

Enhancement of Fracture Healing Using Adenoviral Transfer of the BMP-2 Gene in an Infected Non-union Model

Summary: This study evaluated the use of adenoviral transfer of the BMP-2 gene (Ad-BMP-2) for enhancing healing in an infected non-union rabbit model. The results of this study demonstrated that Ad-BMP-2 enhances healing in infected non-unions. This treatment modality appears to offer great promise of decreasing the healing time of equine fractures.

Non-union and infected non-union are devastating complications following fracture repair in horses and usually result in euthanasia of the affected animal. Novel methods to enhance healing of complicated fractures are required. Gene transfer of growth factors, particularly the bone morphogenetic proteins (BMPs), has been shown to enhance fracture healing in many animal models. Horses with long-bone fractures usually have a lot of damage to the soft tissue, blood supply and bone. The fracture site is often contaminated, leading to infection. Previously, however, there had been no studies evaluating gene transfer of growth factors for enhancing healing in infected non-union models. Therefore, the purpose of this study was to evaluate the use of adenoviral transfer of the BMP-2 gene (Ad-BMP-2) for enhancing healing in an infected non-union model.

This research project was in fulfillment of a PhD project for Dr Louise Southwood. Drs. Frisbie, Kawcak and McIlwraith were integral in the study design, as well as analysis and interpretation of data. Rabbits were used as the model because this was a pilot study. A femoral fracture defect with plate and

screw fixation was used as the basic model. A sclerosing agent was used on the ends of the proximal and distal fragments to cause damage to the ends of the bone and facilitate development of infection. Rabbits were inoculated at the fracture site with *Staphylococcus aureus* and injected in the defect with either Ad-BMP-2 (Treated) or Ad-LUC (CONTROL). Radiographs were taken postoperatively (0), and 4, 8, 12, and 16 weeks after surgery. The weeks to initial and bridging callus formation, radiographic callus grade (0 to 4), and the percentage defect ossification were evaluated from the radiographs. Dual energy x-ray absorptiometry (DEXA) was used to measure the bone mineral density (BMD) in the defect. Following euthanasia, histomorphometry was performed to determine the percentage of fibrous tissue, cartilage, and bone in the defect.

Rabbits treated with Ad-BMP-2 had earlier initial and bridging callus formation and a higher overall callus grade (Figure 1). There was no difference between Ad-BMP-2 and Ad-LUC in the percentage defect ossification, BMD, or the percentage of fibrous tissue, cartilage or bone in the defect.



A. Non-Union Ad-Luc B. Non-Union Ad-BMP C. Infected Non-Union Ad-Luc D. Infected Non-Union Ad-BMP-2

Figure 1 showing radiographic evaluation of excised femurs at 16 weeks, and illustrating the amount of proliferation in the rabbits in the non-union and infected non-union groups treated with Ad-BMP-2 compared to the controls (Ad-LUC).

Continued Development of Novel Therapies for Traumatic Synovitis, Capsulitis and Osteoarthritis in the Horse

Although the results of this study demonstrated that Ad-BMP-2 enhances healing in infected non-unions our results were not as favorable as previous studies of infected non-union or just non-union. The reasons for this may be that the sclerosing agent damaged the adenoviral vector or that there was enough damage to the cells at the fracture site that there were no viable cells for the adenoviral vector to transduce. Therefore further studies are being

performed to evaluate the effect of the sclerosing agent on adenoviral transfer of genes. Future studies are required to evaluate other forms of gene therapy. This being said, this treatment modality appears to offer great promise to decrease the healing time of equine fractures.

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