A SINGLE INJECTION OF rhBMP-2 ENHANCES FRACTURE HEALING IN A RABBIT ULNAR OSTEOTOMY MODEL

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Introduction: It is estimated that healing is delayed or impaired in 5-10% of all fractures in the US (1). Thus, non-invasive treatments to enhance fracture healing would be clinically useful in many situations. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a potent differentiation factor that induces bone formation in a variety of animal models. When delivered with an absorbable collagen sponge (ACS), rhBMP-2 has been shown to enhance fracture healing in rabbits and goats (2, 3). In addition, Einhorn et al showed that a percutaneous injection of rhBMP-2 in aqueous buffer enhances fracture healing in rats (4). To further evaluate the feasibility of rhBMP-2 therapy via percutaneous injection, we determined whether a single injection of rhBMP-2 in a buffer carrier would enhance osteotomy healing in rabbits. In addition we determined the in vivo retention profile of rhBMP-2 delivered in buffer using \(^{125}\)I gamma scintigraphy and compared it to that of rhBMP-2 delivered with an ACS onlay.

Methods: Bilateral mid-ulnar osteotomies (0.5 – 1 mm) were created using an oscillating saw in 9 adult (8 months old), male rabbits. In each rabbit, one limb was treated with a single injection of rhBMP-2/buffer (0.2 mg/mL rhBMP-2, total dose of 40 \(\mu\)g rhBMP-2) three hours after surgery, and the contralateral limb was left untreated. The injection was made under fluoroscopic control into the region of the fracture using a 21 g needle. The limbs were bandaged and splinted for 4 days following surgery. Radiographs were obtained immediately after surgery, and weekly thereafter. The biodistribution and retention of rhBMP-2 was determined using scintigraphic imaging of \(^{125}\)I-labelled rhBMP-2. Images were obtained following the injection, three hours after the injection, and at 1, 2, 3, 4, 7, and 10 days after surgery. The rabbits were euthanized 4 weeks after surgery. Following euthanasia, the excised limbs were radiographed and scanned using peripheral quantitative computed tomography (pQCT) to assess callus area (mm\(^2\)), mineral content (BMC, g), and density (mg/cm\(^3\)). After biomechanical testing, the specimens were faxitron radiographed to determine the location of the fracture. The effect of rhBMP-2 treatment on healing was assessed using paired t-tests. Tests were two-tailed and differences were considered significant at \(p<0.05\). The protocol was approved by the IACUC of Genetics Institute and all procedures were carried out according to AAALAC guidelines.

Results: Gamma scintigraphy of \(^{125}\)I-rhBMP-2 indicated that 82% ± 7% (mean ± SD) of the administered dose was initially retained at the fracture site, and that 40% ± 10% and 13% ± 5% of the initial dose remained at the site 1 and 3 days after surgery, respectively (Figure 1). One week after surgery only 4% of the administered dose was retained at the fracture site. The area under the retention-profile curve (AUCp) was 56.6 ± 14.6 \(\mu\)-g-days. Area of the mineralized callus was on average 55% greater in the rhBMP-2 treated ulnae than in the untreated, contralateral ulnae (27.4 ± 7.1 mm\(^2\) vs. 17.7 ± 3.5 mm\(^2\), \(p=0.002\)). The mineral content of the callus was 63% greater in the rhBMP-2 treated ulnae (13.5 ± 3.8 g vs. 8.3 ± 1.6 g, \(p=0.001\)). Bone density of the mineralized callus was 4.8% greater in the rhBMP-2 treated limbs, although this difference did not reach statistical significance (\(p=0.10\)). The biomechanical properties of the healing osteotomy were 70-80% greater in the rhBMP-2 treated ulnae compared to their contralateral, untreated ulnae (Table 1).

Discussion and Conclusions: In the rabbit ulnar osteotomy model, ulnae treated with a single percutaneous injection of rhBMP-2/buffer had larger calluses (+50-60%) and greater biomechanical properties (+70-80%) compared to ulnae left untreated. These differences are similar, albeit somewhat smaller, to those observed between untreated ulnae and those treated with an rhBMP-2/ACS onlay (3). The biodistribution data indicated that approximately 80% of the administered dose (~32 \(\mu\)g rhBMP-2) was initially retained at the fracture site, with 4% (1.6 \(\mu\)g rhBMP-2) remaining one week after implantation. In comparison, when rhBMP-2 was delivered in an ACS onlay, approximately 74% of the delivered dose was retained initially, with nearly 40% retained one week after surgery (3).

In conclusion, these data indicate the a single injection of rhBMP-2/buffer at the fracture site is able to enhance healing. These findings provide rationale for further evaluation of rhBMP-2/buffer treatment in higher species. Delivery of rhBMP-2 through percutaneous injection would allow treatment of closed fractures and non-surgical intervention for those fractures with signs of delayed union or nonunion.


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Table 1. Biomechanical Properties, 4 weeks after treatment (mean ± SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>rhBMP-2/Buffer</th>
<th>Untreated</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>TQ (N-m)</td>
<td>0.39 ± 0.16</td>
<td>0.23 ± 0.05</td>
<td>p=0.006</td>
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<tr>
<td>STF (N-m/deg)</td>
<td>0.020 ± 0.008</td>
<td>0.011 ± 0.004</td>
<td>p=0.002</td>
</tr>
<tr>
<td>ETF (N-M*deg)</td>
<td>4.68 ± 2.47</td>
<td>2.74 ± 0.88</td>
<td>p=0.03</td>
</tr>
</tbody>
</table>

Figure 1. In vivo retention of rhBMP-2 as a percent of initial dose delivered. Error bars represent one standard deviation.