BMP-2-induced bone formation and neural inflammation

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ABSTRACT

Bone morphogenetic protein-2 (BMP-2), a potent osteoinductive cytokine from the transforming growth factor beta (TGF- β) family, is currently the most commonly used protein-based bone graft substitute. Although clinical use of BMP-2 has significantly increased in recent years, its prominence has also highlighted various adverse events, including induction of inflammation. This review will elucidate the relationship between BMP-2 and inflammation, with an emphasis on peripheral nerve inflammation and its sequelae. As well, we review the potential additive roles of nerve released factors with BMP2 in the context of bone formation.

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1. Introduction

A member of the transforming growth factor beta (TGF- β) superfamily, bone morphogenetic protein-2 (BMP-2) is an osteoinductive growth factor that was first identified in 1965 by Marshall R. Urist, who discovered its ability to induce osteogenesis when implanted into extraskeletal soft tissue.^{1,2} Currently the most commonly used protein-based bone graft substitute,³ BMP-2 was first introduced for single-level anterior lumbar interbody fusion (ALIF) procedures in 2002.^{4,5} Subsequently, BMP-2 was approved for tibial nonunions in 2004 and oral maxillofacial reconstructions in 2007.⁶ Correspondingly, the use of BMP-2 as a clinical alternative to autograft bone increased from 0.7% in 2002 to 25% of all primary spine fusions and 40% of all revision spine fusions in 2006 in the United States alone.⁷ Likewise, another epidemiological study using national administrative data found a 4-fold increase in the annual number of procedures involving BMP from 23,900 to 103,194 between 2003 and 2007.⁸ In addition, 85% of these procedures used BMP-2 in an off-label manner.⁸ However, this rapid growth of BMP-2 use has been accompanied by an emerging list of side effects and complications, including induction of inflammation, fibrosis and heterotopic ossification.³ This review

paper will focus on BMP-2-induced inflammation, focusing on inflammation associated with peripheral nerves and its sequelae.

2. BMP-2 and inflammation

Various studies have elucidated the relationship between BMP-2 and inflammation in both the preclinical and the clinical setting. In addition, several methods to curtail BMP-2 induced inflammation have also been investigated, which will be discussed below.

2.1. Preclinical studies of BMP-2 induced inflammation

Recently we reviewed the side effect profile of BMP-2.³ Both *in vitro* and *in vivo* preclinical studies show that BMP-2 induces inflammation, as evidenced by increased levels of the inflammatory cytokines interleukin (IL)-1b, IL-6, IL-10, IL-17, IL-18, and tumor necrosis factor(TNF)- α .^{9–14} For example, Zara et al. evaluated the dose-dependent effects of BMP-2 in both a rat femoral segmental defect model and a minimally traumatic rat femoral only model.⁹ Whereas low BMP-2 concentrations (5 and 10 μ g/mL) was unable to induce fusion in the femoral segmental defect model, both mid-range (30 μ g/mL) and high BMP-2 concentrations (150, 300, and 600 μ g/mL) resulted in fusion of the defect.⁹ However, while administration of a mid-range BMP-2 concentration fused the femoral segmental defect without adverse effects, use of high BMP-2 concentrations led to the formation of cyst-like bony shells filled with histologically confirmed adipose tissue.⁹ Similarly, a dosage of 4 mg/mL in the femoral onlay model induced

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significant tissue inflammatory infiltrates and exudates that were accompanied by increased osteoclasts at 3, 7, and 14 days compared to control.⁹

In a similar study, Lee et al. observed dose-dependent release of IL-6, IL-10, and TNF- α following subcutaneous and intramuscular implantation of rhBMP-2 into rats.¹¹ Additionally, soft-tissue edema and hematoma formation was also dose-dependent, as inflammatory volumes directly and positively correlated to rhBMP-2 concentrations.¹¹

