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Original Research

Autologous Platelet Concentrate as a Treatment for Horses with Refractory Fetlock Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is one of the most common musculoskeletal pathologies in horses and is a disease capable of halting their athletic career. Clinically, OA can be a difficult problem to deal with, particularly when there is no longer a positive response to corticosteroids or rest. In order to avoid further articular tissue degeneration, which could lead to the loss of the joint function, novel therapies are focusing not only on controlling inflammation and pain but also on tissue healing and repair. Autologous platelet concentrate (PC) growth factors are known to have anabolic and angiogenic properties and a positive effect on synovial epithelium, cartilage, and pain. The aim of this study was to analyze the clinical outcome of horses with OA treated with PC. Selected cases were treated only with PC, and they all belong to the same sport activity. We show here the method for PC preparation, follow-up evaluation, and outcome of 20 cases with refractory OA treated exclusively with autologous PC. After a 1-year follow-up, 80% of patients were able to resume work. According to our results and observations, PC can be used as a safe and low-cost intra-articular therapy for refractory OA in the horse.

1. Introduction

Chronic osteoarthritis (OA) is the most prevalent cause of diminished performance and halts athletic activity in racing horses [1]. This degenerative disease is characterized by progressive cartilage degeneration, subchondral bone remodeling, synovitis, osteophyte formation, and eventually loss of function [2,3]. Proinflammatory cytokines are induced during OA, particularly interleukin-1beta (IL-1beta), tumor necrosis factor-α, and matrix metalloproteinase, and these cytokines disrupt the anabolic/catabolic balance to the detriment of growth factors such as insulin-like growth factor-1, transforming growth factor beta (TGF-beta), and basic fibroblast growth factor, provoking a chronic degenerative process of the subchondral bone, cartilage, and/or synovial membrane [2,4,5]. Therefore, applying a successful treatment can be a real challenge, particularly in the long term.

Several treatments can be adapted and applied according to the seriousness of the pathology, with intra-articular corticosteroids being the main therapeutic choice [6-8]. However, repetitive administration of corticosteroids leads to negative secondary effects such as cartilage necrosis and further degeneration [4,8]. Consequently, other approaches have been developed mainly to avoid repetitive or abusive use of corticosteroid and to allow the tissue to heal. Autologous platelet concentrate (PC), seen as a growth factor source, has been reported as an alternative treatment [9,10] with even better results than corticosteroids at 1 year after injection [11].

We describe here the outcome of 20 cases of fetlock OA treated with autologous PC and the treatment protocol used. According to synovial fluid analysis, lameness

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evaluation, and owners' perceptions, PC can be used as a successful and safe treatment for OA that no longer responds to intra-articular corticosteroids or rest.

2. Materials and Methods

2.1. PC Preparation

PC was prepared as reported previously [9,12] with some adaptations. After aseptic preparation of the jugular vein, using a butterfly catheter, 80 mL of blood was drawn into sodium citrate (0.129 mol/L) tubes (Vacutainer system; BD). Blood was immediately centrifuged (Eppendorf 5810R) at 120 × *g* for 5 minutes. Then, the 50% of plasma adjacent to the red cell clot was recovered and centrifuged a second time at 260 × *g* for 5 minutes. Subsequently, 25% of plasma, at the bottom of the tube, was recovered, and a final centrifugation at 1000 × *g* for 10 minutes was performed to concentrate platelets and resuspend them in 3 mL of phosphate-buffered saline plus 23 mM $CaCl_2$. The remaining platelet-poor plasma (PPP) was frozen at −80°C for control analysis, in order to compare growth factor levels with those of PC. Platelets were counted using an automated cell counter (Countess model; Invitrogen); PC quality was assessed with a FACSCalibur flow cytometer (Beckton Dickinson) in terms of whole white blood cell and platelets counts. Platelets were activated using $CaCl_2$ (Cooper) at a final concentration of 23 mM for 1 hour prior to the injection [13].

