# Effects of Recombinant Human Bone Morphogenetic Protein 2 on Surgical Infections in a Rabbit Posterolateral Lumbar Fusion Model

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## Abstract

**Recombinant human bone morphogenetic proteins (rhBMPs) are often used during spine surgery, but their effects on postoperative infections have not been well elucidated. Long-bone studies suggest that BMPs may limit local infection and facilitate bone formation. Until now, rhBMP-2 had not been evaluated in the setting of infected spinal arthrodesis. In the study reported here, we evaluated the safety and efficacy of rhBMP-2 and autograft in inducing fusion in the setting of surgically acquired infection.**

**Sixty rabbits underwent fusion with autograft or rhBMP-2 with coadministration of *Staphylococcus aureus* or sterile saline. In the noninoculated groups, 4/15 autograft and 13/13 rhBMP-2 rabbits fused ($P<.001$). In the inoculated groups, 0/14 autograft and 3/12 rhBMP-2 rabbits fused ($P = .085$). There were 4/14 early deaths caused by infection in the autograft group and 0/12 in the rhBMP-2 group ($P = .1$). Although the difference in fusion rates and early deaths from infection for rhBMP-2 and autograft did not reach our predetermined $\alpha$ error threshold, the data were trending toward significance.**

**Our results demonstrated no increase in morbidity or mortality associated with use of rhBMP-2 in the setting of local infection. Although BMP use with infections remains controversial, these results indicate that rhBMP-2 could be used in a contaminated environment.**

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In spine surgery, postoperative infections can be devastating. These complications may increase the risk for developing systemic disease, wound dehiscence, neurologic sequelae, and pseudarthrosis, all of which may compromise clinical outcomes.[1] Despite advances in maintaining sterility and the increasing prevalence of prophylactic antibiotic regimens, postoperative wound infections are still reported in 1% to 12% of spine surgery cases.[2,3] In addition to the acute morbidity that may result from these infections, there is a decreased rate of biological fusion.[4-7]

Many bone graft substitutes have been used for orthopedic procedures in an attempt to enhance bone formation while avoiding many of the adverse events associated with procurement of autogenous bone, such as fracture and intractable pain.[8,9] In particular, recombinant human bone morphogenetic proteins (rhBMPs) are being used with increasing frequency for spinal fusions because of their potent osteoinductive activities, which have been documented in multiple preclinical and clinical studies.[10-17]

Although the osteoinductivity of rhBMPs has been well established, the effects of rhBMPs on surgical site infections have not been fully elucidated. Using a rat model, Chen and colleagues[18] demonstrated that delivery of recombinant human osteogenic protein 1 (rhOP-1, also known as rhBMP-7) into chronically infected segmental femoral defects gave rise to significantly more callus formation and higher fusion rates compared with delivery of collagen carrier alone, though their investigation was limited by lack of autograft control animals. In addition, the same investigators[19] showed that callus formation in rats was also enhanced by concomitant administration of systemic antibiotics with rhBMP-2 in chronically infected femoral defects. Recently, a large prospective, randomized, multicenter clinical trial confirmed that implantation of rhBMP-2 into open tibial fractures lowered the incidence of infection, accelerated both osseous and soft-tissue healing, and minimized the number of secondary interventions.[20]

With regard to spinal applications, Aryan and colleagues[21] described a series of 15 patients who received rhBMP-2 as part of their treatment regimen for vertebral osteomyelitis and achieved solid fusions by 5 years with no evidence of recurrent infection; in their retrospective review, however, the authors failed to include an autograft

control group for direct comparison. Although these studies are promising, the efficacy of rhBMP-2 as a substitute for autograft in the setting of an infected spinal arthrodesis has yet to be appropriately evaluated.

It has been proposed that rhBMPs may influence the spread of local infections by up-regulating various cytokines and other biologically active proteins that collectively may facilitate recruitment of lymphocytes and other cells of the immune system to the surgical site.[5] In support of this hypothesis, our group recently reported that the expression of angiogenin, vascular endothelial growth factor (VEGF), and other angiogenic factors is increased (over the levels recorded with use of autograft) in rabbits that undergo spinal fusion after administration of OP-1.[22] Similarly, rhBMP-2 has also been shown to exhibit angiogenic potential as a result of its stimulation of VEGF-A production.[23]

In the present study, we examined how rhBMP-2 compares with autograft with regard to (1) induction of bone fusion in the setting of surgically acquired infection, (2) systemic immune response as evidenced by complete blood cell (CBC) count and erythrocyte sedimentation rate (ESR) tests, and (3) morbidity and mortality of the infection. At time of sacrifice, fusion was determined by manual palpation and histologic techniques. Blood for CBC count and ESR tests was drawn weekly from each animal to examine the systemic response. At the time of sacrifice, any abscess was evaluated to determine morbidity of the infection.

