Effect of intravenous tiludronate disodium administration on the radiographic progression of osteoarthritis of the fetlock joint in Standardbred racehorses

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OBJECTIVE
To compare the effects of tiludronate disodium and 3 other medical treatments on clinical and radiographic findings and biomarkers of disease progression in horses with osteoarthritis of the fetlock joint.

ANIMALS
100 Standardbred racehorses with spontaneous traumatic injury of the fetlock joint.

PROCEDURES
Horses were retrospectively grouped by whether they received tiludronate IV or triamcinolone acetonide and hyaluronan, polysulfated glycosaminoglycan, or interleukin-1 receptor antagonist protein intra-articularly. Data were collected on clinical, radiographic, and ultrasonographic findings and results for serum and synovial samples obtained before and 6 months after treatment. Lameness score, joint flexion test response, radiographic score, serum concentrations of tumor necrosis factor-α and carboxy-terminal telopeptides of collagen types I and II (CTX-I and II, respectively), and synovial fluid concentrations of interleukin-1β, prostaglandin E2, and CTX-II were compared among treatments.

RESULTS
All treatments resulted in a significant improvement in lameness score and joint flexion test response at 6 months. In horses that received triamcinolone acetonide and hyaluronan, synovial fluid interleukin-1β, prostaglandin E2, and CTX-II concentrations decreased after treatment, suggesting this treatment inhibited progression of hyaline cartilage degeneration and inflammatory processes. Horses that received tiludronate were the only group that had a decrease in radiographic score and serum CTX-I concentration after treatment, supporting the effect of tiludronate on bone metabolism. Tiludronate treatment was also followed by increases in serum and synovial fluid concentrations of CTX-II, a marker of cartilage damage.

CONCLUSIONS AND CLINICAL RELEVANCE
Tiludronate appeared to inhibit the radiographic progression of osteoarthritis in high-motion joints of racehorses at 6 months after treatment by inhibiting subchondral bone remodeling. Whether this effect was associated with a worsening of progressive cartilage damage remains to be ascertained. (J Am Vet Med Assoc 2021;259:651–661)

Abbreviations
ACS  Autologous conditioned serum
CTX-I  Carboxy-terminal telopeptide of type I collagen
CTX-II  Carboxy-terminal telopeptides of type II collagen
IA  Intra-articular
IL  Interleukin
IRAP  Interleukin-1 receptor antagonist protein
PGE2  Prostaglandin E2
PSGAG  Polysulfated glycosaminoglycans
TA-HY  Triamcinolone and hyaluronan
TNF  Tumor necrosis factor

Racchorses that have sustained impact injuries in high-motion joints can develop osteoarthritis as a result of repetitive supraphysiological loads at the level of the articular surface.\(^1,2\) Joint damage slowly progresses over time toward degenerative changes that involve the whole joint and lead to pain and dys-

function.\(^3,4\) The exact chronology of the pathophysiological changes occurring in the joints and leading to osteoarthritis in horses with naturally acquired disease has been broadly defined. Various stages of osteoarthritis are recognized, starting with clinically silent disease, followed by active or clinical onset of osteoarthritis, and finally a decompensated stage with overt pain.\(^1\) The interval between the times of the initial event and the clinical manifestation of osteoarthritis is generally accepted to be shorter in racehorses than in humans,\(^5\) suggesting that the strenuous activity of horses might accelerate lesion progression because of the high frequency of mechanical trauma. Racehorses can race for years before degenerative changes of the affected joint induce clinically relevant signs.\(^6\) Because the initial lesions and treatments are generally well characterized in racehorses and...
follow-up information is easily available, large-scale observational studies can be conducted to characterize the pathophysiologic, diagnostic, and therapeutic aspects of naturally acquired osteoarthritis.

