Bone Marrow Aspirate with a subeffective dose of rhBMP-2 in Spinal Fusion: A Quantitative Analysis

ABSTRACT INTRODUCTION:
A well consolidated fusion mass is fundamental to pain reduction and increased functioning which are desired clinical outcomes in a patient after having fusion procedure. Uses of bone morphogenetic proteins (BMPs) contribute to rapid and effective fusion [1]. However, consistent de novo bone formation is still considered a clinical challenge even when using BMPs. For instance, fusion consolidation is particularly difficult in patients who have had a previous surgery at the same anatomical site, have multi-level disease, diabetes, metabolic bone disease, nutritional deficiencies, or are elderly [2]. Other challenges associated with the use of BMPs include high costs [3] as well as dose-dependent risk of adverse events [4].
Bone marrow has emerged as a potential adjuvant to improve the efficiency and effectiveness of BMP in spine fusion. Freshly harvested and clinically readily available bone marrow aspirate (BMA) includes osteoinductive factors in addition to MSCs, which may optimize the environment for both MSCs and BMPs to promote fusion [5].

This study tests the hypothesis that transplantation of freshly harvested BMA together with a subeffective dose of rhBMP-2 optimizes rhBMP-2 efficiency and effectiveness. The resultant fusions were biomechanically tested to determine the quantitative effect of BMA on the mechanical properties of the fusion mass. To our knowledge, no other study has looked specifically at a subeffective dose of rhBMP-2 in combination with BMA to evaluate its potential clinical use.

METHODS:
A L4-L5 posterolateral intertransverse process fusion procedure was performed in Lewis rats.

Dilution Series Dosing Curve (n=53): A subeffective concentration of rhBMP-2 at which 33%-50% of the rats demonstrated posterolateral fusion (ED33-50, 0.006 mg/mL rhBMP-2) was determined.

Treatment Groups (n=36): A subeffective concentration of 0.006 mg/mL of rhBMP-2/absorbable collagen sponge (ACS) plus fresh syngeneic transplants of BMA; 0.006 mg/mL rhBMP-2/ACS; or BMA/ACS.

Determination of Fusion: Rats were sacrificed 8 weeks after surgery. Fusions were evaluated radiographically for evidence of bridging trabecular bone between the transverse processes and by manual palpation by three independent observers.

Biomechanical Testing: Biomechanical testing was performed on 10 of the fused spines (n=6 rhBMP-2: BMA/ACS, n=4 rhBMP-2/ACS only). Each specimen was cleaned of musculature and potted in custom aluminum cups using a two-part epoxy resin on either end of the fusion mass. Afterward each specimen was mounted onto a servo-hydraulic actuator (MTS Bionix 370.02, MTS Corp., Eden Prairie, MN) equipped with a mini load cell (MINI45 Transducer, API Corp., Apex, NC) and tested to failure at an angular deformation rate of 30 deg/min while the corresponding applied moment and rotation were recorded. The strength was measured as the maximum torque to failure. Stiffness was calculated as the slope of the linear regression between the initial and maximum applied torque upon failure.

This study was approved by the IACUC of UCLA and Cedars-Sinai Medical Center administered in compliance with applicable federal, state, and local laws and regulations.

RESULTS:
BMA directly mixed with 0.006 mg/mL rhBMP-2/ACS significantly increased the L4-L5 fusion rate to 89% compared to a rate of 33-50% found for rats implanted with rhBMP-2/ACS only (p<0.05). No fusion/bone formation was observed in rats implanted with BMA/ACS alone.

Biomechanical Testing: The strength of the fusion mass was not statistically different (p>0.79) between the rats implanted with rhBMP-2/BMA/ACS and those implanted with rhBMP-2/ACS alone. There was no significant difference (p=0.41) between the average stiffness of rhBMP-2/BMA/ACS and rhBMP-2/ACS alone, Figure 1.

DISCUSSION:
This is the first study to combine fresh, unmanipulated BMA with a subeffective dose of rhBMP-2. The direct application of BMA mixed with an experimentally determined subeffective dose of rhBMP-2 significantly improved the fusion rate to 89% compared to 33%-50% fusion without the added BMA. BMA alone in a carrier was not sufficient to induce fusion. These results support our hypothesis that transplanting BMA together with a subeffective dose of rhBMP-2 increases osteogenesis and improves the frequency of fusion success. While the rate of bone formation and remodeling appears to be influenced by the addition of BMA, the results of our biomechanical analysis indicate that the mechanical properties of the bony mass are unaffected.

The addition of autologous BMA at the critical time of implantation may initiate the differentiation process simultaneously in a greater number of cells prior to systemic clearance or degradation of rhBMP-2. Furthermore, the addition of autologous BMA also adds a variety of osteoinductive factors which may further increase the osteogenicity of the fusion site, including endogenous BMPs and other cytokines, growth factors, and signaling regulators.

This study is limited by the use of freshly harvested, directly applied BMA which did not allow us to quantitatively evaluate its composition. Also, during biomechanical testing we assumed the geometry and microstructure of the fusion masses were relatively homogeneous allowing comparison of material properties. A more qualitative assessment of the fusion mass is necessary to validate this assumption.

SIGNIFICANCE:
A biologic challenge is posed when using rhBMPs in patients presenting with surgically challenging tissue environments of poorly vascularized tissues, pseudarthrosis repair, metabolically compromised, or elderly patients due to fewer numbers of mesenchymal or other cells and osteoinductive factors. Improving the performance of rhBMP-2 in spine fusion with adjuvant BMA, as demonstrated by the results of this study, has the potential to lower costs, decrease adverse effects, and improve the host environment for better outcomes, especially in compromised patients.

ACKNOWLEDGEMENTS:
rhBMP-2 was provided gratis by Medtronic Sofamor Danek, Memphis, TN.

REFERENCES: