ORTHOPAEDIC RESEARCH CENTER

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Orthopaedic Bioengineering Laboratory

Colorado State University

Evaluation of Autologous Conditioned Serum (ACS) Using an Experimental Model of Equine Osteoarthritis

Take Home Message
Autologous conditioned serum (ACS) significantly improved clinical lameness as well as histologic parameters in the synovial membranes of horses with experimentally induced osteoarthritis. There were no significant side effects noted throughout the study. This is the first controlled study which provides positive evidence for the use of this product in equine osteoarthritis.

Introduction
Interleukin-1 (IL-1) is thought to be the major mediator of joint disease. Various studies in both horses and humans have evaluated the use of a natural antagonist (interleukin-1 receptor antagonist, IL-1Ra also referred to as IRAP) to block IL-1 activity and decrease the progression of joint disease[1-3]. In human clinical trials, recombinant IL-1Ra has been administered subcutaneously on a weekly basis, and is showing beneficial effects in people with rheumatoid arthritis[1]. In another study, IL-1Ra was administered to equine subjects using gene transfer. An adenoviral vector carrying the equine IL-1Ra gene sequence was injected into a joint that had previously undergone experimentally created osteoarthritis (OA). The injection thus caused the horse's own synoviocytes to produce equine IL-1RA within the affected joint[3]. This study demonstrated a potent anti-arthritic or chondroprotective effect of IL-1Ra.

Recently, a new product, ACS[a], is being beta tested in the United States equine market. This product has been shown to stimulate the production of IL-1Ra from cultured peripheral blood of human patients by 140 fold[4]. Furthermore, this product has had anecdotal success in horses with OA[b], although to date no controlled equine studies have previously been published. The purpose of this study was to evaluate ACS compared to placebo treatment.

Materials and Methods
This study was a blinded experimentally controlled randomized block design that utilized 16 horses in an established model of osteoarthritis[5]. On day 0 of the study, arthroscopic surgery was performed, and OA was induced unilaterally in the mid-carpal joint of all horses. On day 14, horses were divided into two treatment groups: placebo control and ACS treated (Figure 1). The placebo control horses had 6ml of saline injected into the chip joint on days 14, 21, 28 & 35, while the ACS treated joints (OA joints) received 6ml of serum prepared as directed by the manufacturer at similar time periods. On day 14 the horses began a strenuous exercise regime 5 days per week for the remaining 8 weeks of the study. Synovial fluid and serum were assessed every other week for total protein concentration, white blood cell count (WBC) and levels of the inflammatory marker, prostaglandin E_2 (PGE_2). Horses were assessed for lameness using the AAEP grading scale every two weeks. At the termination of the study, operated joints were evaluated grossly, and tissues were harvested for biochemical and routine histologic examinations.

Statistical analysis utilized both a Mixed model analysis of variance and a Least Square mean when individual comparisons were made, and p-values <0.05 were considered significant.

Results
All horses completed the study and no adverse events were recorded. Horses treated with ACS were observed to have significantly improved lameness in OA joints, even five weeks after the last treatment when compared to placebo treated horses (1.3 ± 0.2 versus 2.1 ± 0.2, respectively). A significant reduction in synovial membrane hyperplasia was also seen in the treated compared to placebo OA joints at day 70 (0.4 ± 0.3 versus 1.3 ± 0.3, respectively). Trends (p-value <0.10) for improvement in cartilage immunohistochemistry and gross necropsy were noted for OA ACS treated joints when compared to placebo treatment. The levels of IL1-Ra were estimated in both the Orthokine IRAP serum prior to joint treatment, as well as in the synovial fluid throughout the study, with no significant differences observed.

Discussion
The ACS system uses glass beads exposed to chromium sulfate as a method to stimulate peripheral white blood cells to produce an "anti-inflammatory soup"[4]. The current study assesses this product in a blinded placebo controlled experimental model. Importantly, this study did not demonstrate any negative side effects associated

with the Intra-articular administration of ACS for the duration of the study, but was not a designed safety study. Significant clinical improvement was seen following treatment of induced OA at the last point measured during the study. Significant improvement was also noted in synovial membrane parameters, as well as trends for gross improvement, further supporting a therapeutic action of this preparation. Interestingly, no measurable levels of IL1-Ra were found in the prepared serum (ACS). While the commercial IRAP kit[c] used in this study has antibodies designed against human IL1-Ra, previous work has supported the use of this kit for measurement of equine IL1-Ra levels[5]. It is possible that secondary structure of the equine IL-1Ra protein is not being detected by the human based ELSIA kit. Further investigation into this apparent discrepancy is warranted, as well as examination of the specific proteins up-regulated in this treatment modality. It has been shown that when the Orthokine IRAP system is used on human blood up regulation of IL-4, IL-10 fibroblastic growth factor and transforming growth factor beta factor can be seen[6], all of which would be expected to have beneficial effects in joint disease. Again distinguishing if these results are reproducible using equine blood and which factor (s) are responsible for the beneficial effect need to be the focus of future work.

References

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[a] Orthokine IRAP ™, Arthrex Biosystems, 27299 Riverview Center Blvd Suite 108, Bonita Springs, FL 34134.

[b] Personnel communication Thomas Weinberger 2004.

[c] R&D Systems Inc., 614 McKinley Place N.E., Minneapolis, MN 55413.