NITRIC OXIDE MODULATES rhBMP-2 INDUCED CORTICOCANCELLOUS AUTOGRAPH INCORPORATION IN A NOVEL RAT INTERTRANSVERSE FUSION MODEL

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Introduction
Nitric oxide (NO) is a gaseous molecule that is synthesized by the action of nitric oxide synthases (NOS) on L-arginine. NO modulates fracture repair. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a bone-inductive protein that has recently been approved by the Food and Drug Administration (FDA) to enhance human spinal fusion. Progression of fusion involves remodelling of the fusion bone mass with a decrease in the fusion mass size. It is not known whether nitric oxide has a role in spinal fusion or in rhBMP-2 enhanced spinal fusion and remodelling.

Materials and Methods
A Novel Rat Intertransverse Fusion Model: Male Sprague Dawley rats with a mean weight of 345 grams (±45 grams) and an average age of 12 weeks (± 5 days) were used. A 4 cm longitudinal dorsal skin and bilateral muscle splitting approach was performed to expose the transverse processes of L4 and L5. A # 15 blade was used to decorticize the transverse processes. Autograft was prepared by morcellizing several vertebrae obtained from the tail by caudectomy. Collagen type I sponge (Helistat, Integra Life Sciences, Plainsboro, NJ) was cut into uniform 2 mm pieces. Depending on the group, the sponge was soaked in either saline or in 1 ml of rhBMP-2 solution (43 µg/ml). Collagen sponge was then mixed with the autograft. To ensure consistent autograft density in all groups, a custom graft-delivery jig was designed to compress and then deliver graft material via a plunger over the intertransverse membrane between L4 and L5.

Experimental Design: To determine the effect of systemic NOS inhibition on rhBMP-2 induced spinal fusion; four groups of rats underwent posterior lumbar intertransverse fusion while one group (S, n=28) had a sham operation performed. The four groups were: autograft alone group (A, n=28); autograft + systemic inhibition of NOS group (AL, n=28); autograft + 43 µg/ml rhBMP-2 group (AB, n=28) and autograft + systemic inhibition of NOS + 43 µg/ml rhBMP-2 group (ALB, n=28). The groups of rats undergoing NOS inhibition received N⁰-nitro L-arginine methyl ester (L-NNAME; Sigma Chemical Co., St Louis, MO) at a dose of 1 mg/ml ad libidum in their drinking water starting two days preoperatively. In each group, 14 rats were sacrificed at 21 days and 14 rats sacrificed at 44 days by carbon dioxide asphyxiation.

Radiographic Analysis: At sacrifice, lumbar spines were explanted and radiographed with the Faxitron x-ray apparatus and digitized. Radiographs were scored using a 5-point radiographic fusion score (0=no bone to 4=clear external bony cortex with > 75% intertransverse area consisting of bridging trabeculae) by two blinded observers. Using Metamorph Image Analysis System (Universal Imaging Co., Downingtown, PA), total fusion mass area on each digitized radiograph was contained within the SROI measured in mm². For further analysis, a square region-of-interest (SROI) was centred over each bilateral fusion mass and bony area contained within the SROI measured in mm² for all groups.

Biomechanical Analysis: Qualitative manual palpation for relative motion between L4 and L5 was performed by three blinded observers for explanted spines. Spines were then potted in epoxy resin and a novel mechanical testing device was used to cyclically load the specimens in flexion and extension to forces of ±1.5N and -1.5N. Forces were measured by a load cell and displacement measured by a linear variable differential transducer (LVDT) for a total of 120 cycles. A representative cycle for each specimen was extracted and range of motion (mm) and hysteresis (N/mm) were calculated.

Histological Analysis: Specimens underwent decalcified histological preparation and consecutive 5 µm sections were stained with H&E and Goldner’s trichrome. Images were recorded and the fusion segment was demarcated into two zones; 1) the distal and proximal transverse process-fusion mass zones and 2) the centrum of the fusion mass. These zones were independently scored by a 5-point histological score (0=no bony contiguity to 4=bone on bone, consolidated fusion with marrow channels. Sections were also analysed for percent fibrous tissue as a reciprocal index of fusion mass maturity.

Statistics: All data are presented as mean ± SE. Differences between experimental groups were assessed using unpaired two-tailed Student’s t-tests and analysis of variance (ANOVA). The level of significance was set at p<0.05.

Results (Table 1)
Effect of NOS inhibition on spinal fusion: There was 5 times more range of motion in group AL compared to the group A on day 44 following surgery. (AL/ A; *p<0.05)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S (mm)</th>
<th>A (mm)</th>
<th>AL (mm)</th>
<th>AB (mm)</th>
</tr>
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<tbody>
<tr>
<td>Range of motion (mm)</td>
<td>0.4±0.2</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
<td>0.1±0.0</td>
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<tr>
<td>Hysteresis (N/mm²)</td>
<td>0.1±0.0</td>
<td>0.5±0.2</td>
<td>0.3±0.1</td>
<td>0.2±0.1</td>
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Discussion: In this novel rat model, the results indicate that 1) nitric oxide synthase inhibition leads to poor biomechanical fusion six weeks after surgery, 2) rhBMP-2 induces intertransverse process fusion as early as three weeks and remodelling is completed by six weeks and 3) lack of nitric oxide synthase during rhBMP-2 induced spinal fusion leads to poorer radiographic, biomechanical and histologic spinal fusion. We conclude that nitric oxide pathways play a modulatory role in rat intertransverse process spinal fusion.