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Bone Morphogenetic Protein Binding Peptide Mechanism and Enhancement of Osteogenic Protein-1 Induced Bone Healing

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Study Design. In vitro and *in vivo* evaluation of BBP interactions with BMP.

Objective. To explore bone morphogenetic protein-binding peptide (BBP)'s mechanism of action, investigate an extended repertoire for BBP applications, and evaluate the usefulness of BBP as a surgical adjuvant when used with recombinant human osteogenic protein-1 (rhOP-1).

Summary of Background Data. Bone morphogenetic proteins (BMPs) are osteoinductive proteins that provide a potential alternative to autograft. Their utility is limited by cost, and potential dose-dependent risks, such as local inflammatory reactions and ectopic bone formation. BBP, a cyclized synthetic peptide, avidly binds recombinant human BMP-2(rhBMP-2) and has been shown to accelerate and enhance its osteogenic qualities.

Methods. BBP binding with 4 growth factors from the transforming growth factor -beta family were assessed using surface plasmon resonance. The *in vivo* retention of rhBMP-2 was quantified by comparing the percentage of retained [^{125}I]-labeled rhBMP-2 in absorbable collagen sponge implants with or without BBP at 1, 3, and 7 days postimplantation. The adjunctive effect of BBP with rhOP-1-induced bone growth was evaluated by comparing time to fusion and fusion rates in a rodent posterolateral fusion model with 2 different doses of rhOP-1 with or without BBP.

Results. BBP bound all 4 growth factors with an intermediate affinity. The *in vivo* retention of rhBMP-2 alone ranged from about 40% on day 1 to about 30% on day 7, whereas, the retention of rhBMP-2 in the presence of BBP was about 85% on day 1 and about 55% on day 7. The addition of BBP to rhOP-1 resulted in significantly earlier and greater fusion rates than achieved with rhOP-1 alone.

Conclusion. The mechanism of the BBP enhanced osteoinductive properties of BMPs involves the binding and retention of the growth factor, resulting in a prolonged exposure of BMP to the desired fusion site. The use of BBP in conjunction with BMPs may prove to provide satisfactory fusion outcomes, while reducing the costs and side effects associated with BMP use.

Key words: bone morphogenetic protein, bone morphogenetic protein binding peptide, osteogenic protein, bone graft alternative. **Spine 2010;35:2049–2056**

The use of autogenous bone graft is the current gold standard in the 1.5 million bone-grafting surgeries performed annually in the United States.[1] Although this practice has resulted in high rates of fusion success, it is associated with increased operative time and blood loss, along with a significant degree of donor-site morbidity.[2–4] Additionally, in certain settings such as revision cases, multilevel constructs, or in patients with medical comorbidities, autogenous bone graft may exist in limited quantity and quality. This significant need for a suitable alternative to autogenous bone graft has stimulated great interest in the exploration of bone graft substitutes and extenders.

One avenue of extensive research involves the use of bone morphogenetic proteins (BMPs). BMPs are osteoinductive proteins in the superfamily of transforming growth factor-beta (TGF-β). Since their discovery by Marshall R. Urist in 1965,[5] several BMPs have been identified and are currently being produced in mass quantities using recombinant technologies. Recombinant human osteogenic protein-1 (rhOP-1), also known as rhBMP-7, and rhBMP-2 are the only 2 BMPs currently approved for orthopedic procedures. Both have shown to be effective osteoinductive proteins in preclinical and clinical trials.[6–18] These growth factors provide a potential alternative to autogenous bone graft and are capable of overcoming the suboptimal biologic environment for bone formation seen in revision surgeries and in patients with risk factors for pseudarthrosis.[19–23] Unfortunately, the utility of these alternatives is limited by their cost,[24–26] and associated risks, such as local inflammatory reactions,[27–30] ectopic bone formation,[27,31–33] and

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the theoretical risks of carcinogenicity, and teratogenicity.[27] The risk for these adverse factors increase in a dose-dependant manner and are present at the doses currently used for orthopedic procedures.