## Materials and Methods

This was a prospective randomized, controlled animal study. Figure 1 is a schematic overview of the experimental design. The primary experimental variables were type of bone graft implanted and whether the surgical site was inoculated with bacteria. The surgical protocol used is similar to those used in previous studies conducted by our group[16,24,25] and was approved by our institution's animal care and use committee.

The New Zealand white rabbit is a well-accepted animal model for assessing biological processes underlying posterolateral spinal arthrodesis.[26,27] A reliable method for generating spinal infections in these animals—directly inoculating the surgical site with *Staphylococcus aureus*—was described by Poelstra and colleagues.[28]

### Surgical Procedure

Sixty adult female New Zealand white rabbits weighing between 3 and 5 kg were included in this study. For each animal, a 25-μg fentanyl patch was placed on the dorsal skin of the neck 1 day before surgery to ensure adequate perioperative analgesia. Surgical anesthesia was induced with a combination of intramuscular ketamine 35 mg/kg and xylazine 2.5 mg/kg. Buprenex 0.04 mg/kg was also injected subcutaneously at time of induction to provide postoperative analgesia. A coaxial nose cone was used to titrate the inhalational isoflurane 0.5% to 3.5% administered during the procedure.

Once anesthesia was acceptable, a scalpel was used to make a dorsal midline incision through the skin overlying the lumbar region and then bilateral paramedian fascial incisions consistent with a Wiltse approach. Once the correct vertebral levels were identified by referencing the sacrum, the L5 and L6 transverse processes were exposed laterally to their tips and decorticated. Depending on the randomization results, either rhBMP-2 or autograft was subsequently placed in the posterolateral gutters. No instrumentation or metal implants of any kind were incorporated into these fusion constructs.

For the autograft groups, corticocancellous bone graft (~3-4 $cm^3$) was harvested from the iliac crests through separate fascial incisions. These incisions were closed with No. 1 Vicryl suture before the posterolateral fusion was performed. Given the concentrations reported in similar studies,[16,17,29,30] animals in the rhBMP-2 groups were implanted with rhBMP-2 1.29 mg per side (3 mL containing 0.43 mg/mL) with an absorbable collagen sponge. The rhBMP-2 with sponge was prepared at least 20 minutes before implantation to allow sufficient time for activation before being placed in the posterolateral gutters and positioned across adjacent transverse processes.

All animals were randomly assigned to receive either the bacterial inoculum or sterile saline. A total of 1 mL of the appropriate solution was applied to the graft material bilaterally. The fascial incisions were closed with No. 1 Vicryl suture, and the skin incision was stapled. None of the rabbits in any of the groups received antibiotics during the perioperative or postoperative period.

The fentanyl patch was maintained on postoperative days 1 and 2, and all rabbits were monitored for clinical signs of systemic or local infection. At 5 weeks, all rabbits were sedated with subcutaneous ketamine 35 mg/kg and euthanized with intracardiac phenobarbital 2 mL (Euthasol; Virbac Animal Health, Fort Worth, Tex). Any animal that did not survive the full 5 weeks of postoperative follow-up was submitted to our institution's Veterinary Clinical Services (VCS), which performed necropsy to assess cause of death.

### Inoculum of Bacteria

The inoculum of methicillin-sensitive *S aureus* (MSSA) used in these experiments represents a modification of the methodology reported by Poelstra and colleagues.[28] Each

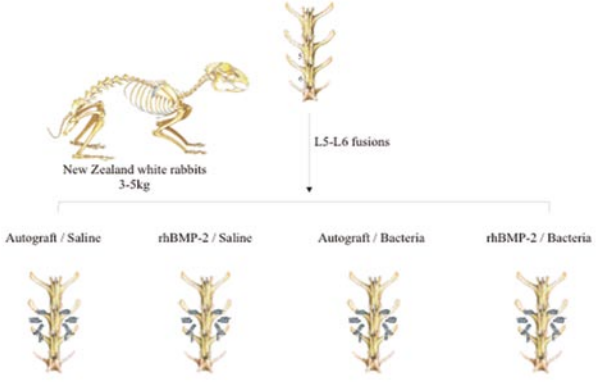


**Figure 1.** Experimental design.

inoculum contained 500 to 1,000 colony-forming units (CFUs), which were determined to be the lowest bacterium dose that consistently generated local surgical site infections without inducing sepsis.[28]

