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The Use of Recombinant Human Bone Morphogenetic Protein 2 (rhBMP-2) to Promote Spinal Fusion in a Nonhuman Primate Anterior Interbody Fusion Model

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Abstract

Study Design. A study on the efficacy of recombinant human bone morphogenetic protein 2 (rhBMP-2) in a nonhuman primate anterior interbody fusion model.

Objectives. To investigate the efficacy of rhBMP-2 with an absorbable collagen sponge carrier to promote spinal fusion in a nonhuman primate anterior interbody fusion model.

Summary of Background Data. RhBMP-2 is an osteoinductive growth factor capable of inducing new bone formation *in vivo.* Although dosage studies using rhBMP-2 have been performed on species of lower phylogenetic level, they cannot be extrapolated to the primate. Dosage studies on nonhuman primates are essential before proceeding with human primate application.

Methods. Six female adult Macaca mulatta (rhesus macaque) monkeys underwent an anterior L7-S1 interbody lumbar fusion. All six sites were assigned randomly to one of two fusion methods: 1) autogenous bone graft within a single freeze-dried smooth cortical dowel allograft cylinder (control) or 2) rhBMP-2-soaked

absorbable collagen sponges within a single freeze-dried smooth cortical dowel allograft cylinder also soaked in rhBMP-2. The animals underwent a baseline computed tomography scan followed by 3- and 6-month postoperation scans. Anteroposterior and lateral radiographs of the lumbosacral spine were performed monthly. After the monkeys were killed, the lumbar spine fusionsites were evaluated. Histologic evaluation of all fusion sites was performed.

Results. The three monkeys receiving rhBMP-2-soaked collagen sponges with a freeze-dried allograft demonstrated radiographic signs of fusion as early as 8 weeks. The control animals were slower to reveal new bone formation. The computed tomography scans revealed extensive fusion of the L7-S1 lumbar vertebrae in the group with rhBMP-2. A pseudarthrosis was present in two of the control animals.

Conclusions. This study was able to document the efficacy of rhBMP-2 with an absorbable collagen sponge carrier and a cortical dowel allograft to promote anterior interbody fusion in a nonhuman primate model at a dose of 0.4 mg per implant site (1.5 mg/mL concentration). The rate of new bone formation and fusion with the use of rhBMP-2 and cortical dowel allograft appears to be far superior to that of autogenous cancellous iliac crest graft with cortical dowel allograft.

Keywords:

interbody fusion; primate; recombinant human bone morphogenetic protein; spine fusion

Spinal fusion is a common surgical procedure used to manage a variety of disorders. Lumbar fusion ranks as the second most common lumbar spine procedure, with 25 lumbar fusions performed per 100,000 persons.[10] Between 1988 and 1990, more than 62,000 lumbar fusions were performed annually in the United States, with a failure rate of 20% to 40%.[31]

Currently, spinal fusion usually involves harvest of bone autograft from the pelvis. Donor site complications and morbidity have been estimated at 8% to 25%.[7,9,30,39] Documented donor site complications have included pain, nerve and arterial injury, peritoneal perforation, sacroiliac joint instability, and herniation of abdominal contents through defects in the ilium.[13] In addition, the volume of bone extracted from the donor site often is insufficient.

Methods used to achieve spinal fusion include posterior (intertransverse process, facet, lamina) techniques and anterior (intervertebral body) techniques.[36] Although posterolateral intertransverse process fusion is the most common type of fusion performed in the lumbar spine, the nonunion rate reportedly has ranged from 5% to 35% for a single-level fusion.[29] The advantages of anterior interbody arthrodesis include stabilization of the anterior (anterior halves of the vertebral bodies) and middle (posterior halves of the vertebral bodies) columns of the spine, whereas a posterolateral arthrodesis stabilizes only the posterior column.[25,36]Additionally, anterior interbody fusion may address directly the problem of intervertebral disc degeneration and related pain.