Many medical treatments are available for the short-term management of osteoarthritis in horses. In only a few studies has the ability of these treatments to control the long-term progression of the disease been compared. Corticosteroid drugs, administered IA alone or in combination with hyaluronan, are the most commonly prescribed anti-inflammatory agents for the treatment of high-motion joint injuries and osteoarthritis in horses.7,8 A large body of evidence supports their short-term effects with respect to lameness improvement and inflammation control.9 In the long term, however, their effects are more controversial. Intra-articular corticosteroid administration has time- and dose-dependent effects on articular cartilage, with beneficial effects occurring at low doses and brief treatment durations and detrimental effects occurring at high doses and long treatment durations.10,11 Polysulfated glycosaminoglycans preserve cartilage integrity and proteoglycans content in the matrix, contributing to an appropriate balance between anabolism and catabolism in the articular tissues.12,13 A biological approach involving ACS as a source of IRAP is also possible, with significant clinical and histologic improvement observed in horses with experimentally induced osteoarthritis following ACS versus placebo treatment.14,15 The long-term effects in horses with naturally acquired osteoarthritis require further investigations, given that the half-life of IRAP in the joint compartment after IA administration appears to be brief.16 One must also acknowledge that different phenotypes of osteoarthritis exist (eg, inflammatory mediated, subchondral bone turnover driven, and trauma driven),17 and horses with osteoarthritis primarily characterized by subchondral bone injury are potentially poor responders to all types of joint medications.

Non-nitrogen bisphosphonates act as antiresorption drugs, reducing the ability of the osteoclasts to degrade bone matrix. Three studies have demonstrated a positive clinical effect of tiludronate in horses with navicular syndrome,18 bone spavin,19 and degenerative changes at the thoracolumbar vertebral facets,20 but no evidence exists to support tiludronate administration for the treatment of osteoarthritis in high-motion joints. The IA use of non-nitrogen bisphosphonates has been proposed, but the transient increase of proteoglycan degradation observed in horses has deemed this approach questionable.21 Important concerns have been raised about the increased use of bisphosphonates in young racehorses.22 In this population, subchondral bone is a common source of pain. Besides the lack of scientific data demonstrating a positive effect of bisphosphonates in racehorses, their effect on bone metabolism could reduce bone turnover23 and thus increase bone stiffness, increasing the risk of microcrack propagation in the presence of stress fractures.24-26 Lastly, the analgesic properties of bisphosphonates, mediated by a combination of osteoclast inhibition and osteoclast-independent mechanisms, raise ethical concern in the racehorse industry. For these reasons, the real benefit of bisphosphonates use in equine practice is debated, and treated racehorses should be exercised with caution when bone bruising or stress fractures are suspected.

The main hypothesis of the study reported here was that IV tiludronate disodium administration inhibits the progression of initial-stage osteoarthritis of the fetlock (metacarpophalangeal or metatarsophalangeal) joint in Standardbred racehorses in training. Our objective was to describe and compare the progression of naturally acquired osteoarthritis in racehorses treated with tiludronate versus TA-HY, PSGAG, or IRAP. Specifically, the clinical and radiographic presentation of the disease as well as biochemical markers of joint disease were assessed before and 6 months after treatment in a cohort of active Standardbred racehorses.

Materials and Methods

Study design

A retrospective observational study was conducted by use of data and samples previously collected (from November 2012 through July 2016) from an open cohort of 567 Standardbred racehorses at 5 training centers in northern Italy during clinical procedures and their systematic follow-up.6 Per standard procedure, the horses included in this cohort had clinical data recorded and underwent follow-up examinations before and 6 months and 1, 2, 3, 4, and 5 years after any exercise-related orthopedic injury sustained in their career or until retirement. To maximize the number of horses available, 6 months was chosen as the maximum follow-up period for this study. Radiographic and ultrasonographic examination as well as serum and synovial samples were obtained as part of follow-up. Clinical data were first reviewed, and the availability of the samples was then verified. The research protocol was approved by the Ethical Committee of the University of Turin (protocol No. 69 01/10/2020), and informed consent was obtained from all horse owners.

All horses included in the study had a diagnosis of initial-stage osteoarthritis at the fetlock joint, and they were treated with 1 of the following 4 treatments: TA-HY, tiludronate, PSGAG, and IRAP. Clinical and imaging data collected at baseline (at diagnosis, before treatment began) and 6 months after treatment was completed were assessed. Results for samples of synovial fluid and serum obtained at the same time points were used to quantify markers of inflammation as well as cartilage and subchondral bone metabolism.

Inclusion criteria

To be included in the study, horses were required to fulfill the following criteria: ≥ 4 years old at the mo-
ment of fetlock joint injury, in active training with the same driver at the moment of the injury and 6 months after treatment, diagnosis of initial-stage osteoarthritis of the fetlock joint at the time of examination for lameness, treatment with 1 of the 4 treatments of interest, and available serum and synovial samples (≥ 2 aliquots for each condition evaluated). The age limit was introduced because tiludronate is labeled for use in horses ≥ 4 years old.22

The diagnosis of initial stage of osteoarthritis at the fetlock joint was made if the horse had lameness that improved approximately 10 minutes after IA analgesia administration (5 mL of 2% mepivacaine solution) as well as mild or very mild radiographic and ultrasonographic findings for the affected joint as measured by use of a composite scoring system (Appendix). Horses were required to have mild to moderate synovitis, capsulitis, or both without obvious signs of cartilage and bone lesions and only focal or diffuse initial cartilage damage (fibrillation and wear lines) observed during arthroscopy, without any other lesions. Horses that underwent joint arthroscopy to remove an osteochondral fragment at the level of the dorsoproximal border of the proximal phalanx were still included in the study. Because the aim of the study was to capture changes in the initial stage of osteoarthritis, horses were excluded if they had developmental osteochondral disease, sesamoiditis, apical sesamoid fracture, notable osteophytes or enthesisophytes at the articular margins or capsular insertions, subchondral bone cysts, and major fractures of the proximal phalanx affecting the fetlock joint. Once horses were selected, details of the administered treatment and exercise program were reviewed to verify the homogeneity of the cohort.

Treatments

Horses that received tiludronate were first sedated with xylazine hydrochloride (0.1 mg/kg, IV). Tiludronate disodium (1 mg/kg) was diluted in 500 mL of saline (0.9% NaCl) solution and IV administered once over a 30-minute period. This treatment regimen was chosen on the basis of results in a previous clinical trial in which 2 regimens for tiludronate administration were compared in 73 horses, revealing the superior efficacy of IV administration.

For horses that underwent IA treatment, PSGAG (250 mg) were administered every 7 days for 4 treatments, TA-HY (10 mg of triamcinolone acetonide and 51 mg of hyaluronan) was administered once, and IRAP (1 aliquot) was administered every 7 days for 4 treatments. The IRAP was prepared from the ACS system, and the aliquots were frozen at –20°C until administration. All IA injections were aseptically performed through the lateral and palmaro(plantaro) proximal recess of the fetlock joint, with the distal portion of the limb maintained in a flexed position. A light bandage was applied to cover the injection site immediately after injection and maintained in place for 24 hours to reduce the risk of synovial contamination. Horses that received IRAP or PSGAG injections were rested in box stalls and hand walked during their entire treatment period (4 weeks).

Clinical and radiographic examination

Two investigators (AB and NBB), blinded to treatments that the horses received, evaluated 3 outcomes. Severity of lameness (range, 0 [no perceptible lameness] to 5 [most severe lameness]) per the lameness scoring system of the American Association of Equine Practitioners28 and response to digital flexion tests (0 = negative, 1 = very mild response, 2 = response consistently elicited, and 3 = markedly positive response) were scored by use of video recordings of lameness examinations. Archived radiographic images and ultrasound scans of the affected fetlock joint were also scored for severity of abnormalities. There were 11 categories of radiographic and ultrasonographic changes that were each graded from 0 to 3 to yield a total score (ie, radiographic score) ranging from 0 to 33 (Figure 1; Appendix). Final scores were determined by consensus between the 2 investigators.

During lameness examinations, each horse had been video recorded at trot in a straight line over a hard surface. The digital flexion test had been performed for 60 seconds. The horse had then been trotted in a straight line. Radiographic examination of the affected fetlock joint had been performed with a digital radiology system and a portable radiography unit (exposure parameters: 72 kV, 30 mA, and 0.004 seconds) and included 4 views: lateromedial, 15° dorsoproximal-palmaro(plantarlo)distal, 45° dorsomedial-palmaro(plantarlo)lateral, and 45° dorsolateral-palmaro(plantarlo)medial. Ultrasonographic examination had been performed with a portable unit and 10- to 13-MHz frontal linear array and included evaluation of transverse and longitudinal scans of the dorsal aspect of the joint and longitudinal scans of the medial and lateral aspects of the joint.

Biomarkers

Blood and synovial fluid samples had been obtained from horses after they had been withheld from exercise for 24 hours. Blood was obtained by jugular venipuncture into 10-mL tubes containing EDTA and placed on ice. Synovial fluid was obtained by arthrocentesis of the fetlock joint. The first sample was obtained when the initial diagnostic analgesia was performed. To this aim, the skin was clipped of hair and aseptically prepared, with the horse unsedated. The fetlock joint was slightly flexed, and the sample was obtained with 19-gauge, 4-cm-long needles and the lateral suspensory branch as a landmark. Approximately 6 mL of synovial fluid was withdrawn from the palmar(plantar)-proximal synovial recess into EDTA-containing tubes and placed on ice. Within 1 hour after collection, synovial fluid samples were centrifuged at 1,600 X g and 4 °C for 20 minutes to remove cells and debris. Samples were then mixed with 1mM...
phenylmethylsulfonylfluoride, an inhibitor of serine proteases. Blood samples were centrifuged at 3,500 X g and 4 °C for 12 minutes. Aliquots (0.4 mL) of serum and synovial fluid were collected in plain tubes and frozen at –80°C for subsequent analysis. After thawing, synovial fluid samples were treated with hyaluronidase (20 U/mL) for 30 minutes at 37 °C to reduce viscosity and diluted 1:2 with 0.1375% Tween buffer solution. Sample concentrations of the following biomarkers were measured by use of commercially available, validated ELISA kits: CTX-II (serum and synovial fluid), IL-1β (synovial fluid), TNF-α (serum), PGE2 (synovial fluid), and CTX-I (serum). The ELISA results (absorbance) were measured at 450 nm with a microplate reader. Each sample was analyzed in duplicate. Positive, negative, and blank control substances were included on each plate. The intra- and interassay coefficients of variability were calculated for each series of assays.

**Exercise program**

On the day of treatment, or for the entire duration of treatment, all horses were rested in box stalls. They progressively resumed exercise as prescribed, starting with hand walking for 5 to 10 min/d. As a general rule, horses treated with tiludronate were hand walked for 8 weeks, those treated with PSGAG and IRAP for 5 weeks, and those treated with TA-HY for 2 weeks. Light trotting was then introduced, starting with 5 to 10 min/d, followed by 5- to 10-minute increases every week to a maximum of 50 min/d. This regimen was maintained for an additional 3 to 4 weeks before exercise was resumed (5 d/wk of light trotting and 2 d/wk of strenuous exercise training).
Statistical analysis

The target sample size for the study (25 horses/treatment group) was determined a priori on the basis of previously reported values for biomarker concentrations. Statistical analyses were performed with statistical software. Repeated-measures 2-way ANOVA with the Bonferroni post hoc test was used to assess the effects of time and treatment and the interaction between these 2 variables on the 3 outcomes of interest. Continuous data are summarized as mean ± SD and discrete data as median. Values of P < 0.05 were considered significant.

Results

Animals

A total of 100 Standardbred racehorses (48 females, 39 sexually intact males, and 13 geldings) with a median age of 5 years were included in the study. Osteoarthritis had been diagnosed in the left hind limb (n = 26), right hind limb (26), right forelimb (25), and left forelimb (23). Signalment and clinical characteristics of horses within each treatment group were summarized (Table 1).

Clinical and radiographic examination

At baseline (ie, before treatment began), all horses had grade 2 or 3 lameness (median score, 2/5). At 6 months after treatment was completed, lameness ranged in severity from grade 0 to 2 (median score, 0/5). Scores for the digital flexion test response at baseline ranged from 1 to 3 (median score, 2/3), whereas at 6 months, they ranged from 0 to 2 (median score, 1). A significant (P < 0.001) effect of time was detected for both outcome variables in all treatment groups. Results of post hoc comparisons further confirmed that all treatments were associated with a significant decrease in the lameness score and flexion test response after 6 months (Figure 2).
At baseline, the median value for radiographic scores was 8 of 33 in all 4 treatment groups. At 6 months, it was significantly \((P < 0.001)\) higher than the baseline value for the PSGAG, TA-HY, and IRAP groups but not significantly \((P = 0.40)\) higher for the tiludronate group. Significant effects of time \((P < 0.001)\), treatment \((P = 0.001)\), and their interaction \((P < 0.001)\) were detected. At 6 months, horses in the tiludronate group had significantly \((P < 0.001)\) lower radiographic scores than did those in the TA-HY, PSGAG, and IRAP groups.