A suspension of MSSA strain ATCC 49230 (American Typed Cell Culture Collection, Manassas, Va) that had been maintained at –80°C was initially plated on Mueller-Hinton agar. Bacteria were applied again 1 day before surgery to allow for their recovery from their storage conditions. At time of surgery, inoculums containing the requisite number of bacterial CFUs were generated using serial dilutions derived from 0.5 McFarlane solutions determined by using a calibrated Dade MicroScan turbidity meter (Dade Behring, Deerfield, Ill). Each rabbit in the infection groups was implanted with a single bacterial dilution prepared fewer than 30 minutes before delivery.

The exact bacterial concentration (CFU/mL) of each inoculum was determined by plating the final dilutions onto multiple Mueller-Hinton agar plates, which were incubated overnight. The colonies were quantified the next day, and the mean of these values was used to calculate the number of CFUs administered to each animal.

## Serum Analysis

Serum markers for infection were followed at weekly intervals throughout the entire postoperative course. All blood was acquired from the central artery of the ear, except for the final sample, which was drawn directly from the heart at time of sacrifice, before the intracardiac injection of phenobarbital.

**CBC Count.** Blood (2 mL) was collected in a vial coated with ethylenediaminetetraacetic acid, or EDTA (CapiJect; Terumo Medical Corporation, Elkton, Md). All specimens were analyzed by Antech Diagnostics (Boston, Mass).

**ESR.** The Westergren method was used to calculate ESR. Blood (0.5 mL) was collected in a vial coated with EDTA, 0.24 mL of the sample was transferred into a standard ESR reservoir (FisherBand Dispette 2; Fisher Scientific, Pittsburgh, Pa), and the final value was calculated with a MicroSed ESR System pipette (HypoGuard; Fisher Scientific).

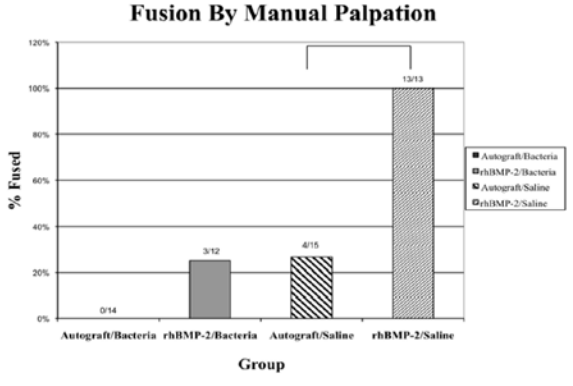


**Figure 2.** Fusion percentage by rabbit group. Values indicate what percentage of group had successful fusion as determined by manual palpation, and hash mark above column indicates significant ($P<.05$) difference between groups.

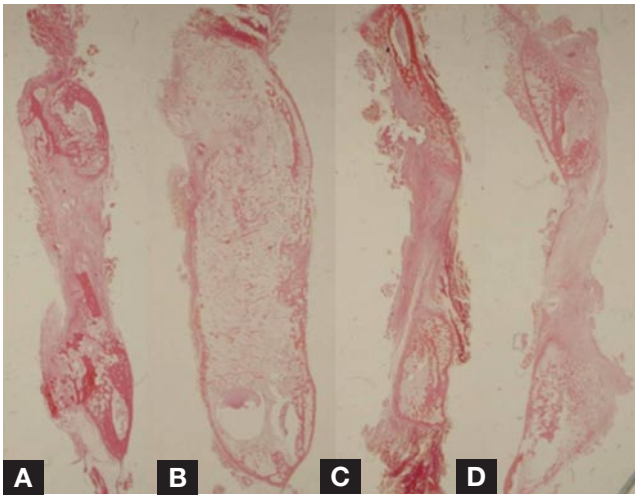


**Figure 3.** Representative hematoxylin-eosin–stained histologic slides for 4 rabbit groups: (A) autograft/saline, (B) recombinant human bone morphogenetic protein 2 (rhBMP-2)/saline, (C) autograft/bacteria, (D) rhBMP-2/bacteria.