Bone morphogenetic proteins (BMPs) are a group of related proteins originally identified by their presence in bone-inductive extracts of demineralized bone.[36] Recently, they have been synthesized via recombinant techniques using Chinese hamster ovarian cells.[34] At least six related members of the BMP family have been identified via molecular cloning (BMP-2 to BMP-7). These BMPs are part of the transforming growth factor beta (TGF-β) superfamily.[22,38] They have the biologic capacity to induce the differentiation of perivascular mesenchymaltype cells into cartilage, which then is replaced by bone through a process of enchondral ossification.

The predominant component of BMP has a molecular weight of 17,500 Kd. It behaves as a physiologic entity and transfers across membranes according to the laws of diffusion of small molecules.[33] Osteogenic proteins such as BMP, with complete host acceptance and biologic integration, are an attractive alternative to autogenous bone

grafts.[1] In general orthopedics, BMP has clinical potential as a bone graft substitute and for use in spinal surgery.[15]

Currently, little lumbar spinal fusion data on nonhuman primates is available for comparison. A recent study by Boden et al[5] showed that an eightfold increase in the bovine-derived osteoinductive growth factor concentration (μg bone protein/g demineralized bone matrix carrier) was needed to induce adequate bone formation in the rhesus monkey spine (posterolateral lumbar intertransverse spine fusion) compared with that used in previous rabbit studies. Previous studies achieved bone induction in baboons using osteogenic proteins placed intramuscularly,[20,21,23] within the tibia,[2] and in calvarial defects.[24]

Although dosage studies using recombinant human bone morphogenetic protein (rhBMP) have been performed on species of lower phylogenetic levels including rats,[14] rabbits,[28] sheep,[12] goats,[6] and dogs,[8,19]they cannot be extrapolated to the primate.[1] On the basis of the previous work by Boden et al,[5] the current study investigated the efficacy of recombinant human bone morphogenetic protein 2 (rhBMP-2) with an absorbable collagen sponge carrier and a dowel allograft in promoting spinal fusion in rhesus macaque monkeys using an anterior interbody fusion model. The protocol was approved by the Animal Care Committee at William Beaumont Hospital, Royal Oak, Michigan.

Materials and Methods

Six female adult Macaca mulatta (rhesus macaque) monkeys underwent an anterior L7-S1 interbody lumbar fusion. The animals weighed 4 to 6 kg. All six sites were randomly assigned to one of two fusion methods: 1) autogenous iliac crest bone graft within a single freeze-dried smooth cortical dowel allograft cylinder (control-no addition of rhBMP-2) or 2) rhBMP-2-soaked absorbable collagen sponges (11 mm × 24.5 mm × 3.5 mm) soaked in 1.5 mg/mL of rhBMP-2, with a single freeze-dried smooth cortical dowel allograft cylinder also soaked in rhBMP-2. This resulted in a delivery of 0.4 mg of rhBMP-2 per implant site.

The materials used for implant preparation included rhBMP-2 (Genetics Institute Lot PC4579-135), a Helistat collagen sponge consisting of sterilized bovine tendon-derived ethylene oxide (Integra Life Sciences Lot 5001410), a custommachined cortical dowel allograft (MD-I) from a killed rhesus monkey (University of Florida Tissue Bank), and Calcein, a fluorescein derivative from Sigma (C-0875).

Recombinant Human Bone Morphogenetic Protein-2 Sponge-Allograft Implant Preparation.

A vial containing 8.0 mg of lyophilzed rhBMP-2 was reconstituted with 1.8 mL of sterile water for injection to obtain a 4.45 mg/mL solution. The vial was swirled gently until a clear solution was obtained. A 1.8-mL volume of the 4.45 mg/mL rhBMP-2 solution then was mixed with a 3.5-mL volume of MFR 906 Buffer (Genetics Institute, Cambridge, MA) for a final volume of 5.3 mL, with an rhBMP-2 concentration of 1.5 mg/mL.

Using a 1-cc syringe with a 22-gauge needle, 1.0 mL of the 1.5 mg/mL rhBMP-2 solution was placed in a vial containing an allograft cylinder. A 11 × 24.5-mm strip of absorbable collagen was placed in a sterile petri dish, and 0.270 mL of the 1.5 mg/mL rhBMP-2 solution was applied. The allograft and sponge were removed from the solution after 1 hour and placed in a sterile petri dish. Sterile forceps were then used to roll the strip lengthwise. It was thereafter placed in the sterile, rhBMP-2-soaked allograft cylinder, which was implanted (Figure 1).