**Synovial biomarkers**

At baseline, no significant differences in mean synovial fluid IL-1\(\beta\) concentration were identified among the 4 treatment groups. The interaction between treatment and time for this variable was significant \((P < 0.001)\). The TA-HY group was the only one in which mean synovial fluid IL-1\(\beta\) concentration was significantly \((P < 0.001)\) lower at 6 months versus baseline. Contrarily, in the PSGAG group, a significant \((P = 0.008)\) increase was observed in the analyte over the same period. At 6 months, mean synovial fluid IL-1\(\beta\) concentration was significantly \((P = 0.003)\) higher in the PSGAG group than in the TA-HY group (Figure 3).

At baseline, mean synovial fluid CTX-II concentrations was higher in the TA-HY group than in the tiludronate or IRAP group \((P = 0.003\) for both comparisons). Values for this analyte differed significantly by time \((P < 0.001)\) and treatment \((P = 0.002)\). Mean CTX-II concentration at 6 months was significantly \((P < 0.001)\) higher than at baseline in the tiludronate, PSGAG, and IRAP groups. At 6 months, horses in the tiludronate group had a significantly \((P = 0.04)\) lower mean synovial fluid CTX-II concentration than did those in the TA-HY group (Figure 3).

Mean synovial fluid PGE2 concentration was significantly higher in the TA-HY group than in the tiludronate group \((P < 0.001)\), PSGAG group \((P = 0.01)\), and IRAP group \((P = 0.005)\) at baseline. Values were also influenced by treatment \((P = 0.02)\) and the interaction between time and treatment \((P < 0.001)\). Compared with baseline values, mean PGE2 concentration at 6 months was significantly \((P < 0.001)\) higher for horses in the PSGAG group and significantly \((P < 0.001)\) lower for horses in the TA-HY group. At 6 months, horses in the PSGAG group had higher PGE2 values than did horses in the tiludronate group \((P = 0.002)\) and TA-HY group \((P < 0.001);\) Figure 3).

**Serum biomarkers**

Mean serum TNF-\(\alpha\) and CTX-II concentrations were higher in the TA-HY group than in the tiludronate group at baseline \((P = 0.002\) and \(P = 0.001\), respectively). Values for both proteins were significantly influenced by time \((P < 0.001\) for both) and treatment \((P < 0.001\) and \(P = 0.02\), respectively). A significant \((P = 0.04)\) increase in serum TNF-\(\alpha\) concentration following treatment was observed only in the PSGAG group. Mean serum CTX-II concentration was significantly higher at 6 months versus baseline in the tiludronate group \((P < 0.001)\), PSGAG group \((P < 0.001)\), and IRAP group \((P = 0.04);\) Figure 4). At 6 months, mean serum TNF-\(\alpha\) concentration was lower in the tiludronate group than in the PSGAG group \((P = 0.01)\) and TA-HY group \((P < 0.001)\). Mean serum CTX-I concentration was similar among all treatment groups at baseline. A significant effect on CTX-I values was identified for time \((P = 0.02),\)
treatment ($P < 0.001$), and their interaction ($P < 0.001$). Mean serum CTX-I concentration decreased significantly ($P < 0.001$) with time in the tiludronate group, whereas values in the PSGAG and TA-HY groups increased significantly ($P = 0.003$ and $P = 0.008$, respectively). At 6 months, mean serum CTX-I concentration was significantly ($P < 0.001$) lower in the tiludronate group than in the PSGAG, TA-HY, and IRAP groups (Figure 3).

Discussion

A primary, but still unmet, clinical need in equine medicine is the identification of treatments able to inhibit the molecular mechanisms that cause joint damage in horses. In the present study, the long-term (6 months) effects of non-nitrogen bisphosphonates (tiludronate) versus 3 other medical treatments for osteoarthritis of the fetlock joint (TA-HY, IRAP, and PSGAG) were compared in a uniform cohort of Standardbred racehorses in training. The main finding was that, although all 4 treatments were followed by improvements in lameness score and digital flexion test response, only tiludronate appeared to effectively inhibit the radiographic progression of osteoarthritis over the study period. On the other hand, synovial biomarkers of joint inflammation such as PGE2 and IL-1$β$ concentration significantly decreased only for horses receiving an IA injection of TA-HY. None of the 4 treatments were followed by decreases in serum or synovial fluid CTX-II concentrations, suggesting they could not effectively inhibit the cartilage damage that occurred in affected joints. A marker of bone metabolism, CTX-I appeared to be effectively inhibited only by tiludronate.