Huang et al. hypothesized that inflammatory environments contribute to decreased BMP-2 osteoinductive efficacy.¹⁵ In order to recreate pre-operative and post-operative inflammatory responses, thirty-seven Sprague Dawley rats were administered lipopolysaccharide (LPS) injections and were treated with BMP-2/absorbable collagen sponge (ACS) implantation.¹⁵ LPS administration resulted in significant elevation of serum levels of pro-inflammatory cytokines such as TNF- α and IL-1 β and infiltration of inflammatory cells around the BMP-2/ACS implants. Additionally, the bone volume, mineral content, and mineral density of the implants decreased significantly, in conjunction with decreased expression of osteocalcin (OCN), increased osteoclast count, and inhibition of osteoblastic differentiation. These results indicate that LPS induced an inflammatory environment which inhibited the osteogenic effect of BMP-2.

2.2. Clinical studies of BMP-2-induced inflammation

rhBMP2-impregnated bone grafts have been in clinical use since 2006. Since then, case series have been published to describe complications.^{16–23} To determine the cause of complications among patients who had received BMP2, we used the Food and Drug Administration's Manufacturer and User Facility Device Experience (MAUDE: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfmaude/search.cfm>), searching for the INFUSE Bone graft.²³ In total, 10,260 reported adverse incidents were reported since that time. Of these, 16.5% (n = 1698) were due to inflammation/edema/swelling, 15.5% (n = 1588) were due to ectopic bone formation, 59.1% (n = 6071) were due to pain, and 7.5% (n = 768) were due to infection. These findings suggest that inflammation and ectopic bone formation related to BMP2-impregnated bone grafts is a serious clinical concern which deserves further attention.

Clinical observations have confirmed the pro-inflammatory effects of BMP-2. The most common clinical manifestations of BMP-2-induced inflammation include seroma formation and cervical spine swelling. Case studies have shown that BMP-2-induced seroma formation usually occurs during the first postoperative week,^{24,25} and is usually subclinical until impingement upon nearby organs results in either pain or dysesthesias.²⁶ Specifically, one study found that lumbar seroma occurs in 1.2% of rhBMP-2 patients, compared to 0% among control patients.²⁷ Similarly, another study found formation of painful seromas and edema following rhBMP-2 use in posterolateral lumbar spine fusions among 4.6% of patients.¹⁹ Another case study described postoperative seroma formation in the cervical region following BMP-2 use, leading to bilateral paresthesia of the upper extremities which required drainage. The seroma was also accompanied by 3000-fold, 5000-fold, and 34-fold levels of IL-6, IL-8, and TNF- α respectively.²⁴

Another serious side effect of BMP-2 use is cervical spine swelling, which can cause patients to experience dysphagia and difficulty breathing or speaking. However, in the most severe cases, cervical spine swelling can be fatal; the first fatality was reported in 2006, and since then, six more have been reported.²⁸ In 2008, the FDA also received 38 reports of complications due to off-label use of BMP-2 in anterior cervical surgery.²⁹ Consequently, the FDA

issued a black box warning for BMP-2 use in the same year addressing the increased risk of cervical spine swelling and death.²⁹ Thus, the inflammatory side effects associated with BMP-2 use are a substantive area of concern, and novel mechanisms to inhibit BMP-2 induced inflammation are needed.

2.3. Inhibition of BMP-2-induced inflammation

Several studies have shown that BMP-2 associated inflammation can be reversed by the co-administration of anti-inflammatory molecules or modulation of BMP-2 release kinetics. For example, Shen et al. described the suppression of BMP-2-induced inflammation by the osteoinductive growth factor Nel-like protein 1 (NELL-1) in a femoral onlay rat model.¹² Specifically, absorbable collagen sponges saturated with various reagents were implanted into 74 male Lewis rats randomly divided into four experimental groups: (1) phosphate buffered saline (PBS), (2) 0.8 mg BMP-2, (3) 0.8 mg NELL-1, and (4) 0.8 mg BMP-2 + 0.8 mg NELL-1.¹² Evaluation for inflammation was subsequently performed at 3, 7, and 14 days post-operative via ELISA. Whereas BMP-2 treatment increased levels of TNF- α and IL-6, NELL-1 treatment alone did not induce changes in serum levels of these inflammatory cytokines.¹² However, NELL-1 significantly inhibited BMP-2-induced increases of peak serum levels of TNF- α and IL-6 at 7 days post-operative.¹² Mechanistically, NELL-1 inhibits BMP-2-induced transcriptional factor activity of nuclear factor kappa-light-chain-enhancers of activated B cells (NF- κ B), which are located downstream of TNF- α and regulate immune and inflammatory responses.¹² Moreover, attenuation of BMP-2-induced inflammation was also associated with reduced generation of reactive oxygen species (ROS), which can also trigger inflammatory pathways through myeloid cell recruitment.¹²