BMP-binding peptide (BBP) is a cyclized synthetic peptide comprised of 19 amino acids that avidly binds rhBMP-2 and has been shown to accelerate and enhance the osteogenic qualities of rhBMP-2 in rodent hindquarter and posterolateral fusion models.[34,35] The hypothesized mechanism of the enhancement of BMP's action involves the slow and sustained release of the growth factor. The use of such binding peptides has the potential to decrease the concentrations of BMP necessary to achieve bone growth and fusion, subsequently decreasing the expense and risk for associated adverse events. The goals of this study were to further explore BBP's mechanism of action by quantifying its BMP-releasing properties in an *in vivo* model, to investigate an extended repertoire for BBP applications by studying the binding of BBP to other members of the TGF-β family of proteins, and to evaluated the usefulness of BBP as a surgical adjuvant when used in association with rhOP-1 in a rodent model of spinal fusion.

■ Materials and Methods

Characterization of BBP Binding With Surface Plasmon Resonance

The dynamic and equilibrium binding of BBP and the parental protein, spp24 (secreted phosphoprotein, 24 kD), with several growth factors from the TGF-β superfamily were determined using surface plasmon resonance with a Biacore X instrument (GE Healthcare, Piscataway, NJ). Surface plasmon resonance is an optical technique in which the binding of an analyte (*e.g.*, BBP) to a covalently-immobilized ligand (*e.g.*, rhBMP-2) on a glass chip is measured as an electrical signal proportional to the mass of analyte that binds to the chip as the running buffer containing the analyte flows over the surface. RhBMP-2, rhOP-1 (rhBMP-7), rhTGF-β, and recombinant mouse growth and differentiation factor-5 (GDF-5) (0.4 μg each; R&D Systems, Minneapolis, MN) were dialyzed into 10 mmol/L acetate buffer (pH 5.0) and amine coupled to a CM-5 sensor chip using the reagent kit supplied by the manufacturer (Biacore, GE Healthcare, Piscataway, NJ). BBP (GeneMed, South San Francisco, CA) and the related protein were dissolved in HEPES-EP running buffer (10 mmol/L HEPES, pH 7.4; 150 mmol/L NaCl; 3 mmol/L EDTA; and 0.005% surfactant P-20) at 1×10^{-5} M to 1×10^{-4} M. To ensure precise quantitation of protein concentrations, which are essential for kinetic calculations, samples were centrifuged at 12,000 rpm in a microfuge for 1 minute before use. The supernatant was decanted and used for analyses. Protein concentrations were determined using UV spectroscopy at an absorbance of 280 nm. Extinction coefficients were calculated using ProtParam tool (www.expasy.ch/tools/protparam.html). Flow rates ranged from 5 to 50 μL/min, and injection volumes were 20 to 100 μL. To ensure uniformity, an effort was made to react sufficient ligand to the chip to give a baseline value of about 2500 RU (response units). The kinetic constants k_a (on rate, association constant, "recognition") and k_d (off rate, dissociation constant, "stability") as well as the equilibrium constant K_D (equilibrium constant, "affinity") were determined by a Langmuir analysis using BIAevaluation software (version 3.2) installed on the instrument by the manufacturer.

Effect of BBP on BMP Retention In Vivo

The effect of BBP on the *in vivo* retention of rhBMP-2 was studied in 4 to 6 week old female Sprague-Dawley rats following a protocol approved by the IACUC of the University of Alberta, Edmonton, Canada and using methods previously described in detail.[36–41] Briefly, 5 mg of cold rhBMP-2 and about 1×10^5 cpm of [^{125}I]-labeled rhBMP-2 in 50 mL were applied to absorbable cross-linked collagen sponges (ACS; $14 \times 14 \times 3$ mm; Helistat, Integra Life Sciences, Plainsboro, NJ) in the presence of vehicle or 0.5 mg BBP. After approximately 10 minutes of incubation at room temperature the preparation was implanted by inserting it into a blind pouch created by gentle blunt dissection in the abdominal musculature. There were 4 samples for each treatment/time point (2 samples per rat and 2 rats per time point). The tracer retention was measured at 1, 3, and 7 days postimplantation by recovering the implants and using a γ-counter.[36–41] Aliquots of the application fluids were counted at the time of implantation to determine total cpm at t_o. The percent of applied rhBMP-2 retained was calculated at each time point by dividing the explant-associated cpm with the cpm implanted at t_o.

