ADENO Viral Transfer of the BMP-2 Gene for Enhancing Fracture Healing in an Infected Non-Union Rabbit Model

Introduction: Infected non-union is a common and devastating complication following fracture repair, and novel methods for treatment are needed. The bone morphogenetic proteins (BMPs) have been shown experimentally and clinically to enhance healing of non-unions. The BMPs are usually delivered to the fracture site as recombinant proteins, but there are several limitations associated with this type of delivery. Gene therapy has the potential to overcome many of these limitations. Although adenoviral transfer of the BMP-2 gene (Ad-BMP-2) was found to enhance fracture healing in a rabbit non-union model, there have been no studies evaluating Ad-BMP-2 for enhancing healing in infected non-unions. The objective of this study was to test the hypothesis that Ad-BMP-2 would enhance fracture healing in the infected non-union model.

Methods: Sixty-four skeletally mature New Zealand White rabbits were used. A 11mm femoral defect stabilized with plates and screws was the basic model. Experimental groups were: (1) non-infected Ad-Luciferase control (NON-LUC), (2) non-infected Ad-BMP-2 treated (NON-BMP), (3) infected Ad-LUC control (INF-LUC), and (4) infected Ad-BMP-2 treated (INF-BMP). Rabbits in the infected groups were inoculated with Staphylococcus aureus, and quantitative aerobic culture was performed following euthanasia to confirm the presence or absence of infection. A sclerosing agent (sodium morrhuate) was used on the ends of the bone at surgery to facilitate the development of osteomyelitis and to induce a non-union. Fracture healing was evaluated radiographically immediately after surgery, and at 4, 8, 12, and 16 weeks. Initial- and bridging-callus formation, percentage defect ossification, and external callus grade were recorded. Histomorphometry was performed following euthanasia at 16 weeks. The percentages of fibrous tissue, cartilage, and new bone formation were measured. Data were analyzed using an ANOVA. The level of significance was p<0.05.

Results: Radiographically, rabbits treated with Ad-BMP-2 had an earlier initial- and bridging-callus formation, and a higher overall external callus grade compared to rabbits in the Ad-LUC groups (Fig 1 and 2). Histological analysis was unable to demonstrate significant effect of BMP-2 treatment based on new bone formation at 16 weeks; however, rabbits in the Ad-BMP-2 group that were euthanized at 2 and 4 weeks after surgery had more new bone and cartilage compared to rabbits in the Ad-LUC group.

Discussion: The results of this study suggest that Ad-BMP-2 may enhance healing of infected non-unions. The results of our study were not as favorable as previous studies in part because animals healed by a large bridging-callus and not by defect ossification. This could have been a result of the sclerosing agent, which may have either damaged the adenoviral vector or caused damage to the cells in the defect leaving inadequate cells for the adenovirus to transduce. In an in vitro study the sclerosing agent did not affect the transduction efficiency of the adenoviral vector but did decrease bone formation in an in vivo experiment. Based on a small number of rabbits euthanized prior the end of the 16 week study period BMP-2, may have had larger effect on histologic parameters early in this fracture healing model. While Ad-BMP-2 appears to be valuable in infected fractures, in vivo gene transfer may not be ideal for use in fractures associated with severe tissue and cell damage. Using a method that ensures viable cells at the fracture gap such as ex vivo gene transfer or in vivo gene transfer in combination with bone grafts may provide better results. Future studies comparing different methods of gene transfer as well as different growth factors for enhancing healing of infected non-unions are required.