## Evaluation of Fusions

After explantation of the lumbar spine, the L5–L6 segment was evaluated by manual palpation. Specimens were considered solidly fused when no motion was detected between the vertebrae. Manual palpation was used to determine fusion because this method has excellent correlation with biomechanical analysis of fusion masses and is significantly better than histologic analysis.[24] This more functional approach is less likely to miss bridging bone passing from the plane of study, which may limit the accuracy of histologic techniques.

All the explanted spines were subjected to histologic analysis. The L5–L6 spinal segment was split longitudinally and fixed in 10% phosphate-buffered formalin. These specimens were immersed in Decalcifier II solution (Surgipath, Richmond, Ill) and agitated until they were fully decalcified (3-8 days). The spines were dehydrated with graded ethanols, cleared in toluene, infiltrated with paraffin using a Tissue Tek VIP 1000 tissue processor (Sakura Finetek, Torrance, Calif), and embedded in paraffin (Paraplast X-tra; Fisher Scientific) in preparation for sectioning. Microsagittal sections were generated with a Leica 2125 microtome (Leica, Heidelberg, Germany) and disposable knives (C.L. Sturkey, Lebanon, Pa), after which they were stained with hematoxylin-eosin.

All the specimens were graded by 3 independent observers using a scale proposed by Huo and colleagues.[31] With this scale, bony healing is assessed on the basis of the histologic ratio of immature callus formation, mature bone, cartilage, and fibrous tissue present in each section (Table). The reviewers underwent brief group training on how to use the histologic scoring system and scored sample sections in parallel. When scoring, they were blinded to treatment groups and used number codes for the samples.

**Table. Histology Scoring System**

| Score | Associated Finding |
|---|---|
| 1 | Fibrous tissue |
| 2 | Predominantly fibrous tissue with small amount of cartilage |
| 3 | Equal mix of fibrous and cartilage tissue |
| 4 | Predominantly cartilage with small amount of fibrous tissue |
| 5 | Cartilage |
| 6 | Predominantly cartilage with small amount of bone |
| 7 | Equal mix of cartilage and immature bone |
| 8 | Predominantly immature bone with small amount of cartilage |
| 9 | Union of transverse processes by immature bone |
| 10 | Union of transverse processes by mature bone |

### Infection Classification

Each rabbit was classified on the basis of presence or absence of abscess at the time of dissection and early death. Presence or absence of abscess was determined by the researcher who performed the dissection. Early-death animals were defined as those with infections that required early sacrifice and those that died from bacterial sepsis. Need for early sacrifice was determined by VCS pathologists on the basis of abscess drainage, significant weight loss, development of neurologic deficits, overall appearance of animal, and other indicators of animal health. The pathologists were blinded to the treatment groups.

### Data Analysis and Statistical Methods

A priori analyses were performed to determine the interobserver reliabilities of the histologic scores provided by the independent examiners for each specimen. Intraclass correlation coefficients (ICCs), representing an interobserver reliability measure that expresses the relative homogeneity among raters with respect to the total variation, were calculated with a 2-way random effects model and the consistency definition. ICC strength was estimated with the classification scheme <0.40 (poor), 0.40 to 0.59 (fair), 0.60 to 0.74 (good), and >0.74 (excellent).[32] Fisher exact tests were used to compare the number of rabbits that received rhBMP-2 versus autograft that were excluded, the number of autograft and rhBMP-2 rabbits in the different infection classifications, and the fusion rates by manual palpation of the rhBMP-2 and autograft groups.

Bacterial inoculum concentrations and infection/abscess grades were evaluated with *t* tests for independent samples. Analyses of variance and post hoc Bonferroni analyses were used to compare the mean CBC counts and ESRs of all the groups. Statistical calculations were performed with SPSS 7.0 (SPSS Inc., Chicago, Ill), and results were considered significant at $P<.05$.

## Results

Mean initial and final weights of all the animals were 3.85 kg (SD, 0.36 kg) and 3.56 (SD, 0.35 kg), respectively. Complete clinical and histologic data were available for 54 rabbits (13 rhBMP-2/saline, 12 rhBMP-2/bacteria, 15 autograft/saline, 14 autograft/bacteria). Of the animals included in the study, only 4 died or were sacrificed early because of infection complications. All of these were autograft/bacteria rabbits (1 died from sepsis; the other 3 were sacrificed because of neurologic or wound complications). None of the rabbits in the other groups that completed the study died early.