In Vivo *Posterolateral Fusion Study Design*

This portion of the study was approved by the University of California, Los Angeles Chancellor's Animal Research Committee. A total of 120 male Lewis rats (8 weeks of age, 200–260 g, Charles River, Wilmington, MA) were divided into 8 groups. Groups differed only by the materials added to the ACS ($5 \times 5 \times 13$ mm) that were applied to the surgical sites. The preparation of the implants was previously described in detail.[34] Implanted rhOP-1 was obtained from Stryker Biotech, Hopkinton, MA. The control groups were: (I) Decortication alone (n = 10); (II) ACS only (n = 10); (III) ACS with 1000-μg BBP (n = 10); and (IV) ACS with 10-μg rhOP-1 (n = 10). The experimental groups were: (V) ACS with 3-μg rhOP-1 (n = 20); (VI) ACS with 3-μg rhOP-1 plus 1000-μg BBP (n = 20); (VII) ACS with 1 μg rhOP-1 (n = 20); (VIII) ACS with 1-μg rhOP-1 plus 1000-μg BBP (n = 20) (Table 3). Animals were housed and cared for in a temperature-regulated animal facility exposed to a 12-hour light/dark cycle. Animals were killed 8 weeks after surgery with CO_2.

Surgical Technique

Animals were anesthetized with 2% to 2.5% isoflurane administered in oxygen (1 L/min) and the surgical site was shaved and disinfected with alternative Betadine and 70% alcohol. Animals were premedicated with 0.15-mg buprenorphine and after surgery received tapered doses every 12 hours for 2 days.

The posterolateral intertransverse process spinal fusion at L4–L5 in the rat is a well established model in our laboratory.[34,42–45] The iliac crest was used as a landmark to locate the body of the L6 vertebra. A 4-cm longitudinal midline incision was made through the skin and subcutaneous tissue over L4–L5 down to the lumbodorsal fascia. Then a 2 cm longitudinal paramedial incision was made in the paraspinal muscles bilaterally. The transverse processes of L4–L5 were exposed, cleaned of soft tissue, and decorticated with a high-speed burr. The surgical site was then irrigated with sterile saline and identical treatment materials were placed bilaterally, taking care to apply the implant to fully cover the transverse processes. The

Table 1. Kinetic Analysis of the Binding of BBP and spp24 to 4 Growth Factors of the TGF-β Family

Analyte	Ligand	K_D (k_d/k_a) "Affinity" M	k_a "Recognition" M^{-1} s^{-1}	k_d "Stability" s^{-1}
BBP	rhBMP-2	5.33×10^{-8}	3.17×10^{4}	1.69×10^{-3}
BBP	rhOP-1 (rhBMP-7)	1.16×10^{-6}	3.18×10^{3}	3.69×10^{-3}
BBP	rhTGF-β	6.8×10^{-8}	2.77×10^{4}	1.75×10^{-3}
BBP	rmGDF-5	7.77×10^{-7}	4.53×10^{3}	3.52×10^{-3}
spp24	rhBMP-2	1.77×10^{-8}	3.11×10^{5}	5.5×10^{-3}

BBP indicates bone morphogenetic protein binding peptide; rhBMP, recombinant human bone morphogenetic protein; rhOP-1, recombinant human osteogenic protein-1; rhTGF-β, recombinant human transforming growth factor-beta; rmGDF-5, recombinant mouse growth and differentiation factor-5; spp24, secreted phosphoprotein-24.

paraspinal muscles were then allowed to cover the implants and the lumbodorsal fascia and skin were closed with 4–0 Prolene sutures (Ethicon Inc., Somerville, NJ). Animals were allowed to ambulate, eat, and drink *ad libitum* immediately after surgery.

Radiographic Analysis

Posteroanterior radiographs were taken on each animal at 4, 6, and 8 weeks using an AMX-3 portable radiograph instrument (GE Healthcare, Piscataway, NJ). Radiographs were evaluated by 3 independent observers employing the following standardized scale: 0: no fusion; 1: incomplete fusion with bone formation present; and 2: complete fusion. The scores from the observers were added together and a score of 5 or 6 was considered "fused."

Manual Assessment of Fusion

Eight weeks after surgery, the spines were removed and evaluated by 3 blinded independent observers for intersegmental motion. Any motion on either side between the facets or transverse processes, including unilateral movement, was considered nonunion. The bilateral absence of movement was considered fusion. Spines were scored as either fused or not fused. Unanimous agreement was required to consider a spine to be "fused."