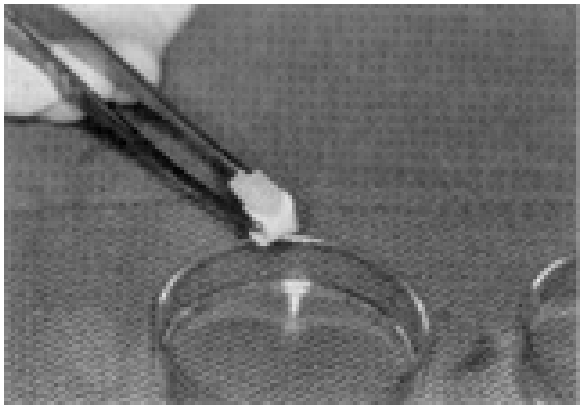

Figure 1

Autograft Implant Preparation. Iliac crest cancellous autograft was obtained under sterile conditions in routine surgical fashion using a small curette. After the bone was collected, it was placed into the allograft cylinder and implanted.

Calcein Solution Preparation and Administration. A calcein solution was prepared by adding 0.3 g of Calcein to 30 mL of a 2% sodium bicarbonate buffer solution. The solution was sterilized by passage through a 0.22-µm syringe filter. Forty-eight hours before the animal was killed, 1 mL of the solution was injected for every kilogram its body weight.

Surgical Procedure. The animal was anesthetized using intramuscular and intravenous ketamine, diazepam, and isoflurane 2%. The animal then was shaved and prepared with betadine. The table was placed in a slight Trendelenburg position to allow the abdominal contents to shift out of the pelvis. A 5-cm incision was then made over the L7-S1 vertebral bodies. The rectus abdominis and fascial layer were incised and retracted. The peritoneum then was carefully entered and retracted. The abdominal contents were retracted superiorly, thus revealing the aortic bifuraction. The sacral prominence was then identified. A 21-gauge needle was then placed between the presumed L7-S1 disc space. Intraoperative radiographs confirmed the correct disc space. A guide wire was placed centrally in the L7-S1 disc space to a depth of 10 mm followed by a trephine placed 12 mm deep. The trephined hole was cleaned using a pituitary rongeur. The posterior longitudinal ligament was not violated. Distraction of the disc was followed by single-barrel outer-sleeve placement, thus maintaining disc space distraction. The prepared cortical dowel allograft was then placed and gently tapped into position (Figures 2A and 2B). The peritoneum was closed. The wound was irrigated and then closed in routine fashion.

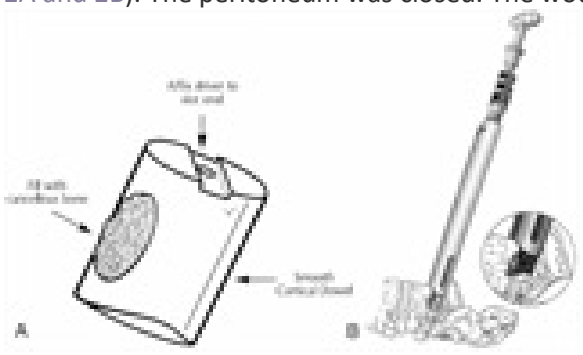

Figure 2

Histology. Each animal was injected with Calcein solution 48 hours before it was killed. The specimens were packaged in a 10% formalin solution and shipped to the Medical College of Wisconsin where histologic analysis was performed. All spinal levels containing a cortical dowel allograft cylinder were bisected sagittally to produce

right and left halves. These halves then were labeled and processed for decalcified and undecalcified histologic analysis. One half of the spinal level of each animal was randomly chosen for undecalcified histologic analysis. The other half was processed by decalcified histologic analysis.

Analysis of the slides determined 1) the type and quality of tissue ingrowth in the threaded allograft cylinders and the amount in contact with the cylinders, 2) the presence or absence of histologic fusion, 3) the amount of bone formed inside the threaded allograft cylinders by qualitative optical microscopy as well as ultraviolet fluorescence of fluorochrome bone label, 4) the cytologic and histologic response to the allograft cylinder, and 5) the cellular characterization of any response, inflammatory or noninflammatory.

