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Original Research

Levels of Cytokines and Matrix Metalloproteinases 2 and 9 in the Synovial Fluid of Osteoarthritic Horses Treated With Pamidronate

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ABSTRACT

The aim of this study was to evaluate the effect of pamidronate on the clinical score and the secretory profile of inflammatory biomarkers (interleukin [IL]-6, tumor necrosis factor alpha [TNF-α], matrix metalloproteinase [MMP]-2, and MMP-9) in the synovial fluid in clinically healthy horses and in horses with joint disease. Healthy horses and horses with joint symptoms were examined and subjected to a standardized clinical evaluation of the locomotor system. The clinical condition was evaluated by a global score. Matrix metalloproteinases 2 and 9 were measured by gel zymography. The concentration of cytokines (IL-6 and TNF-α) in synovial fluid was determined by enzyme linked immunosorbent assay (ELISA). Pamidronate treatment significantly improved the clinical condition of horses with osteoarthritis (OA). Values of IL-6 (pg/mL) were similar (ns) in the healthy control group (102.2 ± 26.94) and at day 3 of treated (TD) group (113.9 ± 18.33). Tumor necrosis factor alpha level, at day 3 of treatment, was significantly lower in TD groups than in untreated osteoarthritis. Treated group registered a fast increase in MMP-9 activity but till days 21 and 60 it was not detectable. No significant differences were found in the MMP-2 activity between groups. We concluded that treatment with pamidronate has a beneficial effect on the clinical score of horses with OA and can reduce proinflammatory cytokines (IL-6 and TNF-α) and MMP-9 at different stages after treatment.

1. Introduction

Osteoarthritis (OA) is an inflammatory disease that evolves to painful and degenerative joint damage. Osteoarthritis usually occurs as a result of physical overtraining, dietary imbalances, or growth disorders—including osteochondrosis—during development [1,2]. Osteoarthritis is one of the major causes of economic loss in sport horses because it results in either temporary or permanent, premature retirement from sports competitions and racing [3]. The aforementioned risk factors lead to the occurrence of repeated and incompletely resolved articular microtrauma that maintain and increase the severity of the inflammatory process. Furthermore, an abnormal maturation of the cartilage might result in the formation of cartilage flaps and intraarticular bone fragments that can predispose to articular damage [2]. Osteoarthritis is characterized by joint damage that affects the articular cartilage, the adjacent

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subchondral bone, and the synovial membrane. Pain is another important factor associated with the inflammatory process leading to progressive loss of joint function and turning into a performance-limiting factor.

During the onset of OA, the changes in the properties of normal cartilage impact on subchondral bone, causing an increase in the resorptive activity of osteoclasts and bone turnover [4]. In addition, the production of anabolic factors by chondrocytes decreases, together with an increase in the release of proteases and other catabolic factors involved in joint and bone damage [5]. Vascular changes and upregulation of inflammatory cytokines and nitric oxide production in the subchondral region and synovial fluid are also observed in OA [6,7].

During the initial phase of OA, macrophages and neutrophils participate in the cascade of inflammatory response by secreting proinflammatory cytokines, tumor necrosis factor alpha (TNF-α), and interleukin (IL)-6 [8–11]. In turn, the inflammatory cytokines induce the release of matrix metalloproteinases (MMPs) [4,12], which are known to be involved in articular cartilage degradation [13].

Tumor necrosis factor alpha is a key cytokine in the inflammatory process and is known to increase vascular permeability by inducing the endothelium to express adhesion molecules and cellular migration factors that promote leukocyte diapedesis [14]. Moreover, IL-6 is involved in the degradation of proteoglycans in the articular cartilage [15].

Although MMPs are zinc-dependent endopeptidases involved in tissue repair, they are also associated with the development of arthritis in humans [16] and in horses [17]. Matrix metalloproteinases 2, 3, 9, and 13 are especially involved in cartilage matrix degradation [18].

