The Effect of Noggin Interference in a Rabbit Posterolateral Spinal Fusion Model

ABSTRACT INTRODUCTION:
Posterolateral lumbar fusion is a standard surgical treatment for many spinal disorders including spondylolisthesis, disc degeneration, and traumatic instability. The current gold standard to achieve a solid arthrodesis is the use of autogenous iliac crest graft combined with modern instrumentation. However, even under ideal conditions the rate of pseudarthrosis may approach 3% to 25.5% in the intertransverse plane. Growth factors, including bone morphogenetic proteins (BMPs), have been investigated to improve these fusion rates without the need for iliac crest bone harvesting. The Food and Drug Administration has approved the use of BMP-2 bound to a collagen matrix as a bone graft substitute for intradiscal spinal fusions. Despite this, most BMP-2 use is off-label in the posterolateral spine, and involves using supraphysiological levels of BMP. BMP activity is regulated at several distinct levels during osteoblast differentiation, most notably by BMP antagonists such as noggin, DAN, and chordin. Little is known about the interplay between these endogenous proteins. Previous work in our laboratory demonstrated that noggin expression is significantly elevated in muscle during spinal fusion. However, other research has suggested that knockdown of noggin may lead to improved fusion in conjunction with exogenous BMP-2. However, the inhibition of noggin expression and its affect on the overall fusion is unknown. We sought to investigate whether siRNA-mediated suppression of noggin in paraspinal muscle could enhance endogenous BMP and enhance the rate of spinal fusion.

METHODS: siRNA Selection for noggin knockdown R9ab cells (rabbit fibroblasts) were cultured in Eagle’s MEM with 10% FBS. Five rabbit noggin targeted siRNAs were synthesized as Stealth RNAi™ duplexes. Plasmid expressing rabbit noggin was co-transfected with siRNA oligos.

Skeletal Fusion: The experimental protocol was reviewed and approved by the UC Davis IACUC. Twenty-six skeletally mature New Zealand White rabbits underwent single-level, bilateral, posterolateral, intertransverse process fusions at L5-L6, based on the model established by Boden et al[1], using 2ml. cortico cancellous bone grafts from each iliac crest.

In-vivo Noggin siRNA Delivery The noggin siRNA construct was injected into the paraspinal muscle between L5 and L6 on each side. Electric pulses (8 cycles/second rectangular current at 200V for 20msec with 200msec intervals) were applied using a two transfection apparatus and a BTX ECM 830 electroporator. The exposed L5 and L6 transverse processes were decorticated with an electric burr, harvested iliac crest graft was placed in the intertransverse plane, and the wound closed.

Sample Groups and Harvest Short-term effects of siRNA on gene expression and protein levels were investigated at days 2, 4, and 7. Long term noggin expression and spinal fusion was investigated at weeks 2, 4, and 6. At sacrifice, paraspinal muscle tissue adjacent to the fusion bed was collected, and the entire L4-L7 section of spine was excised. Muscle was immediately lysed in Buffer RLT from the RNaseasy mini kit for fibrous tissue and homogenized with a Polytron rotor-stator tissue homogenizer. Samples were divided for protein and RNA extraction.

Western Blot Samples for protein extraction were acetone precipitated. 5 µg protein was loaded into each lane and probed with antibodies against Noggin, BMP-2, BMP-7, GAPDH.

Radiographs Fusion was assessed using weekly posteroanterior radiographs. The radiographs were graded in a blinded fashion using the following scale to assess the amount of intertransverse bone graft: Grade 0, no bone between the transverse processes; Grade 1, small islands of bone between the transverse processes; Grade 2, bridging bone with two or more radiolucent lines or one large gap within the fusion mass; Grade 3, bridging bone with one radiolucent line in the fusion mass; Grade 4, bridging bone with no gaps or lucent lines.

Statistics Statistical comparisons were done using Student’s t-test with significance set at p<0.05.

RESULTS: In vitro knockdown of overexpressed noggin was observed with all siRNAs by western blotting, while the scrambled siRNA had no effect on noggin protein (Fig 1). The most potent siRNA was developed into a fluorescently-labeled and targeted to the rabbit noggin mRNA stage 2000. In short-term in-vivo experiments, the fluorescent siRNA was detected in paraspinal muscle at days 1, 2, and 4, but not after day 7 after electro-