Undecalcified Histologic Processing. The spinal level containing the threaded allograft cylinder was labeled and processed via sequential dehydration in alcohol, clearing in xylene, and embedding in graded catalyzed methyl methacrylate. The entire decalcification process took between 6 and 8 weeks. After polymerization was complete, the samples were sectioned and stained. The blocks were sectioned on a low-speed diamond saw (Buehler Isomet, Lake Bluff, IL) to a thickness of 100 to 300 μm. Sections were then made in the sagittal plane through the spinal level and surrounding tissues. Grinding was performed to obtain the desired thickness, stain dependent. The sections were then measured with a metric micrometer (Fowler, Japan). A trichrome stain was then used to permit histologic differentiation.

Decalcified Histologic Processing. After fixation in 70% ethyl alcohol, decalcification was carried out in a 10% disodium ethylenediamine tetraacetic acid solution at a pH of 7.3 at 37 C. After 2 weeks, decalcification was monitored radiographically using an x-ray unit (Faxitron, Hewlett-Packard, McMinnville, OR). After decalcification, the samples were rinsed for 1 day in running tap water and processed using a paraffin infiltrator (Shandon-Lipshaw, Chicago, IL). The infiltrated tissues were then embedded in paraffin blocks for sectioning. The blocks were cut on a rotary to produce sections between 6 and 8 μm in thickness. Hematoxylin and eosin (H & E), Mallory-Heidenhain, toluidine blue O, and safranin O/fast green stains were then used to evaluate the cellular and histologic response of the allograft cylinders.

Microradiography. Two undecalcified sections from each animal were radiographed using copper K-alpha radiation at 20 kV and 30 mA using a microradiography unit (Kristalloflex-2, Siemens, New York, NY) and spectroscopic plates (Kodak, Rochester, NY). A custom-made camera with an extension tube measuring 22.9 cm in length was used to obtain high-resolution radiographs. The thickness of the sections was measured with a metric micrometer (Fowler, Japan) to determine the exposure time. Sections were exposed for 10 to 12.5 minutes for each 100 μm of thickness. The samples were placed on a spectroscopic plate and a vacuum was applied. The cassette assembly was then inserted into a camera mounted on the x-ray unit and exposed to the radiation. The plates were thereafter developed, fixed, and analyzed for ossification using standard optical microscopy.

Results

Radiographic Analysis

Anteroposterior, lateral, and oblique radiographs of the animals were obtained at 0, 8, 14, and 18 weeks. Computerized tomography (CT) scans with sagittal reconstructions were performed at 2, 12, and 24 weeks.

All three of the monkeys receiving rhBMP-2-soaked collagen sponges with a freeze-dried allograft demonstrated radiographic signs of fusion as early as 8 weeks. The control animals were slower to reveal new bone formation. The 3- and 6-month postoperation CT scans revealed extensive fusion of the L7-S1 lumbar vertebrae in the group with rhBMP-2 as compared with the control group (Figures 3A-D). A solid fusion was obtained in all three of the animals receiving rhBMP-2. A pseudarthrosis was present in two of the control animals. The fusion mass

in the control animals was minimal, occurring only in the cortical dowel allograft cylinder at 6 months after surgery.

