

Review Article

Bone morphogenetic protein-2 and spinal arthrodesis: the basic science perspective on protein interaction with the nervous system

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Abstract

The use and “off-label” indications for recombinant human bone morphogenetic protein-2 (rhBMP-2) in spinal arthrodesis have been significantly expanded over the last decade. New surgical approaches and pathologies treated often place the exogenous protein near the spinal cord or peripheral nerves, yet little data exist to the potential interaction between rhBMP-2 and the nervous system. The current review was undertaken to provide a basic science perspective on the wide-ranging effects that rhBMP-2, a potent growth factor, has on the injured spinal cord and the local dorsal root ganglia (DRG). Results from the early animal studies on neural safety of rhBMP-2 were compared with the more recent in vivo work characterizing protein impact on the injured spinal cord. Potential mechanism of the rhBMP-2–induced radiculitis after lumbar arthrodesis is also discussed.

The original pre-FDA approval animal study did not uncover any interaction between rhBMP-2 and the spinal cord or the nerve rootlets comprising the cauda equina. Recent in vivo work indicated, however, that in a penetrating injury model, rhBMP-2 triggers direct signaling in all spinal cord cells. In the rat, this interaction was deleterious to spontaneous recovery by exacerbating the inflammatory response to injury, increasing the glial scar, and making it more inhibitory to axonal regeneration. With respect to posterolateral lumbar arthrodesis in a noninjury model, rhBMP-2 use contributed to a transient postoperative mechanical hyperalgesia. Potential mechanism of this allodynia is through an observed inflammatory response within and around the local DRG.

In summary, contrary to the original beliefs in the clinical community, rhBMP-2 does elicit a profound signaling response within the spinal cord and the peripheral ganglia. Recent preclinical studies indicate that rhBMP-2, if provided direct access to the spinal cord parenchyma or the DRG, can trigger significant inflammation and morphologic changes within these tissues that could be deleterious to neurologic recovery. © 2011 Elsevier Inc. All rights reserved.

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Introduction

Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been used in clinical practice as an alternative to autologous bone grafting since its original Food and Drug Administration (FDA) approval in 2002 [1]. Despite the FDA approval of only a specific indication for its use in an anterior lumbar interbody fusion with a specific threaded cage, some surgeons have expanded rhBMP-2 indications to assist with obtaining spinal arthrodesis [2,3]. Specifically, a recent review of the Nationwide Inpatient Sample database by Ong et al. [4] indicated that in 2007 over 85% of all surgeries using the product were for apparently “off-label” applications. However, with the growing

volume of surgeries involving rhBMP-2, along with an increasing number of spine surgeons using the product in a variety of surgical approaches and spinal pathologies, additional complications have emerged. Among them are apparent neurological complications that were not reported during the early stages of rhBMP-2 development and clinical application [5–8]. Surprisingly, there are still limited basic science data concerning the direct effects of exogenous application of rhBMP-2 around the spinal cord and nerve roots.

Early work appeared to indicate little perceptible morbidity of rhBMP-2 on neurologic tissues. In 1999, Meyer et al. [9] performed a study in the dog model and reported on the safety of rhBMP-2 when used in a laminectomy defect in the lumbar spine. A critical review of the study reveals, however, that the authors only evaluated undecalcified plastic-embedded histological sections from the lumbar spine, that is, a histological examination of the roots or cauda equina using modern immunohistochemical (IHC) techniques was not reported. Furthermore, the authors applied a total of 0.24 mg of bone morphogenetic protein-2 (BMP-2), which would be equivalent to a human dose of 1.68 mg of rhBMP-2 (for a 70 kg patient based on the weight/dose ratio). This amount is significantly less than the clinical dosage used during spinal arthrodesis in adult patients undergoing lumbar surgery.

Meyer et al. acknowledged that BMP receptors are present in various central and peripheral nervous system cells. Use in the lumbar spine will more typically expose roots and cauda equina to rhBMP-2, as the spinal cord most often terminates at the midbody of the L1 vertebra in humans. The nerve rootlets comprising the cauda equina are technically part of the peripheral nervous system, leaving the effects on the spinal cord fully unknown.

At the time of publication, Meyer et al. concluded that although rhBMP-2 elicited some bone formation at the laminectomy site, it did not cause any clinically significant neurological sequelae for the animals. Since that report, the vast majority of preclinical studies have focused on the efficacy of rhBMP-2 in eliciting bone formation and enhancing fusion rates, whereas little work has been done characterizing its potential interaction with the spinal cord, nerves, or surrounding tissues [10–16].

Today, a number of postoperative complications including soft-tissue swelling, edema, heterotopic bone formation, and radiculitis have been described in the literature [17–20]. An increased rate of retrograde ejaculation was noted in the original FDA trial on rhBMP-2 use in anterior lumbar interbody fusion [21]. Similarly, FDA documents noted that back and leg pain adverse events were markedly higher in the early postoperative period after posterolateral fusion with both INFUSE and AMPLIFY (Medtronic Biologics, Inc., Memphis, TN, USA) formulations of rhBMP-2 [22].

As most trials of “off-label” use of rhBMP-2 have been for the treatment of degenerative spinal disorders, little is known about the potential hazards of using this product

in other clinical circumstances. Spinal column trauma/fractures, similar to the FDA-approved use in open tibial fractures, could be a potential indication for rhBMP-2. Fractures and dislocations to the vertebral column are often associated with varying degrees of spinal cord, dural, or nerve root injury. Disruption in the mechanical barriers could expose the spinal cord parenchyma and other neurological tissues to the exogenous rhBMP-2 protein if placed in close proximity during surgical intervention. Similarly, in a non-trauma induced cervical myelopathy, there is always some degree of spinal cord damage, which may also affect barrier integrity, thus providing a potential access route for rhBMP-2 to the spinal cord if used in surgical management of this condition.

Driven by the paucity of basic science data on this subject, our group initiated a series of *in vivo* studies that aimed to ascertain the effects of rhBMP-2 on neural tissues when used during spinal arthrodesis after a spinal cord injury (SCI).

Spinal cord injury and acute response with rhBMP-2 exposure

In our initial study, we evaluated whether rhBMP-2 applied on an absorbable collagen sponge could diffuse within the spinal cord parenchyma and elicit a functional signaling cascade [23]. After a penetrating SCI, we performed a posterolateral arthrodesis, with or without rhBMP-2, at intervals ranging from 30 minutes to 21 days after injury. The extended time line allowed us to evaluate whether rhBMP-2 could enter the spinal cord and if so, whether this is dependent on the integrity of the blood spinal cord and meningeal barriers. Through immunohistochemical analysis, we explored whether there was BMP-specific signaling (phosphorylated Smad1/5/8 proteins) in neurons, astrocytes, oligodendrocytes, macrophages, and activated microglia, as well as in invading meningeal fibroblasts.

Overall, we observed a profound increase in the number of pSmad 1/5/8-positive cells within the spinal cord when rhBMP-2 was introduced to the spine at 30 minutes, 24 hours, or 7 days after an SCI. Bone morphogenetic protein-specific intracellular signaling activation confirmed our hypothesis that the protein can diffuse out of the carrier sponge, enter the spinal cord, and trigger functional changes in the central nervous system. In addition, we observed increased pSmad 1/5/8-positive staining in the inflammatory cells surrounding the lesion, which could lead to an exacerbated post-SCI inflammation. Interestingly, rhBMP-2 implantation to the spine at 21 days after SCI did not elicit an appreciable increase in pSmad 1/5/8 immunoreactivity within the spinal cord when compared with control rats. This time point correlated with restoration of the blood spinal cord barrier; therefore, we believe that significant intraparenchymal rhBMP-2 infiltration is directly dependent on the integrity of the protective barriers.