In another study, Ji et al. elucidated how to deliver and preserve the activity of BMP-2 incorporated into bone grafts more effectively, while also preventing BMP2 induced inflammation.³⁰ Poly(lactic-co-glycolic acid) (PLGA) is an optimal BMP-2 carrier, but is associated with adverse effects including increased inflammation and decreased osteoinductivity.³⁰ Thus, four preparations of composite grafts were created: collagen (control), collagen combined with bone particulates (control), collagen combined with BMP-2/PLGA delayed-release microspheres (control), and collagen combined with both bone particulates and BMP-2/PLGA delayed-release microspheres (experimental) to assess whether these combinations could lead to improved preservation of *in vivo* BMP-2 osteoinductivity.³⁰ Following implantation into the gluteus maximus pockets in rats, ectopic osteogenesis and alkaline phosphatase (ALP) levels were compared among groups as a measure of osteogenic capability. Compared to the three control grafts, the experimental graft composed of collagen, bone particulates, and BMP-2/PLGA delayed release microspheres increased the osteoinductivity of BMP-2, as evidenced by the reduction of required dose of BMP-2 and volume of autologous bone.³⁰ Mechanistically, this was attributed to the ability of collagen to tightly bind both the bone particulates and the BMP-2/PLGA delayed release microspheres to host bone.³⁰ This enabled BMP-2 to adhere to the bone particulates more efficaciously, resulting in increased osteoinductivity.³⁰ Additionally, collagen prevented aseptic inflammation by filling in the gaps between separated microspheres, resulting in isolation of acidic PLGA degradation products.³⁰ Thus, this study showed that the use of collagen plays a critical role in improving the efficacy of composite graft design by increasing BMP-2 osteoinductivity and minimizing aseptic inflammation.

Recently, Agarwal et al. showed that rapamycin, a potent inhibitor of mammalian target of rapamycin (mTOR) reduces the inflammatory response to hyperactive BMP signaling while

preserving the osteogenic capacity of BMP. Consistent with other reports, they found that rapamycin may actually increase osteogenic differentiation. These findings suggest that the osteogenic and inflammatory effects of BMP are potentiated by separate mechanisms which can be differentially targeted.

Furthermore, Xiong et al. utilized human dose equivalent dexamethasone (HDE) in a rodent model of soft-tissue inflammation to treat BMP-2 adverse reactions.³¹ Compared to BMP-2 only positive controls and phosphate buffer negative controls, rodents that received BMP-2 paraspinal implants with administration of 5, 10, or 15 mg of HDE demonstrated significantly less inflammation.³¹ Specifically, MRI showed a 49% decrease in inflammatory edema volume and histopathological analysis demonstrated a 29% decrease in inflammatory cross-sectional area in the 5, 10, and 15 mg groups compared to control.³¹ In addition, immune cellular infiltration decreased 39% in the 15 mg group compared to control, and either 10 or 15 mg of HDE was sufficient to prevent gross anatomical inflammatory exudates.³¹ Thus, although BMP-2-induced inflammation is of concern, these studies show that there are preventative mechanisms that may counteract this adverse effect.

