Treatment of Traumatic Arthritis using Gene Therapy

David D. Frisbie, DVM, PhD, DipACVS

Following a single intra-articular injection of an adenoviral vector capable of driving the protein expression of interleukin-1 receptor antagonist we demonstrated a decrease in lameness and decreased synovitis and osteoarthritis using gene therapy. Author’s address: Equine Orthopaedic Research Laboratory, Department of Clinical Sciences, Colorado State University, 300 West Drake, Fort Collins, CO 80523. © 2000 AAEP.

Introduction

Naturally occurring diseases of the musculoskeletal system are among the most common equine afflictions. In fact, one-half of U.S. horse operations had one or more horses with a lameness problem during a 12-month survey period, and joint or leg problems accounted for the greatest percent of lame horses.1

Most current clinical treatments have been directed toward lowering and then maintaining a decreased degree of inflammation within damaged joints and little attention has been focused on therapeutic agents that actually protect the joint tissues (disease modifying agents). Furthermore, the administration routes, typically systemic or intra-articular, are often not optimal due to low therapeutic concentrations obtained within the joint(s) of interest or procedure related morbidity. In an attempt to circumvent some of these limitations, gene therapy is being investigated to express anti-arthritis and/or disease modifying gene sequences within the joint space. Using laboratory animals, gene transfer has been shown to improve histologic and biochemical parameters of joint disease; however, until the current study a species with naturally occurring joint disease and the ability to monitor clinical disease has not been evaluated. Therefore, the ability to combat experimentally induced synovitis and osteoarthritis in horses was evaluated using gene therapy. Specifically, a molecule with anti-arthritis potential, interleukin-1 receptor antagonist (IL-1Ra), was expressed in diseased joints following intra-articular administration of an adenoviral gene transfer vector (Ad-IL-1Ra) carrying the equine IL-1Ra gene sequence.

Materials and Methods

The evaluation of the Ad-IL-1Ra vector was performed in three separate experiments.2 The first evaluated the ability to construct a vector capable of producing biologically active IL-1Ra protein in vitro. The second experiment, an in vivo dose titration of the vector, was conducted in the equine metacarpal and intercarpal joints to ascertain the concentration of vector that produced the highest transgene expression for the longest period of time without significant deleterious effects. The third experiment evaluated the ability of IL-1Ra delivered using gene therapy to combat the effects of experi-
mentally created synovitis and osteoarthritis in exercising horses.

Sixteen skeletally mature horses, aged 2–5 years, were used in the third study. Horses were in good health and without significant musculoskeletal lesions. Each horse was acclimated to a high-speed treadmill for a 2-minute trot (8–12 mph), 2-minute gallop (25–33 mph) and 2-minute trot protocol to simulate athletic race training. Horses continued a similar exercise protocol 5 days/week beginning 14 days after induction of joint disease until the termination of the study. The horses were divided into two groups (treated and placebo). To experimentally create joint disease all horses had an osteochondral fragment created arthroscopically in one randomly selected intercarpal joint. The “treated” group of horses had $2 \times 10^{10}$ Ad-EqIL-1Ra viral particles/joint diluted to a total volume of 1 ml with Gey's balanced salt solution (GBSS), administered using a direct intra-articular (IA) route into the joint containing the fragment (14 days post creation). The opposite joint (control) received a similar volume of GBSS and the “placebo” group of horses received IA administration of 1 ml GBSS in both intercarpal joints. Synovial fluid was collected from intercarpal joints at 7-day intervals starting prior to fragment creation. Musculoskeletal examinations were also repeated prior to euthanasia, 70 days post fragment creation. At postmortem, gross examination of joint pathology was documented and samples of articular cartilage and synovial membrane were harvested for histologic analyses. Results were analyzed using a mixed model analysis of variance, independent variables included treatment group and when applicable a repeated measures procedure was used. Subject (horse) was considered a random variable when analyzing data from in vivo studies. When individual comparisons were made a Least Squares mean procedure was used, a p-value $<0.05$ was considered significant.

Results

Using the published gene sequence for equine IL-1Ra, an adenoviral vector (Ad-EqIL-1Ra) was constructed that was capable of equine IL-1Ra transgene expression. In the first experiment, this vector was tested in vitro and confirmed to both transduce equine synoviocytes without cytotoxic effects and produce a biologically active IL-1Ra molecule. In the second experiment, the dose titration of the vector suggested a concentration of $2 \times 10^{10}$ particles/joint resulted in IL-1Ra production for the greatest time period without significant deleterious effects.

In the third experiment, clinical examinations of the horses indicated that the therapeutic expression of IL-1Ra significantly decreased signs of joint pain as measured by degree of lameness. The amount of synovial effusion associated with the fragment was also significantly decreased in joints administered Ad-EqIL-1Ra.

Synovial fluid IL-1Ra levels were significantly elevated in joints 7 days post-AdEqIL-1Ra vector administration. Indicating that the vector had transferred the IL-1Ra gene sequence to the joint tissue and subsequent IL-1Ra protein production occurred. IL-1Ra levels were higher than placebo treated joints until 28 days post vector administration.
Postmortem examinations indicated fewer gross pathologic changes existed in fragmented joints administered Ad-EqIL-1Ra compared to joints from placebo treated horses (Fig. 1). The administration of Ad-EqIL-1 was associated with synovial membrane perivascular lymphocytic infiltration and subintimal edema 54 days post administration. Similarly, the creation of a fragment within the joint was also associated with similar changes but not to a similar extent. However, the most severe changes were graded as mild. Furthermore, the administration of Ad-EqIL-1Ra did have beneficial effects on the degree of synovial membrane vascularity in Ad-EqIL-1Ra treated horses. Articular cartilage sections obtained from fragmented joints of placebo treated horses had significantly less safranine-O fast green staining as compared to all other evaluated sections (Fig. 2). This finding suggests that IL-1Ra had a protective effect on osteochondral fragment induced proteoglycan loss.

Discussion
Based on the significant improvements in clinical, gross, and histologic examinations with Ad-EqIL-1Ra treatment, this treatment modality has great promise and is practical for the equine patient. In addition, it also offers future promise for additional anti-arthritic gene sequences in the treatment of equine joint disease as they become available.

Financial support for this research was obtained from the American Association of Equine Practitioners Foundation, Inc, Colorado State University College of Veterinary Medicine, and Biomedical Sciences—College Research Council, and Southern California Equine Research Foundation.

References