Our data showed that all endogenous spinal cord cells, as well as invading macrophages, are functionally

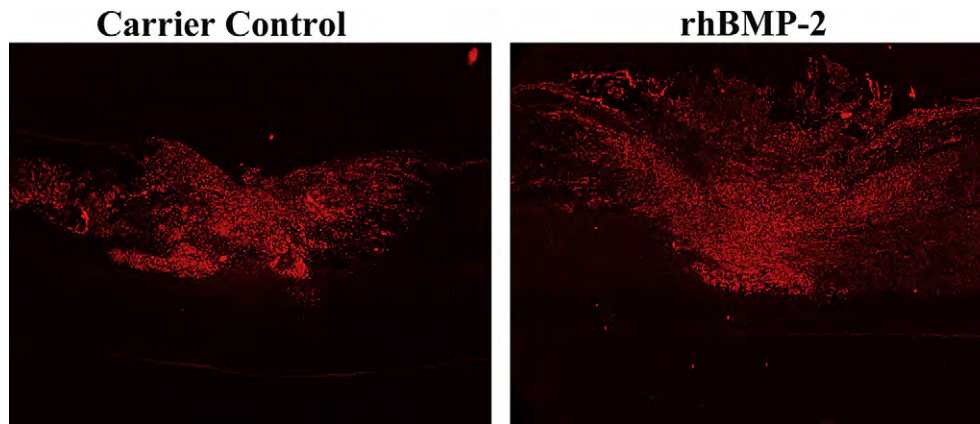


Fig. 1. Longitudinal sagittal sections through the lesioned spinal cord 7 days after SCI and arthrodesis. (Right) Increased macrophage and activated microglia response in the recombinant human bone morphogenetic protein-2–treated rats. Magnification 20 \times .

responsive to rhBMP-2. This initial project did not, however, provide insight into any morphologic or functional changes resulting from rhBMP-2 signaling after an SCI.

Spinal cord injury, morphologic changes, and functional recovery with rhBMP-2 exposure

We, therefore, performed a second investigation using the same rat model of penetrating SCI [24]. In this *in vivo* study, rats received either rhBMP-2 or saline on an absorbable collagen sponge 30 minutes after a spinal cord hemisection and were followed for 1 or 6 weeks post-operatively. The rats' functional recovery was tested, and spontaneous changes in fine and gross motor control were recorded. After sacrifice, spinal cord morphology was examined for the presence of the extracellular matrix proteins, specifically chondroitin sulfate proteoglycans, which are highly inhibitory to axonal regeneration [25]. We were also interested in whether an inflammatory response, previously documented in other soft tissues with rhBMP-2 exposure, would be exacerbated in the central nervous system tissues

examined in this model. In addition, we wanted to evaluate whether any observed changes appeared to affect functional recovery from the experimental SCI.

Postmortem analysis of rats sacrificed at 1 week after lesion indicated a number of morphologic changes in rhBMP-2–treated animals. Specifically, rhBMP-2 implantation triggered a profound inflammatory reaction within the spinal cord parenchyma as evidenced by increased ED-1 staining for activated microglia and invading macrophages (Fig. 1). Compared with the saline control group, ED-1 staining in spinal cords from rhBMP-2–treated animals was 81% greater. Additionally, glial fibrillary acidic protein staining for reactive astrocytes bordering the lesion was increased by 181%, and meningeal fibroblast labeling was 157% higher in these animals. Chondroitin sulfate proteoglycan staining revealed a twofold increase in protein immunoreactivity in the rhBMP-2–treated animals compared with saline controls. Increased astrocyte reactivity contributes to a larger glial scar forming within the spinal cord, which constitutes a physical and chemical barrier to axonal regeneration after injury (Fig. 2). The chemical composition of the scar, including the presence of chondroitin