Microcomputerized Tomography Analysis

The explanted spines were subsequently scanned using high-resolution microcomputerized tomography (micro-CT), using 9 to 20 μm resolution technology (μCT40, SCANCO Medical, Basserdorf, Switzerland) to further assess the fusion rate and observe the fusion mass. Fusion was defined as the bilateral presence of bridging bone between the L4 and L5 transverse processes. Micro-CT data were collected at 55 kVp and 72 μA and reconstructed using a cone-beam algorithm supplied with the micro-CT scanner. Visualization and data reconstruction were performed using μCT Ray T3.8 and μCT Evaluation Program V6.0 (SCANCO Medical), respectively. The reconstructed images were judged to be fused or not fused by an experienced independent observer.

Histologic Analysis

After the rats were killed, the spines were dissected and fixed in 10% formalin, then transferred to 70% denatured ethanol. When imaging was completed, the specimens were decalcified using a commercial reagent containing 10% HCl (Cal-Ex, Fisher Scientific, Fairlawn, NJ), washed with running tap water, and then transferred to 70% denatured ethanol. Serial sagittal sections were carefully cut at the level of the transverse process. The specimens were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Each sample which displayed any fusion was processed for histologic examination to ensure that the fusion mass represented true bone formation and not dystrophic calcification or some other clinically disadvantageous process. Samples with no fusion mass were not examined.

Statistical Analysis

All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS, V17, Chicago, IL). RhBMP-2 retention was compared using Student *t* test. Fusion rates were compared in sequential 2-group comparisons using Fisher exact test. Interobserver reliability was assessed by computing the κ-statistic. Agreement was graded as follows: poor, $\kappa = 0$ to 0.2; fair, $\kappa = 0.21$ to 0.4; moderate, $\kappa = 0.41$ to 0.60; substantial, $\kappa = 0.61$ to 0.8; and excellent, $\kappa > 0.81$. A value of 1 indicates absolute agreement, whereas a value of 0 indicates agreement no better than chance.

■ Results

Peptide and Protein Binding Studies

The results of the kinetic analyses of the interactions between BBP or spp24 and the various growth factors are shown in Table 1. BBP bound all 4 TGF-β family growth factors and the parental protein with an affinity that was not greatly different. rhOP-1 and GDF had the lowest affinity (highest K_D). The difference in the K_D for the BBP/rhOP-1 interaction and the K_D for the BBP/rhBMP-2 interaction was due mostly to a lower k_a (slower "recognition") in the case of the BBP/BMP-7 interaction.

In Vivo *Retention of BMP-2*

The effect of BBP on BMP-2 retention *in vivo* is shown in Table 2. Retention of BMP-2 alone ranged from about 40% on day 1 to about 30% on day 7. On the other hand, the retention of BMP-2 implanted in the presence of BBP was about 85% on day 1 and about 55% on day 7. A significantly greater percentage of rhBMP-2 was retained in the presence of BBP at all time points ($P \leq 0.0001$).

Table 2. Effect of BBP on the Retention of BMP-2 *In Vivo* (Percent BMP-2 Retained)

Treatment	Day 1	Day 3	Day 7
BMP-2 alone	40.9 ± 1.51	31.9 ± 2.07	27.9 ± 2.48
BMP-2 + BBP	83.3 ± 1.56*	70.6 ± 1.34*	53.0 ± 1.51*

Data is shown as the mean ± SE. n = 4 for all groups.
*Significantly greater percentage of rhBMP-2 retained compared to group without BBP ($P \leq 0.0001$).