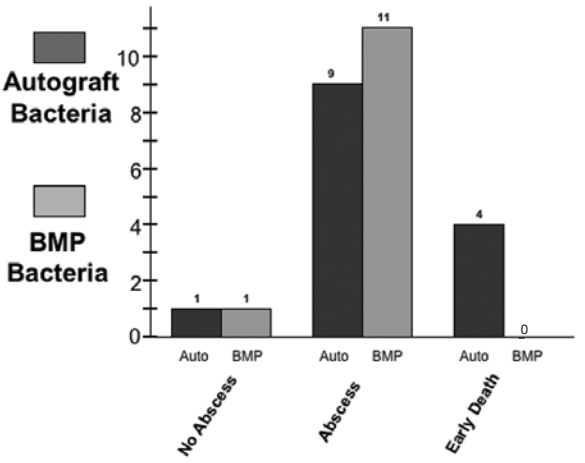


**Figure 4.** Infection classification by group. Early death defined as evidence of sepsis on necropsy (1 rabbit) or significant morbidity prompting early sacrifice by Veterinary Clinical Services (3 rabbits). Abbreviation: BMP, bone morphogenetic protein.

Six rabbits (10%) were lost or excluded from the study. Two (1 rhBMP-2/bacteria, 1 rhBMP-2/saline) died as a result of anesthetic-related complications, and another 3 (2 rhBMP-2/bacteria, 1 autograft/bacteria) expired during the early postoperative period. For the latter 3 cases, the exact cause of death remained indeterminate after necropsy. Although these rabbits exhibited whole-body autolysis, there were no clear pathologic signs of sepsis, such as presence of disseminated bacterial foci or pyogenic inflammation in sites other than the posterolateral lumbar spine. One rhBMP-2/saline rabbit was excluded because of accidental intraoperative contamination. The rate of adverse events in this investigation is consistent with the rates reported in other studies using the same model.[10,16,24,25] There was no significant difference between the number of rhBMP-2 rabbits excluded versus the number of autograft rabbits excluded ($P = .195$).

### Bacterial Inoculums

Mean bacterial inoculums delivered to the autograft/bacteria and rhBMP-2/bacteria groups were 510 CFUs (SD, 149 CFUs) and 467 CFUs (SD, 142 CFUs), respectively. The difference in bacterial concentrations between these 2 cohorts was not significant ($P = .458$).

### Fusion

Manual palpation of the explanted spines was performed by 2 independent reviewers. Interobserver agreement was 100% for all the specimens. For the rhBMP-2/saline and autograft/

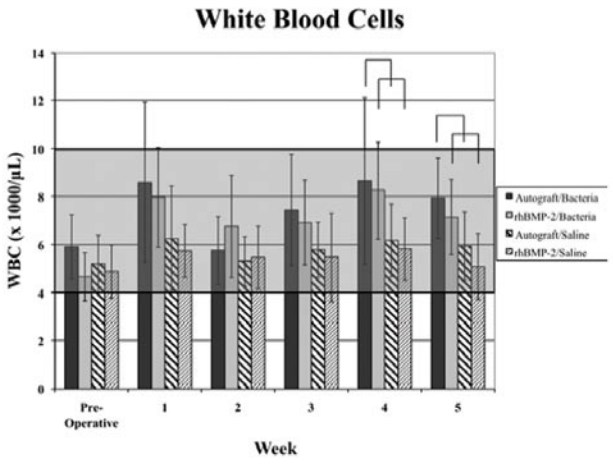


**Figure 5.** White blood cell (WBC) counts over time. Gray box indicates normal range for WBC count, hash mark above column indicates significant ($P$<.05) difference between groups, and error bar indicates SD.

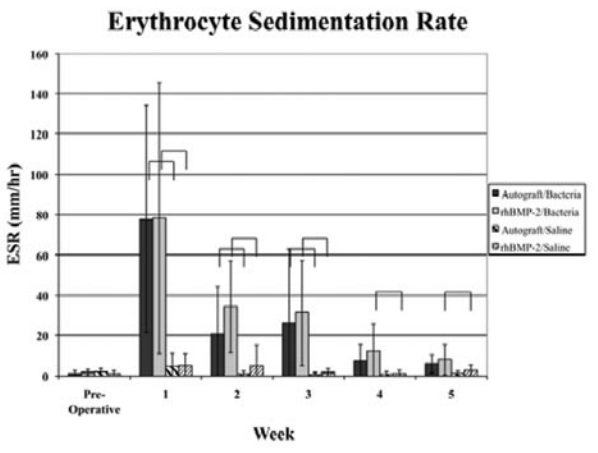


**Figure 6.** Erythrocyte sedimentation rates (ESR) over time. Hash mark above column indicates significant ($P$<.05) difference between groups, and error bar indicates SD.