2.2. Treatment Protocol

Before injection, aseptic preparation of the joint with chlorhexidine soap and solution was performed. Following this, without sedation of the animal, intra-articular injection of 3 mL of autologous PC was done using an 18-gauge needle. No bandages were applied after arthrocentesis, only swab compression was applied for 5 minutes. Three injections were performed with a 15-day interval. During this period, horses were hand walked twice a day without being ridden. Two weeks after the last injection, full work was resumed progressively. At the time of arthrocentesis, a synovial fluid sample was recovered, divided into aliquots, and immediately frozen and stocked at −80°C for further analysis. No adverse effects were notice during or after PC injections.

2.3. Analysis of Platelet-derived Growth Factor Isoform BB in PC and IL-1 in Synovial Fluid

Platelet-derived growth factor isoform BB (PDGF-BB) concentration was assessed in PC before being injected, using sandwich enzyme-linked immunosorbent assay (ELISA; human PDGF-BB DY220; R&D Systems) as reported previously [14,15]; PPP was used as control. PDGF-BB is known to have 91.7% homology with the amino acid sequence for human long-form peptide [16]. Synovial fluid analyzed for IL-1beta was done using sandwich ELISA (equine IL-1beta; product no. VS0131E-002; VetSet; Kingfisher Biotech). A plate reader (Tecan) was used at 450 nm for absorbance measurement.

2.4. Statistical Analysis

PDGF-BB concentration in platelet-poor plasma was compared with the concentration in PC. Statistical significance was assessed using the Man-Whitney two-sided test. IL-1beta concentrations were compared among samples from the first to the third PC injections; statistical significance was assessed using the analysis of variance (ANOVA) test doubled with a Brown-Forsythe test. Data from the modified Western Ontario and McMaster Universities Arthritis Index (WOMAC) index and degree of lameness evaluation were analyzed along with PC treatment. Statistical significance was assessed using the two-way ANOVA test doubled with a Tukey test. Confidence interval was calculated at a 95% level. Additionally, we wished to test the relationship between the owners' perceptions and clinicians assessment of lameness evolution and joint status, so a correlation test of these two parameters was done (Supplemental Table 2). Analysis was done with Graph Pad Prism version 6 software.

2.5. Case Selection

Between 2009 and 2012, 20 endurance horses, Arab or English-Arab breed, were entered in this study. Horses were a mean age of 9.5 ± 1.67 years, and there were 15 geldings and 5 females (further details in Supplemental Table 2). Apart from belonging to the same sport activity, inclusion criteria consisted of horses that presented chronic lameness related to chronic front fetlock OA. All horses had received conventional treatments but without resolution of the problem, particularly intra-articular corticosteroids. In this regard, the last corticoid intra-articular injection was performed at least 6 weeks before PC treatment. Additionally, cases that were treated with other medications together with PC, cases with less than 1-year follow-up, and cases where synovial fluid was not recovered for analysis were excluded from this study.

Chronic OA diagnosis was obtained according to the clinical history of the case and the lameness examination, which included chronic lameness, positive flexion test result, and joint distention. All cases selected presented with a significant improvement in degree of lameness after intra-articular anesthesia, and none of them presented with secondary lesions such as desmitis of a collateral ligament, for example. Diagnosis was completed with radiographic and ultrasonographic examinations, where osteophytes, irregular articular surfaces, and/or synovitis was observed.

3. Results

3.1. Synovial Fluid Quality Improvement in PC-injected Joints

Of the 20 cases in this study, 7 were affected in the right front fetlock and 13 on the left. The PC contained a mean of 560 × 10^3 platelets/μL ± 62 × 10^3 platelets /μL, with a majority of platelets and very few leukocytes (Fig. 1). PDGF-BB analysis of PC revealed a mean concentration of 1,280 ± 70.91 pg/mL (Fig. 1). This growth factor represents a major serum mitogen, and it promotes cell migration and proliferation, key events in tissue repair [17]. Analysis of