung objektiver Techniken zur Evaluierung sowohl der Lahmheit als auch der Schmerz auslösenden Prozesse.

**Schlüsselwörter:** Bisphosphonat, Clodronat, Clodronsäure, Strahlbeinsyndrom, Hufrollensyndrom, Pferd