3. BMP-2 and peripheral neuritis

As previously mentioned, BMP/INFUSE by Medtronic was approved by the Food and Drug Administration in 2002 for use in single-level anterior lumbar interbody spinal fusions (ALIF) for patients with degenerative disk disease.³² Since then, the use of BMP2 in spinal fusion surgery has rapidly grown, and in 2009, a study by Cahill et al. reported that rhBMP was used in 40% of all lumbar spinal fusions and 25% of all spinal fusion procedures in the United States.⁷ However, various clinical side effects of rhBMP-2 treatment are apparent during procedures involving the spine and include neuritis resulting in transient leg pain.

Incidences of radiculitis are a common postoperative effect of transforaminal lumbar interbody transfusion (TLIF) procedures due to BMP-2 induced inflammation of the nerve root³³ or leakage into the epidural space, resulting in root nerve compression by reactive edema³⁴ or ectopic bone impingement.^{21,27,35,36} In one study, 14% of patients who received rhBMP-2 experienced radiculitis in comparison with 3% in the control group.²⁷ Similarly, in another TLIF study by Mindea et al., BMP-2 treatment resulted in 11.4% radiculitis incidence compared with 0% in the control group.³⁷ Yet another study in TLIF by Villavicencino et al. reported that 8 out of 48 patients who were treated with rhBMP-2 experienced postoperative radiculitis.²¹

Similar results have been observed in clinical trials for BMP-2 treatment in posterior lumbar interbody fusion (PLIF). In 1999, a clinical trial that compared iliac crest bone grafts with rhBMP-2 treatment was suspended early due to radiographs finding that 75% of patients who received the rhBMP-2 had heterotopic ossification in the spinal canal or neuroforamina, compared with 13% in the iliac crest group.^{38,39} Additionally, the rhBMP-2 group had increased instances of leg pain, consistent with peripheral neuritis.

BMP-2-induced peripheral neuritis can also manifest as post-operative leg pain following spinal fusion.⁴⁰ Rowan et al. reported increased incidence of 25% post-operative leg pain in rhBMP-2 patients compared with 12% non-rhBMP-2 patients.⁴⁰ This pain was independent of nerve root compression in 17.2% of rhBMP-2 patients and only 7.5% of the non-rhBMP2 patients.⁴⁰ Pain reported in these accounts was mostly transient, as follow-up leg pain was only present 11.6% and 7.6% in rhBMP-2 and non-rhBMP-2 groups, respectively.⁴⁰ In conclusion, rhBMP2 administration to spinal fusion patients has been associated with radiculitis, resulting in leg pain.

4. The role of BMP-2-induced nerve inflammation in bone formation

Although BMPs, including BMP-2, are normally expressed in peripheral nerves, BMP signaling is enhanced at the time of peripheral nerve damage.⁴¹ Tsujii et al. found that BMP-2 is normally expressed in nodes of Ranvier in the sciatic nerves of rats at the proximal and distal edges of myelinating Schwann cells, and that other BMPs and BMP receptors such as BMP-7 and BMPR-1 B are also normally expressed in axon-Schwan cell units.⁴¹ Following transection of the nerve, there were changes in BMP-2 levels, which decreased at the distal stump and led to neuronal degeneration at the proximal stump.⁴¹ Thus, these findings imply that BMP-2 plays a role in injury response in peripheral nerves.

Ai et al. described how members of the TGF β family including activin and BMPs induced the expression of calcitonin gene-related peptide (CGRP) in embryonic sensory neurons *in vitro*.⁴² Expressed in dorsal root ganglion neurons, CGRP is a neuropeptide that functions as a vasodilator and mediator of neuroinflammation and pain sensation.⁴² Utilizing a serum-free culture system, Ai et al. showed that activin, BMP-2, BMP-4, and BMP-6 induced *de novo* CGRP expression in a concentration-dependent manner in 60% of embryonic sensory neurons.⁴² Furthermore, dorsal root ganglion neurons express BMP receptors, and embryonic skin and gut produce BMP mRNA, implicating this signaling pathway in the regulation of CGRP expression.⁴² Similarly, Bucelli et al. found that BMP-2 upregulates both CGRP and Substance P (SP), another inflammatory neuropeptide, in a concentration-dependent manner in nerves derived from the dorsal root ganglia of Holtzman rats.⁴³ Moreover, Shih et al. showed that Substance P and CGRP both promote osteogenic differentiation *in vitro*.^{44,45} These studies show that BMP-2 plays a critical role in neuroinflammation.