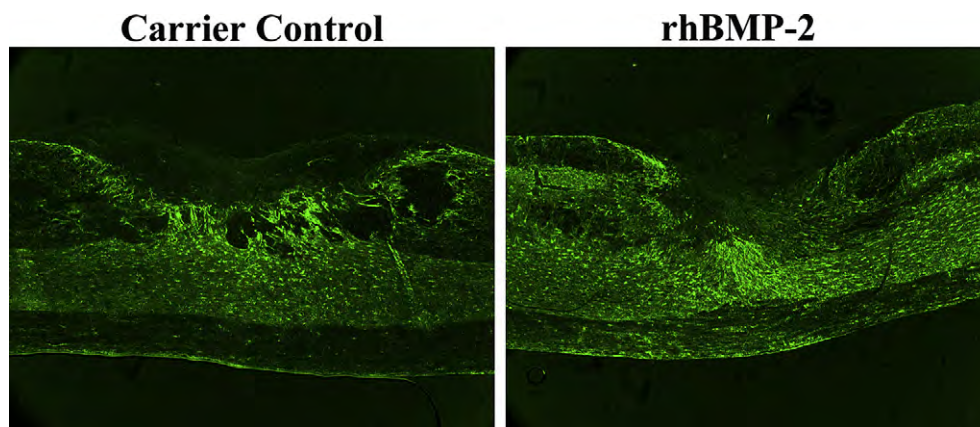


Fig. 2. Glial fibrillary acidic protein staining of longitudinal sagittal sections through the lesioned spinal cord taken 7 days after SCI and arthrodesis. (Right) Recombinant human bone morphogenetic protein-2 treatment contributed to the increased astroglia around the lesion epicenter. Magnification 20 \times .

sulfate proteoglycans, adds to the nonpermissive nature of the scar and can further limit spontaneous recovery.

Proinflammatory effects of rhBMP-2 on the surrounding musculature have been recently reported [6,8,26]; however, our study was the first to document a similar response within the spinal cord [24]. In the central nervous system, the extent of postinjury inflammation has been directly correlated with the severity of persistent neurologic dysfunction [27]; therefore, the rhBMP-2–induced macrophage response observed in our study could serve as an impediment to neurologic recovery. In fact, at 1 week after lesion, we observed a pronounced worsening of functional recovery in rats treated with rhBMP-2 in both fine motor control and open-field ambulation.

Postmortem evaluation of the 6-week group revealed similar trends in morphologic differences between the spinal cords of rhBMP-2 and control-treated rats. These observed alterations appeared to result from a direct deleterious action of rhBMP-2 on the injured spinal cord after SCI and not secondary to mechanical compression of the spinal cord by the maturing fusion mass. This was demonstrated by controlling for mechanical compression with micro-computed tomography scanning, which revealed no evidence of bone encroaching into the spinal canal.

At 6 weeks after lesion, intraparenchymal inflammation remained elevated in the rhBMP-2–treated rats. Likewise, astrocyte immunoreactivity around the lesion core was more than 50% greater than in the untreated controls, and staining for an inhibitory extracellular matrix protein, NG2, was significantly elevated in the rhBMP-2 group. Functionally, we continued to see differences in the change of hind-paw angle of rotation, which is a measure of fine motor control disruption; however, gross locomotor activity was equivalent between the groups.

An unexpected finding observed in this study was the difference in postoperative onset of autophagia or self-mutilation between the rhBMP-2–treated and control rats. In the rat model of SCI, postinjury autophagia may indicate neuropathic pain [28]. In our study, 56% of rhBMP-2–treated rats, compared with 31% of control rats, required treatment for

autophagia. Intraparenchymal inflammation and astrogliosis are associated with nociception [29,30]; however, additional research is needed to determine whether rhBMP-2 has a direct effect on inducing autophagia.

Future directions of research on rhBMP-2 effects on neurologic tissue

Currently our group is engaged in a further in vivo rat study aimed at evaluating the effects of posterolateral arthrodesis with rhBMP-2 in the lumbar spine on morphologic changes within the dorsal root ganglia (DRG) and spinal cord that could lead to the development of postoperative radiculitis (Table).

Our initial data indicate that rats treated with rhBMP-2 develop heightened mechanical hyperalgesia 3 days after surgery, which persists through day 7 but dissipates by the tenth postoperative day. On the cellular level, postmortem IHC assessment of DRG samples taken from animals treated for 7 days with rhBMP-2 showed increased macrophage staining (ED-1) (Fig. 3).