Tiludronate was approved by the US FDA on the basis of results of 1 clinical study$^{18}$ and is currently used for the treatment of pain related to degenerative conditions of the tarsus (hock), navicular bone, and intervertebral joints in horses.$^{18–20}$ A therapeutic role for tiludronate in osteoarthritis of high-motion joints has not been demonstrated, although recent reports$^{22,36}$ suggest that tiludronate has been increasingly used by veterinarians to treat bone pain in young horses. The use of bisphosphonates in racehorses is controversial given that its positive effects on bone metabolism and inflammation may be counterbalanced by its analgesic effect.$^{22}$ Subchondral bone pain due to palmar or plantar osteochondral disease is likely to be highly prevalent and underdiagnosed among Standardbred racehorses in training.$^{37}$ Structural changes associated with this condition are typically undetectable by conventional imaging modalities, requiring the use of MRI or CT, neither of which was routinely used for horses in the present study owing to financial constraints. Our observations suggested that tiludronate may be a good candidate for the treatment of early-stage osteoarthritis. The favorable radiographic response observed following tiludronate administration might have reflected a high prevalence of subchondral bone pain in the initial stages of osteoarthritis among the included horses. More-specific anti-inflammatory treatments such as the corticosteroid component of TA-HY are likely to be less efficacious for treatment of bone pain than they are for treatment of osteoarthritis-associated, inflammation-based processes such as capsulitis. Unfortunately, challenges exist in differentiating the primary source of pain (eg, subchondral bone vs soft tissues of the joint) in animals. The effects of medical treatments on clinical signs of osteoarthritis are likely to be influenced by the source of joint pain, and this variable should be better investigated in future studies.
Tiludronate acts mainly as an antiresorption drug, reducing the ability of the osteoclasts to degrade bone matrix, although anti-inflammatory and analgesic properties mediated by other mechanisms are also recognized.\textsuperscript{38,39} Bisphosphonates alleviate bone pain in dogs with appendicular osteosarcoma,\textsuperscript{39} an extremely painful condition. Such an effect is believed to be related to the ability of this drug to reduce the acidic environment at the ruffled border of the osteoclasts, inhibiting activation of free nerve endings. The mechanisms by which tiludronate inhibited the radiographic progression of osteoarthritis in the horses of the present study remain to be elucidated. The inhibition of bone remodelling could have resulted from a direct effect on bone osteoclasts, indirect anti-inflammatory-mediated effects on several cell types, or both. Horses receiving tiludronate had fairly constant synovial ILI-\(\beta\) and PGE-2 concentrations and serum TNF-\(\alpha\) concentrations in our study, which did not support a prolonged anti-inflammatory action of this drug. However, other markers of inflammation might have decreased in concentration. We previously found a correlation between serum TNF-\(\alpha\) concentration and the severity of osteophytes and enthesiophytes in Standardbred racehorses with osteoarthritis.\textsuperscript{6} Also, data for swine with experimentally induced osteoarthritis indicate that synovial fluid TNF-\(\alpha\) concentration can predict the gross morphological score for injured joints better than joint instability can.\textsuperscript{40} Although serum TNF-\(\alpha\) concentration did not increase in horses that received tiludronate in our study, baseline values were lower in this group than in the other groups, which might have influenced our results. On the other hand, tiludronate-treated horses had lower serum CTX-I concentrations at 6 months relative to baseline, suggesting that bone-remodelling processes were still inhibited at that time point. The potential long-term adverse effects of IV tiludronate administration on skeletal tissue due to osteoclast shutdown in young racehorses are under discussion. Although not investigated in the present study, the safety profile of tiludronate needs to be established before its use in young racehorses can be considered safe.

The radiographic scoring systems adopted in equine clinical and experimental studies\textsuperscript{41-45} are mainly based on the number and size of osteophytes and periarticular enthesiophytes at the capsular insertion of the joint, as an index of local inflammation. This approach is based on experiments involving mice, in which increased expression of pro-inflammatory cytokine mRNA was identified in osteoblasts isolated from periarticular osteoblasts as the osteoarthritis worsened.\textsuperscript{44} In the present study, horses treated with tiludronate had stable radiographic scores, whereas horses treated with PSGAG, IRAP, or TA-HY had higher imaging scores at 6 months, suggesting poor control of the inflammatory process at the level of periarticular tissues. A few studies have yielded data concerning the temporal progression of osteoarthritis in horses with naturally acquired disease. In horses with experimentally induced cartilage surface damage, radiographic changes become evident 10 to 16 weeks after lesion induction.\textsuperscript{41-43} In our previous study involving Standardbred racehorses in training that sustained exercise-induced injuries, a significant increase in radiographic score was detectable 2 years after the initial diagnosis of osteoarthritis. That finding appears to contradict the present findings. However, horses included in the present study had a worse radiographic score at baseline (median, 8/33 vs 5/33 in the previous study\textsuperscript{46}), similar to the median score observed 1 year after enrollment in the previous study (median 7/33). Because of the systematic approach used to identify horses for inclusion in the present study, we believe this difference might have been attributable to the age of the horses (\(\geq\) 4 years vs \(\geq 2\) years in the previous study).