Various studies have also shown how the dual delivery of BMP-2 and SP promotes osteogenesis. For example, La et al. demonstrated that the dual delivery of BMP-2 and SP onto graphene oxide (GO)-coated titanium (Ti) implanted in mouse calvaria optimized bone formation in comparison to controls.⁴⁶ Specifically, GO-coated Ti induced sustained release of BMP-2 for up to 14 days, and mRNA expression of ALP was significantly increased in Ti/GO/BMP-2 implants compared to Ti/BMP-2.⁴⁶ Moreover, mice with Ti/GO/BMP-2/SP implants showed the most extensive bone formation.⁴⁶ Additional *in vitro* experiments elucidated the role of SP and showed that SP stimulates mesenchymal stem cell (MSC) migration in collagen gel, indicating that SP promotes recruitment of MSCs to enhance bone formation.⁴⁶ Thus, this study shows that the inflammatory neuropeptide SP plays a synergistic role in BMP-2-induced osteogenesis.

In a similar study, Noh et al. also proposed that dual delivery of SP and BMP-2 promotes MSC recruitment to facilitate bone regeneration in a mouse calvarial defect model. Specifically, a heparin-conjugated fibrin (HCF) gel which enabled fast delivery of SP and slow release of BMP-2 was optimal, as this design resulted in rapid recruitment of endogenous MSC to the tissue regeneration site but allotted more time for subsequent osteogenic differentiation.⁴⁷ Compared to the SP and BMP-2 groups, dual delivery of SP and BMP-2 showed significantly higher bone regeneration, as indicated by osteocalcin expression and X-ray analysis of calvarial defects 8 weeks post-implantation.⁴⁷

CGRP is another inflammatory neuropeptide that plays a critical role in osteogenesis. For example, Tian et al. investigated the cross-talk between CGRP and BMP-2 during *in vitro* osteogenesis in human osteoblast-like cells.⁴⁸ CGRP induced osteoblast differentiation of MG-63 cells, as evidenced by significant increased protein expression levels of ALP, osteocalcin, and collagen Ia1.⁴⁸ To determine whether osteogenic differentiation occurred via a BMP-2-dependent pathway, the MG-63 cells were pretreated with

the BMP pathway inhibitor Noggin before incubation with CGRP.⁴⁸ Compared to the CGRP control group, the Noggin-treated CGRP group showed significantly decreased levels of ALP, osteocalcin, and collagen Ia1, indicating that the BMP-2 pathway is needed to mediate CGRP-induced osteogenic differentiation.⁴⁸

5. Conclusion

In summary, various studies have delved into the relationship between BMP-2 and inflammation. For example, both *in vitro* and *in vivo* pre-clinical studies have demonstrated that BMP-2 induces increased levels of the inflammatory cytokines IL-1b, IL-6, IL-10, IL-17, IL-18, and TNF- α . Furthermore, inflammatory environments contribute to decreased BMP-2 osteoinductive efficacy. Various case studies have also highlighted how the most common clinical manifestations of BMP-2-induced inflammation include seroma formation, cervical spine swelling, and radiculitis. Moreover, BMP-2 upregulates expression of the neuroinflammatory proteins CGRP and SP, which then contribute to bone regeneration. In conclusion, despite the dramatic increase in clinical use of BMP-2 as an alternative to autograft in recent years, it is important to recognize its various adverse effects that can arise from induced inflammation, and how this inflammation subsequently impacts BMP-2 osteoinductivity.

Conflict of interest

The authors have none to declare.

Authors contributions

Role for VA, CAM, NY, SA, BL: literature review and manuscript preparation, AWJ: manuscript preparation and final approval.

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