**RHBMP-2 IN AN INJECTABLE GELFOAM CARRIER ENHANCES CONSOLIDATION OF THE DISTRACTED CALLUS IN A SHEEP MODEL OF DISTRACTION OSTEOGENESIS**

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Introduction: Distraction osteogenesis is the process of generating viable osseous tissue by the gradual separation of osteotomized bone edges. A major problem associated with this procedure is the long time period needed for consolidation of the newly formed bone. Several studies have addressed this problem of slow bone consolidation and have tried to enhance callus maturation by mechanical loading (1), systemic growth hormone administration (2), or local cytokine injection (3). Although promising, these attempts have been only partially successful due to deleterious side effects and/or limited improvements in bone consolidation. Recent reports have shown that local administration of recombinant human bone morphogenetic protein-2 (rhBMP-2) consistently promotes regeneration of skull, mandibular and long-bone defects, as well as fusion of vertebrae (4). Therefore, the goal of this study was to determine whether a single percutaneous injection of rhBMP-2 in a collagen carrier (Gelfoam) would enhance bone consolidation following tibial distraction in a sheep model. Specifically we asked whether treatment with rhBMP-2 would lead to greater torsional properties of the callus throughout the consolidation period as well as at the approximate end of consolidation, where the mechanical properties of the callus were approximately 70% of those of the intact contralateral limb.

Methods: An unilateral tibial osteotomy stabilized with an external fixator was performed in 14 adult sheep. The sheep were not restricted in loading the osteotomized leg after surgery. Following a latency period of 4 days, distraction was performed at 1.25 mm/day for 20 days. At day 23, sheep were randomly assigned to receive either an injection of a rhBMP-2 (total dose = 8 mg) / Gelfoam (Pharmacia/Upjohn), or an injection of Buffer / Gelfoam which served as a carrier control. During the following 50 days of consolidation in-vivo torsional stiffness measurements of the osteotomized tibia were obtained weekly. The in-vivo stiffness measurements were performed using a specially designed torsional measuring system (5). After sacrifice both tibiae were removed, embedded in PMMA and tested to failure in torsion. The maximum torsional moment and torsional stiffness, determined as slope of the linear portion of the torque vs. angle curve, were recorded. The effect of rhBMP-2 treatment on in-vivo stiffness measurements was assessed using a multivariate ANOVA. Torsional strength and stiffness from destructive testing were compared using a Mann-Whitney-U-test. All animal experiments were conducted according to US and German animal protection laws.

Results: The in-vivo torsional stiffness measurements in the sheep treated with rhBMP-2 / gelfoam were 29 to 81% higher than those of the carrier control group (Fig. 2). These differences were statistically significant at days 44, 51, 65 (% of intact) 91 ± 9, 71 ± 5.8, 65 ± 1.6 (p=0.18, 0.02, 0.19). Destructive testing showed that the maximum torsional moment was 59.5 ± 3.6 * (n=7) 37.4 ± 10.0 * (n=6) (p=0.03). Torsional stiffness (% of intact) 7.9 ± 0.6 * (n=7) 5.8 ± 1.6 * (n=6) (p=0.02). Table 1. Results of destructive torsion after 20 days of distraction and 50 days of consolidation (mean ± SEM).

**Discussion:** This study shows that a single injection of rhBMP-2/Gelfoam dramatically enhances consolidation of the regenerate in a sheep model of distraction osteogenesis. Quantitative in vivo measurements showed a more rapid increase in torsional stiffness as early as day 44 after surgery. It is important to note that in-vivo stiffness is measured using very low forces and cannot be directly compared to stiffness measurements assessed during the destructive testing. One limitation of this study was the inability to measure weight-bearing in the operated and contralateral, un-operated limbs during consolidation, as differences in weight-bearing could affect the healing and ultimately, the mechanical properties of the regenerate. In conclusion, these data strongly support the potential use of rhBMP-2 in an injectable carrier for accelerating the consolidation phase of distraction osteogenesis.


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**Figure 1.** Examples of post-mortem Faxitron X-ray image: left (#173) BMP-2 / gelfoam injection, right (#155) Buffer / gelfoam injection.

**Figure 2.** Average in-vivo stiffness measurements during the consolidation phase. Statistical differences were calculated by multivariate ANOVA. Error bars indicate SEM.

<table>
<thead>
<tr>
<th>Days</th>
<th>BMP-2 / gelfoam</th>
<th>Buffer / gelfoam</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>91 ± 7 *</td>
<td>59 ± 16</td>
</tr>
<tr>
<td>51</td>
<td>71 ± 4 *</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>65</td>
<td>7.9 ± 0.6</td>
<td>5.8 ± 1.6</td>
</tr>
</tbody>
</table>

*significantly greater than Buffer/gelfoam (p=0.03)