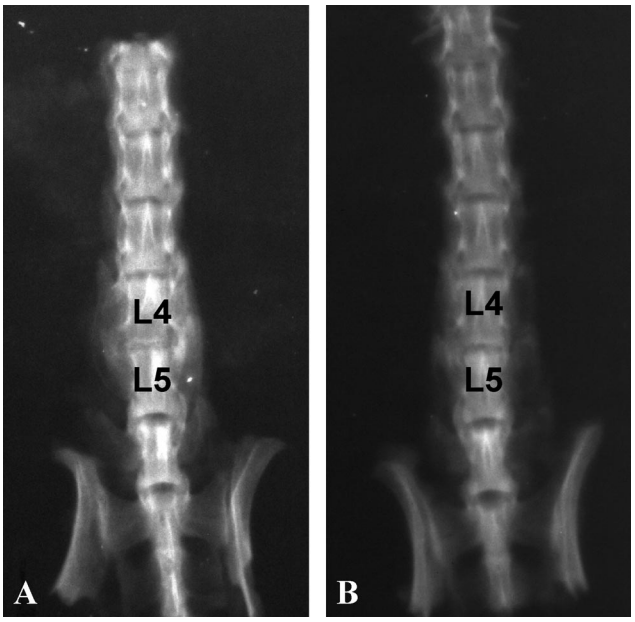

Figure 1. Posteroanterior radiographs of explanted rat spines obtained after 8 weeks of treatment with (**A**) 3 μg rhOP-1 + BBP and (**B**) 3 μg rhOP-1. **A,** Demonstrates a successful arthrodesis with a large intertransverse osseous fusion at L4–L5, whereas (**B**) shows a nonunion at L4–L5.

Surgical Outcomes

No abnormal behavior was noted in the 120 operated rats. None of the rats died before the end of the study. There were no surgical complications and no rats showed any neurologic deficits during the 8-week follow-up period.

Radiographic Analysis

Radiographs of the rat spines were obtained at 4, 6, and 8 weeks (Figure 1). Table 3 shows the proportion of rats in each group that had a total fusion score of 5 or 6 and was, therefore, judged to be fused at the specified time point. The interobserver agreement was substantial to excellent at all time points (κ = 0.763–0.895). None of the animals in the decortication only, ACS only, or ACS with BBP groups showed any sign of bone formation during the 8-week follow-up period. Group VI (3 μg rhOP-1 + BBP) had a higher fusion rate than Group V (3-μg rhOP-1) at all time points. This difference in fusion rate was significant at 6 and 8 weeks (P = 0.048 and 0.031, respectively). Group VIII (1-μg rhOP-1 + BBP) had a higher fusion rate than Group VII (1-μg rhOP-1) at 6 and 8 weeks; however, this difference was only significant at 8 weeks (P = 0.022). There was no statistically significant difference between the fusion rates of Groups IV (10-μg rhOP-1) and VI (3-μg rhOP-1 + BBP).

Table 3. Radiological Evaluation of Treatments. Percents of Subjects Showing Satisfactory Radiological Spine Fusion

Group	Treatment	4 wk (%)	6 wk (%)	8 wk (%)
I	Decortication only	0	0	0
II	ACS alone	0	0	0
III	ACS + 1000 μg BBP	0	0	0
IV	ACS + 10 μg rhOP-1	50	80	100
V	ACS + 3 μg rhOP-1	30	45	55
VI	ACS + 3 μg rhOP-1 + 1000 μg BBP	40	80*	90*
VII	ACS + 1 μg rhOP-1	0	10	20
VIII	ACS + 1 μg rhOP-1 + 1000 μg BBP	0	30	60*

*Significantly greater fusion rate when compared to group without BBP (P < 0.05).
ACS indicates absorbable collagen sponge; BBP, bone morphogenetic protein binding peptide; rhOP-1, recombinant human osteogenic protein-1.

Table 4. Fusion Rate Based on Manual Palpation and Micro-CT at 8 Weeks

Group	Treatment	Manual Palpation (%)	Micro-CT (%)
I	Decortication only	0	0
II	ACS alone	0	0
III	ACS + 1000 μg BBP	0	0
IV	ACS + 10 μg rhOP-1	100	100
V	ACS + 3 μg rhOP-1	55	55
VI	ACS + 3 μg rhOP-1 + 1000 μg BBP	90*†	90*†
VII	ACS + 1 μg rhOP-1	10	10
VIII	ACS + 1 μg rhOP-1 + 1000 μg BBP	50*	50*

*Significantly greater fusion rate when compared to group without BBP (P < 0.05).
†Statistically indistinguishable from high-dose rhOP-1 control group (P = 0.54).
ACS indicates absorbable collagen sponge; BBP, bone morphogenetic protein binding peptide; rhOP-1, recombinant human osteogenic protein-1.

