The Effect of Bone Morphogenetic Protein 2 on Tendon-to-Bone Healing in a Canine Flexor Tendon Model

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SIGNIFICANCE: Outcomes after tendon-to-bone repair are typically unsatisfactory, with a poor healing response and a high rate of failure.

INTRODUCTION: These outcomes are due to a number of factors, including a loss of bone at the site of insertion and a lack of regeneration of the fibrocartilaginous transition between tendon and bone. Bone loss after tendon-to-bone repair, in particular, has been noted in a number of clinical and animal studies coincident with a decrease in repair-site mechanical properties [1]. Suppression of osteoclast and stimulation of osteoblast activity during tendon-to-bone healing has led to some improvement in properties after repair [2], however the strategy of stimulating bone formation has not been fully explored. The osteogenic growth factor bone morphogenetic protein 2 (BMP-2) has shown promise for promoting bone formation, including during tendon-to-bone healing [3]. Based on this previous work, the objective of this study was to improve flexor tendon-to-bone healing by promoting bone formation. We hypothesized that BMP-2, applied at the time of surgical repair, would lead to increased bone mineral density and improved repair site mechanical properties.

METHODS: Animal model: The second and fifth flexor digitorum profundus (FDP) tendons were injured and repaired into bone tunnels in the distal phalanx in 26 canines (i.e., 52 total repairs) [3]. Prior to completing the repair, BMP-2 was applied as follows: In phase I of the study, the two doses of BMP-2 (high dose: 0.688µg/µL and low dose: 0.344µg/µL) were administered in 50µL of calcium phosphate matrix (CPM) to the base of each bone tunnel. Release of BMP-2 from CPM occurs over the course of 5 weeks or longer. In phase II of the study, one dose of BMP-2 (low dose: 0.344µg/µL) was administered in a volume of 58µL using a collagen sponge wrapped around the tendon (COL). Release of BMP-2 from these scaffolds occurs much faster than CPM, with most of the BMP-2 released within 2 weeks of implantation. Post-operatively, forelimbs were subjected to passive motion rehabilitation. Dogs were sacrificed at 21 days. Biomechanical tests: FDP tendon-to-bone specimens were pulled in uniaxial tension until failure at a strain rate of 0.003/s until failure (N=4-8 per group) [3]. From the force-elongation curves we determined maximum force and stiffness (the slope of the linear portion of the curve). From the force-strain curves we determined strain at 20N force and rigidity (the slope of the linear portion of the curve). Bone densitometry: Bone density of the distal phalanges was assessed after biomechanical testing using peripheral quantitative computed tomography (N=4-8 per group) [1,3]. A subset of samples (N=3-6 per group) was also assessed using micro computed tomography to visualize the bone tunnel and the CPM. Histology - Samples for histology (N=2 per group) were processed using standard plastic-embedding protocols and stained with H&E / Von Kossa and Masson’s trichrome. Slides were assessed for osteoid formation, inflammatory cells, vascularity, cellularity, and regeneration of a fibrocartilaginous transition. Statistics - Maximum force results were binned as follows: 0N (i.e., failures), 0-10N, 10-20N, and greater than 20N. Maximum force bins were compared using a Chi-square test. For failure load, stiffness, rigidity, and strain at 20N, group means were compared using an analysis of variance (ANOVA) followed by a Fisher’s least squares differences post-hoc test. Samples with a maximum force (Fmax) less than 10N were considered functional failures and were excluded from the ANOVA analysis. Significance was set to p < 0.05.

RESULTS: Three animals (out of 26) developed post-operative complications and were sacrificed early. These animals were not included in the analysis. Nine samples out of 44 were considered failures or functional failures. Of the remaining samples, mechanical properties were not significantly different when comparing BMP-2 groups with their respective carrier controls (Figure 1). However, stiffness was significantly higher and strain at 20N was significantly lower in the BMP-2/COL group compared to the CPM group (Figure 1). No significant differences in mechanical properties were observed when comparing carrier controls (i.e., CPM vs. COL). BMP-2 did not significantly affect BMD compared to carrier controls. BMD was significantly lower than normal in all injury and repair groups. When comparing binned results for Fmax, a significantly higher percentage of BMP-2 treated specimens had a Fmax < 20N compared to carrier controls (Figure 1). Micro computed tomography did not reveal any qualitative differences between BMP-2 groups versus controls. Histologically, regeneration of a fibrocartilaginous transition between the tendon and bone was not observed in any sample. Large amounts of carrier remained in the tunnels that received that carrier. The CPM was surrounded by osteoclasts in all cases, demonstrating high resorptive activity due to the presence of the mineral matrix. There were more osteoclasts in the BMP-2 groups compared to the carrier control groups. Appositional new bone formation was seen in all BMP-2 treated specimens on trabeculae adjacent to tendon compared to control (Figure 2). While BMP-2 treatment appeared to lead to increased localized new bone formation, no mature bone formation or bone ingrowth onto the portion of the tendon situated inside the bone tunnel was observed. There were no apparent differences between groups when examining inflammatory cells, vascularity, or cellularity.

DISCUSSION:
• BMP-2 treatment failed to measurably improve tendon-to-bone healing in a canine flexor tendon model. Although increases in localized bone formation were seen in the BMP-2 treated groups, there were no increases in overall bone mineral density.
• Failure to improve bone mineral density in this model is likely due to the short time interval between tendon-to-bone repair and the performance of both imaging and biomechanical testing.
• Suppression of osteoclast mediated bone loss (e.g., using bisphosphonates) may be necessary before BMP-2 mediated increases in bone formation can be effective in flexor tendon-to-bone repair [3].
• There was an increase in the number of failures in the treated groups. This may be due to osteoblast-osteoclast cross talk leading to increased localized osteoclast activity after BMP-2 COL treatment [4] or due to excess residual CPM at the base of the bone tunnels [2].
• In conclusion, anabolic bone formation strategies for improving intrasynovial flexor tendon-to-bone healing must consider the osteoclast-mediated bone loss that occurs post-repair and the timing of bone mineralization. It is unlikely that anabolic factors alone can accelerate healing in the critical early period after surgical repair.

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