Triamcinolone has recognized anti-inflammatory effects in joints. However, repeated administration of this corticosteroid drug causes chondrocyte apoptosis in the hyaline cartilage, affecting joint metabolism for a prolonged period.\textsuperscript{10} This raises doubts about the potential adverse effects and long-term safety of triamcinolone, the most common drug administered IA for joint treatment.\textsuperscript{8} Chronic and subacute inflammation due to repetitive joint injury are detrimental in the progression of osteoarthritis and associated with the expression of proinflammatory genes in the hyaline cartilage and noncartilaginous soft tissue.\textsuperscript{45} In the study reported here, only horses that received TA-HY had a reduction in the inflammatory cascade within synovial fluid at 6 months after treatment was completed. This decrease was associated with chondroprotective effects, given that the TA-HY group was the only one in which the synovial fluid CTX-II concentration did not increase significantly, suggesting this treatment did not affect the homeostasis of the extracellular matrix in the hyaline cartilage. Synovial fluid CTX-II concentration is considered a valuable biomarker of cartilage catabolism,\textsuperscript{46} and its usefulness in horses has been validated for monitoring joint disease progression and treatment response.\textsuperscript{47} In agreement with previous findings,\textsuperscript{48} our data confirmed that early intervention with TA-HY can prevent cartilage deterioration in horses with osteoarthritis. However, and in contrast to the beneficial effects on joint cartilage, TA-HY was associated with an increased radiographic score relative to baseline at 6 months after treatment, with values greater than those observed in horses treated with tiludronate and IRAP.

It must be acknowledged that horses treated with TA-HY in the present study had the highest prevalence of arthroscopic surgery because of concurrent osteochondral fragments in the joint, attributable to trauma-driven osteoarthritis. This group had also the highest synovial fluid PGE-2 and serum TNF-\(\alpha\) concentrations at baseline. These observations call into question the weight that we attributed to the presence of osteochondral fragments when assigning scores, suggesting these scores might underestimate the pathological relevance of this type of lesion, thereby introducing selection bias. The TA-HY was
likely systematically administered to horses with the most severe osteoarthritis, resulting in magnification of its anti-inflammatory effect.

In the study reported here, PSGAG and IRAP had significant effect on the clinical manifestation of osteoarthritis but were unable to control radiographic progression and the increase in synovial fluid CTX-II concentration at 6 months. Polysulfated glycosaminoglycans act by inhibiting the degradation of the collagen matrix in the hyaline cartilage. On the other hand, IRAP reduces joint inflammation by selective blockage of IL-1 receptors. Intra-articular treatment with IRAP is recommended to control the acute arthritic inflammatory response, reducing hyaluronic acid breakdown in synovial fluid. However, no information is available regarding its efficacy in the long term. To this aim, a gene therapy approach, ensuring a prolonged production of IRAP in the joint compartment, may be ideal.

The limitations of the present study were mainly attributable to its retrospective and clinical nature as well as to the insidious onset and slowly progressive nature of osteoarthritis. Treatments could not be randomly assigned, and horses in the TA-HY group may have had a worse inflammatory profile at baseline than horses in the other groups, despite a similar clinical presentation. We also acknowledge the lack of a control group of untreated horses, which was due to the unwillingness of racehorse owners to leave a clinical problem untreated. For this reason, we opted to use each horse as its own control, comparing its data before and after treatment. Lastly, in contrast to research involving horses with experimentally induced osteoarthritis, we were unable to determine the precise starting point of osteoarthritis development in the included racehorses, thereby likely introducing intersubject variability and reduced statistical power.

