Evaluation of rhBMP-2/Fibrin Glue-Associated Edema in Rabbit Muscle Tissue

Emily M Lindley, PhD, Aurora, CO, USA (n); Susan Estes, NP, Aurora, CO, USA (n);
Evalina L Burger, MD, Aurora, CO, USA (n);
Vikas V Patel, MA, MD, Aurora, CO, USA (a,d-Medtronic)

Introduction: Recombinant human bone morphogenetic protein type-2 (rhBMP-2) is an osteobiologic often used in orthopaedic surgery to promote bone growth. However, given that rhBMP-2 is a cytokine capable of osteoinduction, it carries the theoretical risk of causing inflammation. In fact, rhBMP-2 use in the cervical spine has led to some reports of soft tissue swelling, which can lead to dysphagia or difficulty breathing in severe instances. The purpose of this study was to quantify edema caused by rhBMP-2 implantation into rabbit muscle tissue. In addition, the edematous effects of different vehicles that are commonly used together with rhBMP-2 were also analyzed.

Methods: Eighteen New Zealand white rabbits were anesthetized and a midline incision was made to expose the dorsal muscles surrounding the spine. Four separate incisions were made into the muscles and the following four materials were implanted into each rabbit: (1) an absorbable collagen sponge (ACS; 1 cm x 1 cm) soaked with 0.1 mL buffer solution (buffer/ACS), (2) ACS soaked with 0.1 mL of 0.43 mg/mL rhBMP-2 (BMP/ACS), (3) ASC soaked with 0.1 mL of 0.43 mg/mL rhBMP-2 and then encapsulated with 0.5 mL fibrin glue material (BMP/ACS/FG), and (4) 0.5 mL fibrin glue material alone (FG). It should be noted that 0.1 mL of 0.43 mg/mL rhBMP-2 solution was considered to be a normal dose of rhBMP-2 for this ectopic rabbit model. The rabbits were then sacrificed at either two, three, or six days postoperative and given an immediate magnetic resonance imaging scan. The STIR images were analyzed to measure areas of edema surrounding the implanted materials using Image J software. Muscle tissue surrounding the implants was also harvested for histological analysis.

Results: Analysis of STIR images revealed that at each of the three time points (postoperative day two, three, and six), muscle tissue implanted with BMP/ACS/FG had significantly larger areas of associated edema than that of muscle implanted with buffer/ACS, BMP/ACS, or FG alone. There were no significant differences between the buffer/ACS and BMP/ACS groups at any time point. Edema surrounding buffer/ACS and BMP/ACS/FG significantly decreased between day two and day six; muscle tissue implanted with BMP/ACS and FG alone also showed a trend of decreased edema over the six postoperative days.

Conclusions: The present findings suggest that edema associated with implanting a normal dose of rhBMP-2 on the ACS carrier is not significantly greater than that caused by the implantation of an ACS soaked in buffer solution. In addition, edema associated with fibrin glue alone was not greater than that of ACS soaked in either rhBMP-2 or buffer solution. However, there appears to be a possible synergistic effect that leads to significantly increased edema when fibrin glue is added to BMP/ACS. Finally, edema in all groups appeared to be transient, decreasing from 2 days to 6 days postoperative.

If noted, the author indicates something of value received. The codes are identified as a-research or institutional support; b-miscellaneous non-income support/miscellaneous funding; c-royalties; d-stock or stock options; e-consultant or employee; n-nothing of value received, and *disclosure not available at time of printing. For full information refer to inside back cover.
Injectable Chitosan for Sustained Protein Delivery to Treat Painful Radiculopathy

Mohammed F Shamji, MD, MSc, Durham, NC, USA (n); Priscilla Hwang, BS, Durham, NC, USA (n); Robert W Bullock, BS, Durham, NC, USA (n); Samuel B Adams, MD, Durham, NC, USA (n); Dana L Nettles, PhD, Durham, NC, USA (n); Allan H Friedman, MD, Durham, NC, USA (n); William J Richardson, MD, Durham, NC, USA (n); Lori A Setton, PhD, Durham, NC, USA (n)

Background: Intervertebral disc herniation may cause painful radiculopathy by both mechanical compression and biochemical irritation of nearby neural structures. Tumor necrosis factor alpha (TNFα) is a proinflammatory cytokine implicated in this pathology, and while effective symptomatic treatment is achieved by systemic anti-TNF therapy, patients are exposed to substantial immunosuppression and associated side effects. There exists a need for a drug delivery vehicle that sustains release of locally-active therapeutics.

Materials: Chitosan is a biocompatible aminopolysaccharide that undergoes thermally-initiated gelation in cosolutions with glycerophosphate (GP). This injectable vehicle may entrap and slowly release therapeutics upon local injection. Gelation time and temperature of chitosan/GP thermogels were evaluated by optical density readings (350 nm), as was the effect of bovine serum albumin (BSA) entrapment on the kinetics of this process. We investigated the in vitro release of BSA and various anti-TNF agents: curcumin (368 Da), sTNFRII (17 kDa), and anti-TNF antibody (150 kDa). In vitro activity of the released drugs was evaluated using an established bioassay.

Results: Turbidity results show the chitosan/GP thermogel achieves gelation at 37°C after 5-10 minutes, independent of protein loading from 0 to 3.75 mg per gel. Sustained BSA release occurred with 50% release at 7 days. All three anti-TNF therapeutics exhibited sustained release, with over 10% of both sTNFRII (Figure 1) and anti-TNF antibody remaining in the gels after 7 days. Each compound exhibited expected activity antagonizing TNFα-cytotoxicity in murine fibrosarcoma cells (Figure 2).

Conclusions: This study demonstrates that thermogelling chitosan/GP entraps and sustains the release of a broad range of anti-TNF agents. Such delivery of disease-modifying therapy could establish a drug depot to treat local inflammation implicated in radiculopathy. The breadth of molecular sizes demonstrates the system’s versatility, and the slow and sustained release could protect the body against toxicities of systemic delivery.

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an “off label” use). See inside back cover for full information.