Manual Palpation

The proportions of rats in each group that were judged to be fused by 3 independent evaluators are shown in Table 4. The interobserver agreement was excellent among these observers (κ = 0.966 to 0.983). The critical comparisons were between rhOP-1 with and without the addition of BBP. Eleven spines in Group V (3-μg rhOP-1) were assessed as fused (55% fusion rate), whereas 18 spines in Group VI (3-μg rhOP-1 + BBP) were considered fused (90% fusion rate). This observed difference was statistically significant (P = 0.031). A similar treatment effect was seen when comparing Group VII (1-μg rhOP-1) with a 10% (2/20) fusion rate to Group VIII (1-μg rhOP-1 + BBP) with a 50% (10/20) fusion rate. This observed difference was also statistically significant (P = 0.014). There was no significant difference between the fusion rates of Groups IV (10-μg rhOP-1) and VI (3-μg rhOP-1 + BBP).

Micro-CT Analysis

Table 4 shows the proportions of subjects in each group judged to be fused. The observed fusion rates were consistent with those determined with the use of manual palpation and radiographic analyses. The spines of the rats in groups VI and VIII exhibited considerable bone formation when compared to their respective control groups without BBP (V and VII). The new bone masses were solidly fused and no gaps were detected between the

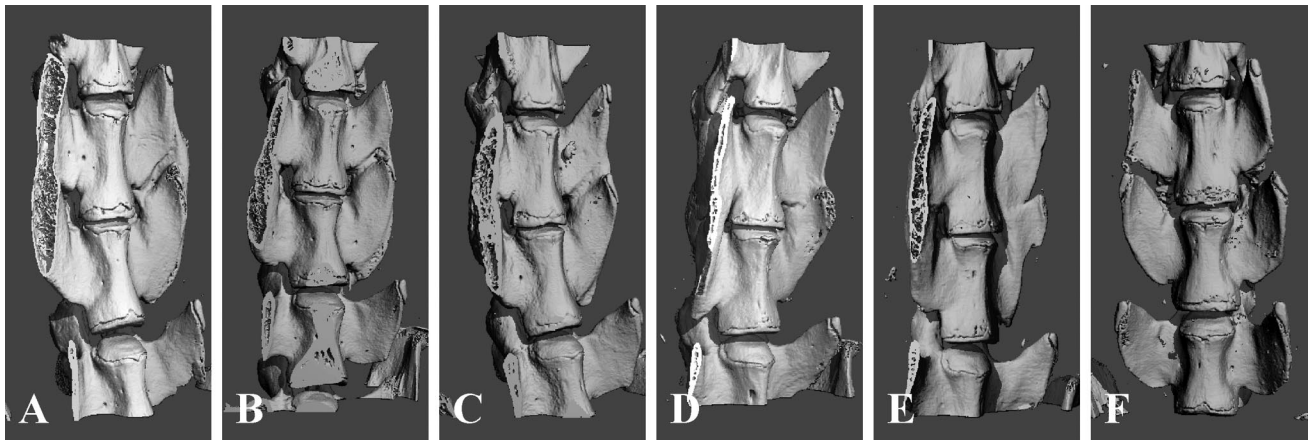

Figure 2. Three-dimensional reconstruction of microcomputerized tomographic images of rat spines (cut-plane images). Fused spines from (**A**) Group IV (10 μg rhOP-1), (**B**) Group V (3 μg rhOP-1), (**C**) Group VI (3 μg rhOP-1 + BBP), (**D**) Group VII (1 μg rhOP-1), (**E**) Group VIII (1 μg rhOP-1 + BBP). **F,** Example of nonunion seen in Group VIII.

transverse processes. Multiple cut sections were reconstructed to evaluate the presence of a bony bridge between the transverse processes. Trabeculae bridging the transverse processes were consistently observed in the cut-plane images of all spine samples deemed to be fused. The bridging trabecular bone was thicker in the groups with BBP when compared to their respective control groups without BBP. Groups I, II, and III which included no OP-1 did not exhibit any bony bridging between the transverse processes. A cleft was observed between the L4 and L5 transverse processes in all spines judged to be not fused (Figure 2).

Histologic Analysis

A representative histologic section from a specimen with a successful fusion is shown in Figure 3. Mature trabecular bone in the fusion mass and extensive remodeling of the mass and the transverse process can be seen. The thickness and maturity of the fusion mass tended to be greater in the groups with BBP as compared to those with the same dose of rhOP-1 alone.

