Evaluation of Adenoviral-Mediated Gene Transfer of hBMP-13 to Improve Rotator Cuff Healing in a Rat Model

Gulotta, LV; Kovacevic, D; Montgomery, S; Ehteshami, JR; +Rodeo, SA
+Laboratory for Soft Tissue Research, Hospital for Special Surgery, New York, NY
RodeoS@hss.edu

INTRODUCTION:
Rotator cuff repair surgery depends on tendon-bone healing that results in scar formation which is weaker than normal tissue(1). The formation of scar tissue makes repairs prone to failure. Biologic therapies may be able to improve healing by limiting the amount of scar formation and promoting formation of the normal fibrocartilagenous transition zone. Previous work in our laboratory showed that the addition of mesenchymal stem cells (MSCs) alone is inadequate to improve healing. Based on these findings, it is postulated that MSCs need a signal in order to induce differentiation. However, the method by which this signal is delivered remains problematic.

BMP-13 may provide such a signal. It has been shown to induce both cartilage and tendon formation and may promote formation of the fibrocartilagenous transition zone seen in the native rotator cuff insertion site(2,3,4). The purpose of this study was two-fold: 1. To determine if a therapeutic agent can be delivered to the tendon-bone interface using genetically modified MSCs, and 2. To determine if Lewis rat MSCs transduced with Ad-rhBMP-13 can improve tendon-bone healing in a rat rotator cuff model.

METHODS:
Part I: Eight Lewis rats underwent unilateral detachment and repair of the supraspinatus. At surgery, 4 received 10^6 MSCs, and 4 received 10^8 MSCs transduced with Ad-Lac-Z (Gift from Chisa Hidaka, MD). Two in each group were sacrificed at 2 and 4 weeks and stained with betagalactosidase to test for gene delivery to the repair site. The amount of betagalactosidase staining was grossly assessed by two independent reviewers to assess gene expression in our in vivo model.

Part II: 60 animals underwent the same surgical procedure and an additional 10 were used for MSC harvest. Animals were randomized into two groups (30 animals/group). The experimental group received 10^5 MSC’s transduced with Ad-hBMP-13 (gift from Gregory Helm, MD, PhD) at 10^5 pfu’s/cell. The second group received untransduced MSC’s. Fifteen animals in each group were sacrificed at two and four weeks. At each time point, 12 animals were allocated for biomechanical testing, and 3 for histomorphometric analysis.

Evaluation of hBMP-13 Gene Expression:
In vitro gene expression was confirmed with RTPCR with the following primers: hBMP-13 forward 5’- TGCCACTCAGAAGACTGTGG3’; hBMP-13 reverse 5’- CCCGCGTCGATGTATAGAAT-3’; rat GAPDH forward 5’- TGCCACTCAGAAGACTGTGG3’, and rat GAPDH reverse 5’- GGATGCAGGGATGATGTTCT-3’.

MSC Preparation and Transduction:
The bone marrow of the long bones of 10 Lewis rats was harvested by lavage. Cells were cultured in DMEM/10%FBS/1%ABAM and passed at 80% confluence for a maximum of 3 times. For the Ad-BMP-13 group, MSCs were transduced 24hrs prior to implantation at 10^5 pfu’s/cell. On the day of surgery, 10^6 cells were suspended in 50µl of Tissue Fibrin Sealant (Baxter, Inc) and added to the tendon-bone repair site(5). Animals in the MSC control group received the same number of cells in an equal amount of fibrin sealant.

Histomorphometric Analysis:
The amount of new cartilage formation was determined by measuring the level of brightness (Gray Scale) under polarized light microscopy of picrosirius red stained slides with ImageJ (NIH).

Biomechanical Testing:
Ultimate load-to-failure, the cross-sectional area, and the calculated stress required for failure were all determined using our established testing protocol(6).

RESULTS:
Part I: At both 2 and 4 weeks, there was more betagalactose staining in the specimens treated with the Ad-BMP-13 MSC’s compared to those treated with MSC’s alone (table below).

<table>
<thead>
<tr>
<th></th>
<th>2 Weeks</th>
<th>4 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSC</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>BMP-13</td>
<td>+++/+++</td>
<td>+++/++</td>
</tr>
</tbody>
</table>

Histomorphometric Analysis and Biomechanical Testing: There were no statistically significant differences between groups at either timepoint in terms of the amount of fibrocartilage formation, the collagen fiber organization, or any biomechanical testing outcomes (Data not shown).

DISCUSSION:
This study showed that genetically modified stem cells in a fibrin glue carrier can exhibit gene expression at the tendon-bone interface as evidenced by betagalactosidase staining as late as 4 weeks after implantation. These results are encouraging for the development of a sustained-release drug delivery system to the healing tendon-bone interface. However, this study failed to show that MSCs transduced with hBMP-13 can improve tendon-to-bone healing following rotator cuff repair at the timepoints examined. Further studies are needed to determine the optimal growth factor, or factors, and its delivery system to improve tendon-to-bone healing.

REFERENCES:

ACKNOWLEDGEMENTS:
The authors would like to thank Liang Ying for her help with the histology for this study. We would also like to thank Chisa Hidaka, MD and Gregory Helm, MD, PhD for providing the adenoviral vectors and Matthew Cunningham, MD, PhD for his technical help.