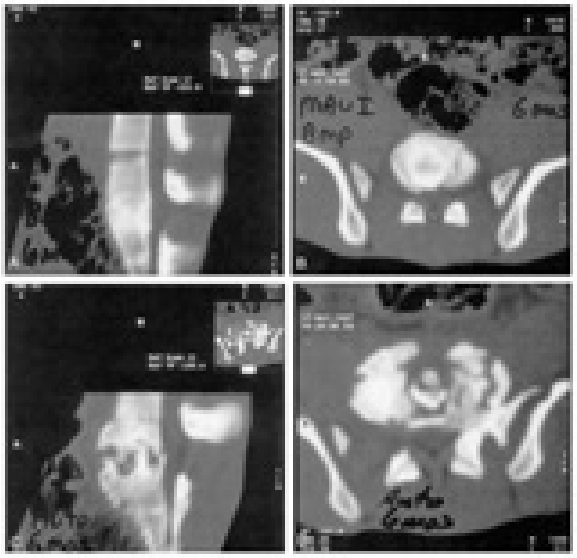

Figure 3

Evaluation of Spinal Segments

One animal from each group was chosen randomly and killed at 3 months, and the L7-S1 interspace was examined. The control animal was noted to have poor new bone growth with abundant fibrocartilage. As the specimen was tested manually, motion at the attempted fusion site was noted. However, the rhBMP-2 fusion site was noted visually to have a larger bone deposition at the fusion site with no evidence of motion on manual testing. The remaining four animals were killed 6 months after surgery. The spinal segments of interest were harvested and tested. No motion was detected on flexion, extension, lateral bending, or rotation in three of the four animals. One control animal was noted to have motion in four planes on manual testing.

Histology

The control animal (no rhBMP-2), which was killed at 3 months, revealed a partial fusion, which existed because of an osseous union in only the medial sections on the right side. The left side revealed no fusion at all. Between the allograft posts, the *de novo* bone was mostly woven with some lamellar character composed of both endochondral and intramembranous ossification. Cytologically, there were a few polymorphonuclear leukocytes (neutrophils) and eosinophils with predominant T lymphocytes and macrophages. Most of the cells on the surface of the allograft were hypertrophied osteoblasts, indicating the process of creeping substitution. Normal hematopoietic tissue was present in the intertrabecular spaces of the newly formed bone.

The histology of the remaining non-rhBMP-2 animals killed at 6 months was similar. One of the animals was noted to have a fusion in the center of the disc through the center of the allograft ring (Figures 4A and 4B). The cortical allograft was not incorporated in one animal, and at higher magnification, some osteoclast resorption of the allograft was observed, whereas hypertrophied osteoblasts were observed in the process of intramembranous osteogenesis on the surface of the allograft. Proximal to the allograft, a low-grade inflammatory response with an acute inflammatory character (predominantly PMNs) was observed. The second non-rhBMP-2 animal killed at 6 months had no osseous union in the anterior, central, or posterior sections (Figure 5). Cytologically, many inflammatory cells consistent with a chronic inflammatory response were evident at the operation site.

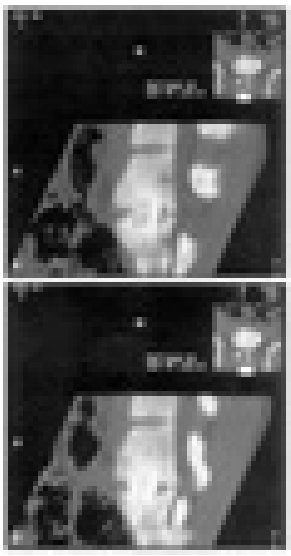

Figure 4

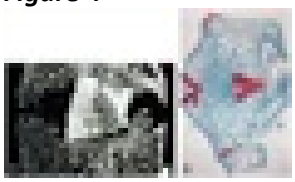

Figure 5

The rhBMP-2 animal killed at 3 months revealed a complete osseous union. The sagittal sections on the right side showed incorporation of the allograft into the fusion mass. The posterior post of the allograft strut was completely covered with lamellar bone. No allograft surface was uncovered. In the sagittal sections, *de novo* bone with no fibrous tissue or fibrocartilage was observed (Figure 6). The bone was mostly lamellar with some woven bone. The foraminal spaces and nerves were maintained. There was no evidence of foraminal encroachment by new bone formation. The medial sections showed no spinal canal stenosis (Figure 7). Cytologically, no inflammatory or immune response was observed. Howship's lacunae were indicative of previous resorptive (osteoclastic) activity similar to that observed in the control animals.

Figure 6

Figure 7

The histology of the remaining rhBMP-2 animals killed at 6 months was similar. A certain fusion existed because of a very complete osseous union in the center of the previous disc space and in the posterior margin on both the right and left sides of the animals. In several of the sagittal sections, no remnants of the allograft were observed in the fusion mass. The bone was mostly lamellar with some woven bone present. The foraminal spaces and nerves were maintained. Cytologically, no inflammatory or immune response was observed.