In conclusion, findings of the present study indicated a superior effect of tiludronate, compared with other drugs commonly used to treat racehorses with naturally acquired osteoarthritis in terms of radiographic progression of the disease at 6 months after treatment. This effect was associated with a long-term impact on bone metabolism, as indicated by a decrease in serum CTX-I concentration, a marker of bone remodelling. However, IV tiludronate administration had no effects on the synovial inflammatory response and was associated with an increase in serum and synovial fluid CTX-II concentrations, a marker of cartilage damage, after 6 months. In light of these findings, prospective studies are needed to ascertain whether the radiographic advantages associated with the use of tiludronate in racehorses counterbalance the observed adverse effects on joint cartilage homeostasis.

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The authors declare that there were no conflicts of interest.

Footnotes

a. Mepivacaine 2%, Galenicca Senese, Monteroni d’Arbia, Italy.
b. Megaxilor 20% (20 mL), Bio 98 Srl, Milan, Italy.
c. Tildren (50 mg), Ceva Vetem SpA, Agrate Brianza, Italy.
d. Adequan (500 mg/5 mL), American Regent Animal Health, Shirley, NY.
e. Kenacort (40 mg/mL), Bristol-Myers Squibb, New York, NY.
f. Hy50, Dechra Veterinary Products LLC, Leawood, Kan.
g. IRAP ProEAS System, Arthrex GmbH, Munchen, Del.
h. Foschi Digital Radiology, Rome, Italy.
i. Gierth X-Ray International GmbH, Riesa, Germany.
j. GE Healthcare, Waukesha, Wis.
k. Hyalurondase from bovine testes (Sigma H3884), Sigma-Aldrich Corp, St Louis, Mo.
l. Sanquin Reagents, Amsterdam, Netherlands.
m. Serum Pre-Clinical Cartilaps (CTX-II) ELISA (code AC-08 F1), IDS, Boldon, England.

References

Equine 2009;41:1149–1160.

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Appendix

Scoring system used for assessment of the severity of fetlock joint osteoarthritis in horses on the basis of radiographic (Rad) views and ultrasound (US) scans.

<table>
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<tr>
<th>Characteristics</th>
<th>Required images</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Joint space narrowing</td>
<td>Rad: DP, DMPL, and DLPM</td>
<td>Normal (2-mm thickness)</td>
</tr>
<tr>
<td>No. of osteophytes</td>
<td>Rad: DMPL and DLPM US: Lmed and Lis</td>
<td>None</td>
</tr>
<tr>
<td>Size of osteophytes</td>
<td>Rad: DMPL and DLPM US: Lmed and Lis</td>
<td>None</td>
</tr>
<tr>
<td>No. of enthesiophytes</td>
<td>Rad: LM US: Ldors</td>
<td>None</td>
</tr>
<tr>
<td>Size of enthesiophytes</td>
<td>Rad: LM US: Ldors</td>
<td>None</td>
</tr>
<tr>
<td>Subchondral bone changes at the palmar or plantar aspect of the metacarpal or metatarsal condyles</td>
<td>Rad: LM</td>
<td>None</td>
</tr>
<tr>
<td>No. of osteochondral fragments</td>
<td>Rad: LM US: Ldors</td>
<td>None</td>
</tr>
<tr>
<td>Size of osteochondral fragments</td>
<td>Rad: LM US: Ldors</td>
<td>None</td>
</tr>
<tr>
<td>Thickening of the soft tissues in the dorsal aspect of the joint*</td>
<td>US: Tdors</td>
<td>None</td>
</tr>
<tr>
<td>Supracondylar bone resorption</td>
<td>Rad: LM</td>
<td>None</td>
</tr>
<tr>
<td>Proximal sagittal crest osteolysis</td>
<td>Rad: LM US: Ldors</td>
<td>None</td>
</tr>
</tbody>
</table>

*Refers to the capsular thickness only, compared with thickness in the contralateral limb.

Each of these 11 characteristics was scored on a scale of 0 (normal) to 3 (pathological). Scores were then summed to yield a total score (ie, radiographic score) with a possible range of 0 to 33.

DLPM = 45° dorsolateral-palmaro(plantaro)medial. DMPL = 45° dorsomedial-palmaro(plantaro) lateral. DP = 15° dorsoproximal-palmaro(plantaro)distal. Ldors = Longitudinal scan of the dorsal aspect of the joint. Lis = Longitudinal scan of the lateral aspect of the joint. LM = Lateromedial. Lmed = Longitudinal scan of the medial aspect of the joint. Tdors = Transverse scan of the dorsal aspect of the joint.