saline animals, rates of successful arthrodesis were 100% (13/13) and 27% (4/15), respectively ($P$<.001) (Figure 2). Fusion rates for the rhBMP-2/bacteria and autograft/bacteria groups were trending toward significant difference at 25% (3/12) and 0% (0/14), respectively ($P$ = .085).

Representative histologic sections of each group are presented in Figure 3. Results of histologic analysis showed excellent interobserver reliability (ICC, 0.978). Although mean histologic fusion scores were significantly ($P$<.001) higher in the rhBMP-2/saline group (8.17; SD, 2.03) than in the autograft/saline group (2.61; SD, 2.14), there was no significant ($P$ = .67) difference between the rhBMP-2/bacteria group (2.83; SD, 2.79) and the autograft/bacteria group (3.33; SD, 2.69).

### Infection Classification

At study completion, no rhBMP-2/saline or autograft/saline rabbits showed evidence of abscess. Of the autograft/bacteria rabbits, 1 showed no evidence of infection, 9 had an abscess in the posterolateral gutters, and 4 died early (3 were sacrificed, 1 died from sepsis). Of the rhBMP-2/bacteria rabbits, 1 showed no evidence of infection, 11 had an abscess in the posterolateral gutters, and none died early (Figure 4). Four of the 14 autograft/bacteria rabbits and none of the 12 rhBMP-2/bacteria rabbits died early ($P$ = .1). Thirteen of the 14 autograft/bacteria rabbits and 11 of the 12 rhBMP-2/bacteria rabbits, which include both the early-death rabbits and the rabbits with abscesses, had disease ($P$ = .65).

### Blood Work

Mean preoperative white blood cell (WBC) count for all the animals was $5.2\times10^3$/µL (normal, $<10\times10^3$/µL). Mean maximum WBC count was $8.7\times10^3$/µL for the autograft/bacteria rabbits and $8.3\times10^3$/µL for the rhBMP-2/bacteria rabbits (Figure 5). For the 2 noninoculated groups, mean WBC count did not exceed $6.5\times10^3$/µL during the entire postoperative follow-up period.

Mean preoperative ESR across all groups was 1.5 mm/h (Figure 6). Mean maximum ESR was 66 mm/h for the rhBMP-2/bacteria rabbits and 77.5 mm/h for the autograft/bacteria rabbits. For the 2 noninfected groups, mean ESR did not exceed 5.5 mm/h for any of the blood draws.

There was no significant ($P$>.05) difference in mean WBC count or differentials or in ESR for the autograft/bacteria and rhBMP-2/bacteria groups or for the autograft/saline and rhBMP-2/saline groups at any of the time points.

## Discussion

Wound infection, a potentially devastating complication of spine surgery, may give rise to multiple sequelae, including local wound problems, pseudarthrosis, and systemic disease. Consequently, any strategy that successfully decreases the incidence of postoperative spinal infections would undoubtedly have a significant impact on patient outcomes. Although studies involving long bones have already shown that rhBMP-2 not only may enhance bone healing but may decrease infection rates (because of its potent osteoinductive and angiogenic properties), reports assessing the effects of this biologically active material in the infected spine are limited.

In the present study, we refined a model of postoperative lumbar infection involving the New Zealand white rabbit[28] (used in the previous experiments) to compare the efficacies of rhBMP-2 and autograft in inducing posterolateral spinal fusion in the setting of infection. We compared fusion rates of rhBMP-2 and autograft in the setting of a surgically acquired infection by testing the spines with manual palpation. We also attempted to evaluate the safety of rhBMP-2 in the setting of infection by comparing inflammatory markers (CBC count, ESR) and infection severity between autograft and rhBMP-2 groups.