Discussion

In this study, the authors were able to document the efficacy of rhBMP-2 with an absorbable collagen sponge carrier and a cortical dowel allograft to promote anterior interbody fusion in a nonhuman primatemodel at a dose of 0.4 mg per implant site with a concentration of 1.5 mg/mL. The rate of new bone formation and eventual fusion with the use of rhBMP-2 and cortical dowel allograft appears far superior to that of autogenous cancellous iliac crest graft with cortical dowel allograft.

Experimental studies using rhBMP-2 to promote spinal fusions have been performed in the rabbit,[17,18,28]dog,[19] sheep,[27] goat,[6] and monkey.[3,4] Recently, there has been much excitement about the potential for osteoinductive growth factors to eliminate the morbidity of autogenous bone graft harvest and to improve the success rate of the arthrodesis. The dosage of rhBMP-2 to be used in the spine has been the subject of recent research. Bone formation outside the intended region could have deleterious results. Miyamoto et al[16] reported ossification of the ligamentum flavum and secondary spinal cord compression in mice while implanting BMP in the lumbar extradural space. A chronic cord compression model using BMP also has been induced in domestic rabbits.[26]

Successful posterolateral intertransverse process spinal fusions have been achieved in the rabbit and rhesus monkey models using osteoinductive growth factor with a bovine demineralized bone matrix carrier at a concentration of 0.3 and 6 mg per implant site, respectively.[5] Numerous studies using rhBMP-2 in various species, sites, and dosages have been performed. However, to the knowledge of the authors, only one study has been performed to assess dosage of rhBMP-2 for anterior spine fusion using an animal of a higher phylogenetic level. Boden et al[3] reported solid fusions in 5/5 rhesus monkeys using 0.75- and 1.5-mg/mL doses of rhBMP-2 (0.3 and 0.6 mg per implant site, respectively) delivered in a threaded titanium interbody fusion cage.

An effective substitute for autogenous bone graft in spinal fusions would obviate the need for bone graft harvesting in patients, decrease the pseudoarthrosis rate, provide adequate initial support, and minimize the risk of disease transmission and infection. In the current study, the cortical dowel allograft and rhBMP-2 combination provided an earlier rate of fusion and a greater fusion mass in than the cortical dowel allograft and cancellous autograft combination. This model represents a worst-case situation because only a single smooth bone dowel was used. Biomechanically, this offers less stability than currently recommended clinically. Typically, two threaded dowels are used in anterior lumbar surgery. In addition, the allograft dowel and cancellous autograft combination may not be the best biologic construct. The actual healing of the allograft bone-host bone interface with regenerate bone or fibrous tissue in humans is unknown.

Allografts reportedly have provided equal or better compressive strength than autografts.[47] They also are readily available, in contrast to autografts, which require an additional procedure to harvest. To be useful, an autogenous bone graft substitute must have similar physical properties, at least with respect to the immediate postoperative mechanical properties.[35] Although titanium has a modulus of elasticity similar to that of bone, the modulus of elasticity is six times greater than that of cortical bone. The altered biomechanics of the lumbar spine from insertion of a metal cage may make it less favorable for use than an allograft or xenograft. Unlike titanium, which is an inorganic material, the allograft may be transformed by the host. Most of the allograft implant in those animals receiving rhBMP-2 was resorbed while surrounding new bone was formed. Avoiding a foreign metal implant may be beneficial in the long term.

The medical and behavioral benefits from primate research over the past several decades have been considerable. In primates, 98% of human DNA can be found.[11] Unfortunately, the high cost of primateresearch limits the number of animals that can be used in a study. In the current study, the number of monkeys was too small to perform statistical analysis and thus to determine statistical significance. However, on the basis of the current findings and those of similar studies,[3,27] the authors believe that the concentration of rhBMP-2 at 1.5 mg/mL is effective in promoting spinal fusion in primates. It is not known whether the dose will need to be

altered to achieve the same results in humans or if the concentration can be extrapolated safely to humans for use in cervical and lumbar spinal fusions.

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