INTERLEUKIN-1 RECEPTOR ANTAGONIST DELIVERY THROUGH ADENOVIRAL MEDIATED GENE TRANSFER AS A TREATMENT FOR EQUINE JOINT DISEASE

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Introduction
The objective of this study was to evaluate the over expression of the equine Interleukin-1 receptor antagonist (IL-1Ra) in the horse, a species with naturally occurring disease using an established model of joint disease.

Methods
Using the published gene sequence for equine IL-1Ra an E1/E3 deleted adenoviral vector (Ad-EqIL-1Ra) was constructed that was capable of equine IL-1Ra transgene expression. In accordance with institutional Animal Care and Use Committee approval, osteochondral fragments were created in one intercarpal joint of 16 horses. On day 14 post fragment creation, one randomly chosen fragmented joint of 8 horses was directly administered the Ad-EqIL-1Ra vector while the remaining joints received placebo treatment. The horses were exercised 5 days per week for the remaining 56 days of the study. Throughout the study synovial fluid was collected every 7 days and on day 70 post fragment creation horses were euthanized following clinical re-evaluation. Gross pathologic changes were documented and synovial membrane and articular cartilage collected for histologic analysis.

Results
Following Ad-EqIL-1Ra administration an approximately 28 day effective upregulation of IL-1Ra expression was demonstrated. This increased level of IL-1Ra was associated with significant improvement in gross articular cartilage lesions (Figure 1), clinical parameters of pain (Figure 2) and disease activity, as well as beneficial effects in histologic parameters measured from synovial membrane and articular cartilage (Figure 3). However, increases in inflammatory cell infiltration into the synovial fluid and synovial membrane were observed in Ad-EqIL-1Ra treated joints.

Discussion
Results of this study suggest that an immunogenic response to the vector may have been responsible for loss of transgene expression. However, the novel use of an equine gene sequence in the horse demonstrated a longer transgene expression period as compared to studies utilizing human gene sequences in non-human species and similar vector systems, in fact levels were similar to those obtained using retroviral vector systems. Significant benefits were seen in parameters of clinical pain and disease activity as well as in histologic changes observed within joint tissues. These results suggest this therapeutic modality may be of clinical benefit.

Figure 1 – Photographs of the 3rd carpal bones from chipped joints of both Placebo (A) and Ad-EqIL-1Ra (B) treated horses. Note more extensive full-thickness articular cartilage erosions in the placebo treated joint especially in areas of the 3rd carpal bone not adjacent to the chip. Photos were taken after aseptic harvest of cartilage from the intermediate carpal bone.

Figure 2 – Day 70 lameness scores plotted by treatment group. Different letters associated with bars indicate a statistical difference (P-value <0.05) between bars. Note significant improvement in lameness scores associated with Ad-EqIL-1Ra compared to Placebo treatment. Chip = joint containing an osteochondral fragment.

Figure 3 - Photomicrograph from 5µm sections of articular cartilage stained with safranin-O fast green. Plate (A) is a representative area of cartilage showing no to slight stain uptake patterns in all areas, the tissue was harvested from a chipped joint of a placebo treated horse. Plate (B) is a representative area showing moderate stain uptake patterns in all areas, the tissue was harvested from a chipped joint of an Ad-EqIL-1Ra treated horse.