The primary limitations of this study are its small size and the limited number of animals in each study group. It is conceivable that, given larger numbers, our results would have shown a significant difference in fusion rates and infection outcomes. In addition, we did not use a perfect replica of the common clinical situation of a preexisting infection that must be cleaned before attempted fusion. It is possible that, had we inoculated the rabbits 2 weeks in advance and then thoroughly débrided and irrigated the site before adding the BMP, results might have been different. Furthermore, these experiments involved injection of a large bacterial load, which in the absence of any type of antibiotic regimen created an extremely challenging environment for fusion. It is possible that the experimental groups would have demonstrated increased divergence had smaller inoculums been introduced or antibiotic therapy been provided. Finally, as with all preclinical investigations, these findings may not be directly extrapolated to the clinical scenarios encountered with humans. Nonetheless, this study made use of a well-established, controlled animal model with a limited number of variables. There was a low incidence of confounding events (only 10% of the animals were excluded from final analysis). In addition, the control data were generally consistent with our previous experience.

According to the experimental design, the 2 groups of animals that were not inoculated with bacteria served as the control groups. Manual palpation confirmed that all the noninfected rabbits treated with rhBMP-2 had successful fusions—a finding consistent with findings from similar studies.[16,17] However, only 27% of the autograft/saline animals had successful fusions according to manual palpation, and this percentage is lower than other published arthrodesis rates (range, 43%-73%).[17,33] The relatively low fusion rate associated with this autograft control cohort may have been influenced by a variety of factors. For instance, although we tried to match the amount of autograft harvested from the iliac crests of each animal, it is conceivable that smaller quantities of bone may have been harvested, resulting in a lower fusion rate.[34] In addition, the rabbits in this infection protocol required more handling for clinical monitoring and regular blood draws—a variable that decreases fusion rates in this model.[35]

In the presence of active local infection without antibiotic treatment, rhBMP-2 induced solid posterolateral fusion in 25% of animals, versus 0% of animals treated with autograft. Although the difference did not reach our predetermined level of significance, the $\alpha$ error was trending toward significance ($P = .085$). This trend is consistent with investigations that have found rhBMPs superior to autograft for promoting spinal arthrodesis in a variety of challenging environments, such as concomitant nicotine use, pseudarthrosis, and doxorubicin infusion.[16,36,37] Our study results also show the devastating potential that common bacterial pathogens have for causing pseudarthrosis.

Two recent human studies have shown that rhBMP-2 used with antibiotics and instrumented fusion can provide a safe treatment for vertebral osteomyelitis.[38,39] These studies found that BMP could achieve high fusion rates, excellent clinical results, and eradication of the infection without adverse side effects. Similarly, Chen and colleagues[18] demonstrated that antibiotics increased bone formation in chronically infected femoral defects in animals. Thus, it is unclear how use of antimicrobial therapy would have influenced the fusion rate in this model, as antibiotics were not administered at any point during the perioperative or postoperative period.

The infection classification (Figure 4) demonstrates that rhBMP-2 was not associated with any additional morbidity or mortality as compared with autograft. None of the 14 rhBMP-2/bacteria rabbits required early sacrifice or died secondary to sepsis; of the 14 autograft/bacteria rabbits, 3 required early sacrifice, and 1 died secondary to sepsis. This discrepancy in mortality, however, did not reach statistical significance (4/14 autograft vs 0/12 rhBMP-2, $P = .1$). Similarly, there was no difference between the 2 groups in number of animals that developed illness (13/14 vs 11/12, $P > .99$). Although there was no significant difference in infection classification, the data does trend toward rhBMP-2 being protective.

Regarding blood work, there was no increase in inflammatory markers or cell counts between the rhBMP-2/saline and autograft/saline groups. Had there been increased inflammation in the rhBMP-2/saline rabbits, presumably it would have been an immune response to the implant itself. As these were control rabbits, we would not expect an increase in immune function directed against any other exogenous factor, such as bacterial inoculation. Thus, these data suggest that there was no appreciable systemic immune response to the rhBMP-2 or collagen carrier.

We evaluated the safety and efficacy of rhBMP-2 and autograft in inducing bone formation in an infected spinal environment. Use of rhBMP-2 did not result in increased fusion or decreased incidence of infectious complications relative to autograft at our predetermined $\alpha$ error threshold; the results, however, did trend toward significance. On the basis of infection classification and blood work, there were no problems associated with use of rhBMP-2 in the setting of a surgically acquired infection, and graft harvest was avoided. As the biological pathways affected by BMPs continue to be elucidated, it is possible that indications for using rhBMP-2 may expand to include clinical situations such as fusion in the setting of active local surgical site infections.

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