This finding coincides with the clinical reports on early postoperative radiculitis [31]. As our study was performed in the rat model, the time line of symptom resolution (10 days postoperatively) is most likely extended in a similar clinical scenario. The rate of peripheral axon regeneration, neurologic recovery, and the associated processes are faster in the rat; therefore, a 10-day course of postoperative allodynia observed in the rat may last for months in human patients [32]. In support of this assumption, the FDA clinical data showed the radiculitis effect to last around 3 months after surgery [33]. Despite the challenges in drawing direct conclusions as to a possible time course of the rhBMP-2–induced hypersensitivity or pain after spinal fusion in humans, our present study successfully replicated the observed phenomenon. This in turn will allow our group and others to further investigate these effects and devise strategies to avoid or manage the complication. Based on our preliminary data, the mechanism of this allodynia

Table

Summary of the basic science findings and possible clinical correlations

Animal procedure	Tissue evaluated	Observed histological effects with rhBMP-2	Observed behavioral effects with rhBMP-2	Clinical correlation
Penetrating SCI	Spinal cord	Increased inflammation, glial scar, and inhibitory extracellular matrix proteins	Decreased open-field ambulation and fine motor control at 1 wk Persistent fine motor deficits at 6 wk compared with SCI+carrier control	Possible impaired or delayed SCI recovery
Lumbar posterolateral arthrodesis	L4–L5 DRG/nerve root	Profound macrophage infiltration in and around DRG	Increased mechanical allodynia between 3 and 10 d postoperatively	Radiculitis after PLF/PLIF/TLIF [31,33]

rhBMP-2, recombinant human bone morphogenetic protein-2; SCI, spinal cord injury; DRG, dorsal root ganglia; PLF, posterior lumbar fusion; PLIF, posterior lumbar interbody fusion; TLIF, transforaminal lumbar interbody fusion.

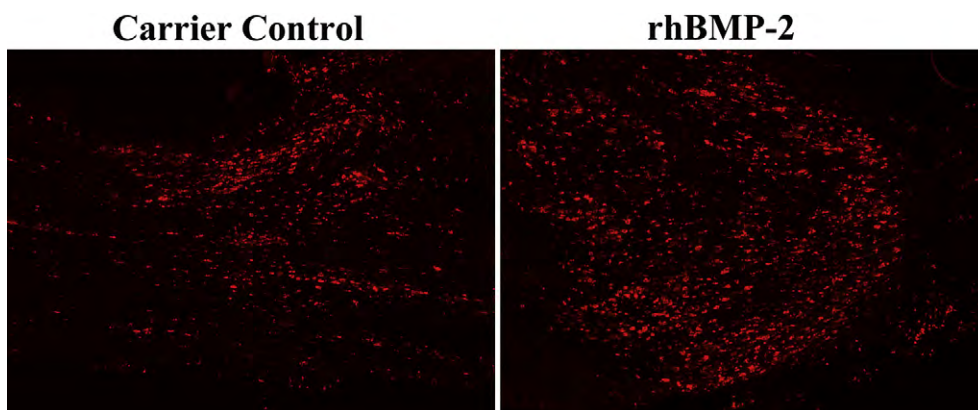


Fig. 3. Immunohistochemical staining of the L5 dorsal root ganglia (DRG) with ED-1. (Right) Upregulated macrophage activity is evident in rats undergoing L5–L6 spinal arthrodesis with rhBMP-2 compared with the DRG from the carrier control group (Left). Magnification 100 \times .

response is potentially through increased inflammation in and around the DRG.

Conclusion

These studies are the first to highlight some of the direct deleterious effects, at the cellular level, of exogenous high-dose rhBMP-2 on the central and peripheral nervous system. Although there is little doubt that rhBMP-2 and similar growth factors may promote bone induction, the relative benefits of rhBMP-2 fusion rates compared with potential and observed complications have not been well reported or analyzed, particularly in “off-label” indications. However, the range of negative or adverse effects with the use of this product has only recently become the subject of systematic research. Although our studies were performed in a rodent model, they raise some very important questions to the true impact of rhBMP-2 when applied around cells of the nervous system. And although rhBMP-2 has a well-established and proven role for certain specific indications, its dosage, delivery route, and carrier materials, and the mechanism of each contributing to observed complications, warrant significant further evaluation.

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