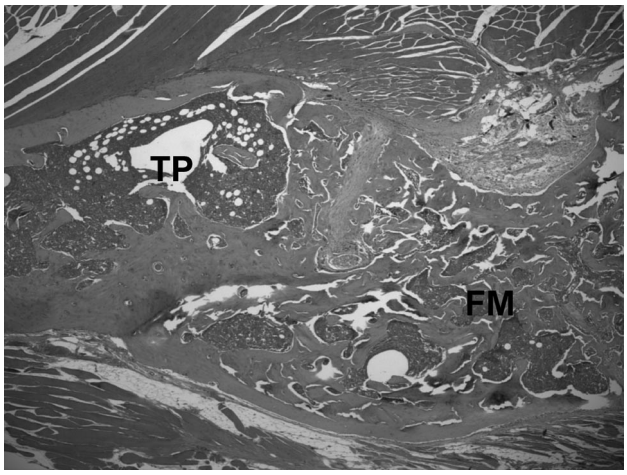

Figure 3. Histologic section from a successful fusion from Group VI (3 μg rhOP-1 + BBP) showing a large fusion mass (FM) with much mature bone and extensive remodeling of the transverse process (TP). Original magnification 25×.

■ Discussion

The use of bone morphogenetic proteins as an alternative to autogenous bone graft in orthopedic applications is a topic of great interest. BMPs have been shown to be equivalent or superior to autograft in numerous studies and have been successful in overcoming suboptimal biologic environments encountered in revision surgeries and in patients with risk factors for nonunion.[19–23] Their use, however, is limited by the substantial associated costs,[24–26] as well as known and theoretical side effects.[27–33] The incidence of these adverse events has been shown to correlate with the dosage of BMP used.[46] Recent efforts have aimed to maximize the efficacy of BMPs, while minimizing the dosages required and incidence of unwanted side effects.[34] One approach to achieve this goal would be the advancement of BMP delivery systems. BMPs have a very short biologic half-life and are rapidly cleared from the body.[47] With this in mind, the ideal delivery system would provide a sustained local concentration of BMP sufficient to induce bone formation, while minimizing systemic concentrations of BMP, and any local or systemic side effects.

BBP is a synthetic, cyclic, 19 amino acid peptide which was previously shown to bind BMP-2 and enhance bone healing when incorporated into a collagen carrier used for the surgical application of BMPs.[34,35] The sequence for this peptide is based on that of a portion of a 18.5 kD protein isolated by Urist and called "bone morphogenetic protein/noncollagenous protein" (BMP/NCP) but which had no independent osteogenic activity.[35] This protein was found to be identical to a fragment of a previously isolated protein, spp24 (secreted phosophoprotein 24 kD).[35] Spp24 shares with other BMP binding proteins, such as fetuin, a cystatin domain which in turn contains a smaller motif, the TRH-1 (TGF-β receptor II homology 1) domain.[35,48] Spp24 binds to BMP-2 and

has been shown to inhibit its osteogenic activity in an ectopic bone formation model and in a transgenic model of bone formation.[49] In order to further the development of BBP as an orthopedic therapeutic, we undertook a series of studies to define the binding of BBP to growth factors in the TGF-β family of proteins and also studies of the effects of BBP on *in vivo* retention of a BMP (BMP-2). We further extended our preclinical studies to evaluate the capability of BBP to enhance bone healing when combined with rhOP-1 in a well-accepted rodent posterolateral intertransverse process fusion model.

The binding constants (K_D, "affinity") for the association of BBP with several TGF-β family growth factors and the parental protein, spp24, are slightly greater (lower affinity) than that for most receptor-ligand interactions (on the order of $K_D = 10^{-8}$–10^{-9} M) and much greater than the most avid interaction in nature (K_D for streptavidin/biotin = 10^{-14} M). An affinity of this magnitude might be useful for providing the "slow release/immobilization" function hypothesized by Wozney *et al* with respect to the action of BMPs in skeletal tissue.[50] The determination of the optimal affinity for an orthopedic therapeutic is an important aspect of on-going research.

The K_D for the BBP/rhOP-1 interaction is greater (less affinity) than that for the BBP/rhBMP-2 interaction. However, the k_d (dissociation, "stability") for each of these 2 interactions is very similar and, therefore, may reflect a kinetic property which has increased importance in the design of therapeutics.