Many different pharmaceutical products have been used to treat OA with the aim of reducing associated inflammation and pain; however, these drugs have adverse effects on the extracellular matrix and bone metabolism. For example, corticosteroids are widely used in the management of equine OA for their highly effective anti-inflammatory properties but are associated with enhancement of cartilage proteolysis, subchondral bone resorption, microfractures, and direct inhibition of osteoblast function. The aim of this article was to evaluate the effects of pamidronate in horses with OA. Although tiludronate was the first bisphosphonate licensed to treat navicular disease in horses [19], pamidronate, an aminobisphosphonate, is widely used in human medicine for the treatment of metabolic bone disease. Information on the anti-inflammatory effects of these drugs in equines is scarce [20].

Pamidronate belongs to the family of aminobisphosphonates and is extensively used for the palliative treatment of cancer metastasis due to its osteoclast inhibitory effect [21–24]. Aminobisphosphonates have a more potent antiresorptive effect compared with other bisphosphonates. Pamidronate has been used in the treatment of osteolysis associated with Paget's disease [25] and is known to have analgesic effects in patients suffering from cancer and other related disorders. In addition, bisphosphonates can inhibit apoptosis of osteocytes and osteoblasts and induce the proliferation of osteoblasts [20,26,27]. Another mechanism by which pamidronate inhibits bone resorption is by stimulating osteoblast inhibitory activity on osteoclasts [28]. Pamidronate acts by inhibiting the mevalonate pathway [29]. Because of its analgesic effect and its antiresorptive capacity, pamidronate could be an alternative treatment for OA [30].

Bisphosphonates might have anti-inflammatory properties, particularly during the onset of arthritis [31]. The aim of this study was to evaluate the effect of pamidronate on the clinical score and the secretory profile of inflammatory biomarkers (IL-6, TNF-α, MMP-2, and MMP-9) in the synovial fluid in clinically healthy horses and in horses with joint disease.

2. Materials and Methods

2.1. Animals, Synovial Samples, and Experimental Design

At the beginning of the study, healthy horses and horses with joint symptoms were examined and subjected to a standardized clinical evaluation of the locomotor system. The clinical condition was evaluated by a global score comprising six items: (1) lameness degree (0–5), (2) tenderness (0–3), (3) presence of pain under forced flexion (0–3), (4) volume of synovial fluid extracted (0–3), (5) color of synovial fluid (0–5), and (6) synovial fluid viscosity (0–4). The score was based on the American Association of Equine Practitioners lameness score [32] with the addition of a wide evaluation methodology focused in the tibiotarsal joint. This joint is an important site of OA prevalence in jumping horses [33,34].

The following groups were considered: (1) control group ($n = 8$) clinically healthy young animals (2–4 years). Young animals were chosen to be certain that since birth they had no history of joint disease or disorders of bone metabolism. The clinical score of this group was $\leq$4. (2) Animals with joint disease that were treated (TD) ($n = 8$, with clinical score $>$5 at the beginning of treatment, aged between 4–8 years). This group was evaluated and analyzed at different stages: baseline or pretreatment group (TD0), day 3 (TD3), day 10 (TD10), day 21 (TD21), and day 60 (TD60). Animals included in TD group were animals with clinical signs and clinical history of chronic recurrent episodes of active OA with poor outcome to conventional treatment modalities.

Radiographic analysis of both tibiotarsal joint was performed in all animals included in the experiment, control, and TD groups. TD group had radiographics alterations of articular cartilage in the joints and subchondral bone resorption.

On days 0 and 9, horses with joint disease were treated with 90 mg of IV disodium pamidronate (Aminomux 90 mg, Gador). There is no detailed information of pamidronate dosage and posology for horses in the bibliography; however, divided doses was recommended. In this article, we used low doses of pamidronate (0.4–0.8 mg/kg intravenously) administered in two doses 9 days apart [20,35]. Synovial fluid samples were obtained on days 0, 3, 10, 21, and 60 by sterile aspiration from the tibiotarsal joint. Samples were centrifuged at 2,000*g* for 10 minutes, and the supernatant was kept at −70°C. The final clinical score in the pamidronate-treated group was considered on day 60.