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An in vitro and in vivo analysis of fibrin glue use to control bone morphogenetic protein diffusion and bone morphogenetic protein–stimulated bone growth

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Abstract

BACKGROUND CONTEXT: Recombinant human bone morphogenetic protein-2 (rh-BMP2) has become popular for augmenting spine fusion in the lumbar and cervical spine. Concerns exist, however, over bone morphogenetic protein (BMP)-stimulated soft-tissue swelling and bone growth stimulation in areas where bone is not desired, especially as the material ''leaks'' into such spaces. The most detrimental effects of such leakage might be airway compromise, while heterotopic bone formation into the spinal canal has been reported in animal and human studies. Fibrin glue has been used as a carrier of many osteoinductive materials; however, its efficacy at modulating the clinical effects of BMP are not known. The amorphous nature of fibrin glue makes it a candidate to control diffusion of BMP and possibly limit bone formation by limiting BMP diffusion to areas where such bone is not desired.

PURPOSE: To evaluate the use of fibrin glue to limit BMP diffusion and BMP-stimulated bone growth.

STUDY DESIGN/SETTING: This is an in vitro basic science study and an in vivo prospective randomized animal study.

STUDY SAMPLE: Eighteen Lewis rats.

OUTCOME MEASURES: In vitro study: Enzyme-linked immunosorbent assay measurement of rh-BMP2 concentration in saline. In vivo study: At day 60, rats were evaluated for neurologic deficits before sacrifice. Spines were harvested, and the following studies were performed: 1) manual testing for fusion and bone growth; 2) X-ray evaluation; 3) Micro–computed tomography (micro-CT) scans.

METHODS: In vitro study: Collagen sponges soaked with BMP at two different concentrations were incubated in saline solution with and without encapsulation by fibrin glue. Saline BMP concentrations were measured at consecutive time points. In vivo study: A rat fusion model using rh-BMP2 for fusion has been developed and tested with resultant100% fusion in over 100 rats. Lewis rats were divided into two groups and treated as follows: I: Exposure of L4–L5 transverse processes, decortication, and placement of BMP sponge in the lateral intertransverse space. II: Exposure and decortication as above and placement of fibrin glue before BMP sponge placement.

RESULTS: In vitro study: Peak rh-BMP2 concentrations in saline were 20% and 45% of the maximum possible for fibrin glue encapsulated sponges and controls, respectively, with a more gradual increase to peak concentration in samples encapsulated in fibrin glue. In vivo study: No rats exhibited any neurologic deficits. X-rays revealed at least partial bone formation in all rats. Manual testing of intertransverse fusion spines revealed 100% fusion in rats treated with BMP only, whereas rats treated with fibrin glue before placement of BMP sponges revealed only one possible fusion.

FDA device/drug status: approved but not for this indication (fibrin glue, rh-BMP2).

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Posterior-lateral bone formation was present on X-ray in both groups, and micro-CT imaging revealed bridging bone from transverse processes to the BMP-stimulated bone in the control groups. In spines treated with fibrin glue before rh-BMP2 placement, bone formation could still be seen within the soft tissues; however, bridging bone connecting to the transverse processes was either significantly decreased or not present.

CONCLUSIONS: Fibrin glue can limit rh-BMP2 diffusion. Also, because it limited bone formation at the transverse processes, it can be inferred that fibrin glue can limit bone formation when used to separate areas of desired bone formation from areas where bone formation is not desired.

Keywords: Bone morphogenetic protein; BMP; Rh-BMP2; Fibrin glue; Fusion; Bone; Bone growth; Spine; Rat; Diffusion

Introduction

Recombinant human bone morphogenetic protein-2 (rh-BMP2) is rapidly becoming popular for augmenting spine fusion in the lumbar and cervical spine. Its use has been reported in anterior and posterior lumbar and cervical fusion surgery, as well as transforaminal lumbar interbody fusion and posterior lumbar interbody fusion type procedures. Bone morphogenetic protein (BMP) may, however, stimulate bone growth in areas where bone is not desired, especially as the material ''leaks'' into such spaces. McKay and Poynton have reported such heterotopic bone growth into the spinal canal and neural foramina [1,2]. More recently, a letter of caution by Medtronic-Sofamor Danek implied that rh-BMP2 use may be related to increased soft-tissue swelling in the cervical spine, the most detrimental effect of which would be airway compromise. Though these complications have thus far only been reported with rh-BMP2, they will likely present as use of additional proteins and osteogenic agents increases. While our ability to stimulate bone growth rapidly increases, methods to control such adverse events have not yet been reported or tested.

Fibrin glue has been used as a carrier of many osteoinductive materials including BMP and demineralized bone matrix [3–7] as well as osteogenic cells [8–10]. It has also been used to improve the material handling of bone graft and bone graft substitutes [11–13]. Thus, it appears to have the ability to temporarily contain such materials before implantation, yet release them in vivo over time while itself being completely absorbed. Indeed Hattori postulated that fibrin glue could control the diffusion of BMP [4]. Conversely, fibrin glue should be able to temporarily control the flow of such biologic agents into areas where bone formation may not be desired. This control of bone formation would depend both on the rate of fibrin glue degradation and on the time course of stimulation of osteogenesis. Fibrin glue resorption occurs over the first 7–14 days after implantation [14], whereas rh-BMP2 concentration decreases to less than 50% concentration 2 days after implantation [15,16]. Thus, fibrin glue could potentially protect neural elements from unwanted bone formation during the peak activity of BMP.

Regarding direct osteoinductive properties of fibrin glue, conflicting reports have shown fibrin glue to augment [11,17] and inhibit [18–21] bone healing and bone formation. Though fibrin glue appears to limit BMP diffusion [4], its efficacy at modulating the osteogenic effect of BMP has not been proven.

The purpose of this two-phase study is to evaluate the use of fibrin glue to limit both the diffusion of rh-BMP2 and its in vivo osteogenic effects. The first phase is an in vitro study that measures diffusion of two different concentrations of rh-BMP2 through fibrin glue. The second, a rat study, uses fibrin glue to block bone formation at transverse process fusion sites. The posterolateral rat fusion model was chosen because this is a proven model of transverse process fusion in rats with robust bone formation after simple decortication and application of rh-BMP2. Thus, if fibrin glue can limit bone formation in this model, it is expected to perform similarly in areas where bone formation is less robust.

Materials and methods

The materials used in all aspects of this study were Tisseel fibrin glue (Baxter Healthcare) and commercially available Infuse brand rh-BMP2 (Sofamor Danek) at a concentration of either 0.032 mg/mL or the standard 1.5 mg/mL with the accompanying collagen sponge cut to appropriate size (5×10 mm or 5×5 mm). The concentration approved for human use, 1.5 mg/mL, was used for one segment of the diffusion study. The reduced concentration of BMP was used for rat fusion as it was found to be the minimum concentration required for 100% successful stimulation of rat intertransverse process fusion (unpublished data from previous dose–response studies in our laboratory). At this concentration, transverse process decortication and placement of BMP sponges stimulates fusion 100% of the time without need for local bone or additional treatments.

In vitro study

Fibrin glue was tested for its ability to limit diffusion of BMP. Collagen sponges were soaked with rh-BMP2 in accordance with the package instructions at both 0.032 mg/mL and 1.5 mg/mL concentrations (Infuse,