The K_D for the BBP/rhBMP-2 interaction is different from the value we previously presented.[35] This is most likely due to previous errors in the calculation of the concentration of BBP used in the analyses. Precise knowledge of the concentration of the analyte is very important in kinetic calculations, but is difficult to obtain with poorly soluble materials such as BBP. Every effort has been made to make accurate and comparable determinations in the present study. The manufacturing practices of different suppliers can also influence the properties of the peptide. These factors remain limitations of our studies.

The results of the BMP retention study support the hypothesis that BBP enhances BMP activity by increasing the retention of BMP at the implant site. The theory is that if a higher concentration of BMP is present for a longer period of time, a greater biologic effect would be expected. BMPs induce a recapitulation of endochondral bone formation, which is to say that the tissue at the implant site goes through a defined and reproducible progression including inflammation, mesodermal stem cell proliferation, cartilage formation, and vascular invasion with replacement of cartilage by bone. This process requires a period of many days and each of the early steps is dependant on BMP. Thus, a delay in the dispersal of BMP will result in a greater concentration of the morphogen many days after implantation and, therefore, an enhancement of processes such as chrondrogenic differentiation of mesodermal stem cells. From these results, one would expect a similar clinical response to a smaller dose of BMP when used in conjunction with BBP. We would infer that the mechanism of action, which is to say an increase in residence time of the cytokine at the implantation site, is the same for OP-1 (BMP-7), TGF-β, GDF-5, and other members of the TGF-β family of proteins that bind spp24 and BBP as it is for BMP-2. However, we have not explicitly demonstrated this.

In the posterolateral intertransverse process fusion portion of this study, the radiographic results demonstrated significantly higher rates of fusion and earlier fusion when rhOP-1 was used in conjunction with BBP than when rhOP-1 was used alone. These findings were further confirmed after harvesting the spines and subjecting them to manual palpation and micro-CT analysis. Although manual palpation is the current gold standard in the evaluation of spinal fusion in this model, the results of manual palpation and micro-CT were identical and correlated well with radiographic outcomes at the end of the study. BBP by itself did not induce any degree of fusion. Nor was ectopic calcification observed in the BBP only treatment groups. These results are consistent with reports by Behnam *et al*[35] who found that BBP alone did not induce ectopic bone formation but who did observe small amounts of dystrophic calcification in association with intramuscular adipose and reports by Alanay *et al*[34] who also did not find any spinal fusion in animals treated with BBP alone. Additionally, the combination of 3-μg rhOP-1 with 1000-μg BBP achieved a fusion rate statistically indistinguishable to that achieved by high dose (10 μg) rhOP-1 alone. This illustrates the potential for BBP combined with a lower dose of rhOP-1 to achieve similar outcomes as a high dose of rhOP-1 alone. It is important to note that the fusion rate (90%) of the lower dose of rhOP-1 (3 μg) plus BBP did not reach the same rate of fusion (100%) achieved by a high dose of rhOP-1 (10 μg) alone. This is consistent with previous findings with BBP in conjunction with low dose rhBMP-2.[34] Studies to determine the optimal dose of BBP to be used with either rhBMP-2 or rhOP-1 will be required in a number of animal models. If these optimization studies demonstrate an equally effective result with a lower dose of rhOP-1, then this material may allow for lower costs and fewer dose-related side effects. Furthermore, BBP may be useful when used in conjunction with a number of growth factors relevant to orthopedic surgery.

■ Conclusion

The mechanism of the BBP enhanced osteoinductive properties of BMPs involves the binding and retention of the growth factor, resulting in a prolonged exposure of BMP to the desired fusion site. Additionally, the addition of BBP to rhOP-1 resulted in significantly earlier and greater fusion rates than achieved with rhOP-1 alone. The use of BBP with BMPs may prove to provide satis-

factory fusion outcomes, while reducing the costs and side effects associated with BMP use.

■ Key Points

- The use of BMPs as alternatives to autograft in spine fusion has resulted in excellent fusion rates and clinical outcomes; however, their use is limited by cost and associated risks, such as significant swelling and ectopic bone formation.
- The incidence of adverse events seems to correlate with the dosage of BMP used.
- BBP, a synthetic cyclic peptide, binds BMPs and other members of the TGF-β family, and slowly releases the growth factors at the desired fusion site.
- The use of BBP in conjunction with rhOP-1 resulted in significantly earlier and greater fusion rates.
- The combination of BMPs with BBP may result in satisfactory fusion outcomes, while reducing the costs and side effects associated with BMP use.

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