# Comparison of two doses of recombinant human bone morphogenetic protein in absorbable collagen sponges for bone healing in dogs

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**Objective**—To determine the effects of 2 doses of recombinant human bone morphogenetic protein-2 in an absorbable collagen sponge (rhBMP-2/ACS) on bone healing in dogs.

**Animals**—27 adult dogs.

**Procedures**—Dogs underwent a mid-diaphyseal (1-mm) tibial osteotomy (stabilized with external skeletal fixation) and received an ACS containing 0.28 mg (0.2 mg/mL) or 0.56 mg (0.4 mg/mL) of rhBMP-2 or no treatment (control dogs). All dogs were examined daily; bone healing was assessed via radiography and subjective lameness evaluation every 2 weeks. After euthanasia at 8 weeks, tibiae were evaluated biomechanically and histologically.

**Results**—Control dogs required antimicrobial treatment for pin-site–related complications more frequently than did rhBMP-2/ACS–treated dogs. At 4 and 6 weeks, weight bearing was greater in dogs treated with rhBMP-2/ACS (0.2 mg/mL) than in control dogs, albeit not significantly. Compared with control treatment, both doses of rhBMP-2/ACS accelerated osteotomy healing at 4, 6, and 8 weeks, and the 0.2 mg/mL dose enhanced healing at 2 weeks; healing at 6 weeks was greater for the lower-dose treatment than for the higher-dose treatment. Histologically, healing at 8 weeks was significantly improved for both rhBMP-2/ACS treatments, compared with control treatment. Among groups, biomechanical variables did not differ, although less osteotomy-site failures occurred in rhBMP-2/ACS–treated groups.

**Conclusions and Clinical Relevance**—In dogs that underwent tibial osteotomy, rhBMP-2/ACS (0.2 mg/mL) appeared to accelerate bone healing and reduce lameness (compared with control treatment) and apparently augmented bone healing more than rhBMP-2/ACS (0.4 mg/mL). Compared with control dogs, rhBMP-2/ACS–treated dogs required antimicrobial treatments less frequently. (*Am J Vet Res* 2007;68:834–840)

Enhancement of bone healing after fracture repair by use of local administration of rhBMP-2 is an established, efficacious, and clinically applicable strategy that has been applied to humans and other animals.[1-11] Bone morphogenetic proteins are a group of signaling molecules that have the potential to cause undifferentiated mesenchymal cells to commit to an osteoblastic phenotype.[10] However, ideal delivery methods and optimal doses of BMPs for use in dogs have yet to be determined. In a previous study[1] performed in our laboratory, it was evident that rhBMP-2 (0.2 mg/mL) delivered within an ACS resulted in improved bone healing and accelerated recovery of limb function after tibial osteotomy, compared with findings in untreated control dogs and dogs treated with rhBMP-2 (0.05 mg/mL). The results of that study indicated that rhBMP-2/ACS had a positive dose-dependent effect on bone healing in dogs and established a lower dose limit.

**Abbreviations**

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|---|---|
| rhBMP-2 | Recombinant human bone morphogenetic protein-2 |
| ACS | Absorbable collagen sponge |

The objective of the study reported here was to compare bone healing in dogs that underwent tibial osteotomy and received treatment with rhBMP-2 (0.2 or 0.4 mg/mL doses) delivered in an ACS or no BMP treatment (control group). The hypotheses were that after tibial osteotomy, bone healing rate and strength in dogs treated with rhBMP-2/ACS would be improved, compared with dogs that received no treatment, and that of the 2 doses of rhBMP-2, the higher would augment overall bone healing more effectively.

## Materials and Methods

The University of Wisconsin Animal Care and Use Committee approved all study procedures prior to subject enrollment. The study was conducted by use of a randomized complete block design involving 3 treat-

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ments and 9 dogs/treatment group. All data were collected and analyzed by personnel who were unaware of treatments.

The 27 adult female mixed-breed dogs used in the study were owned by the university. Allocation of dogs to treatment groups was done in 2 steps. First, dogs were weighed, ranked in descending order of body weight, and grouped into blocks of 3. Each dog in each block was randomly allocated to 1 of 3 treatment groups. In all dogs, mid-diaphyseal osteotomy (1-mm transverse defect) was performed on the right tibia and the bone was stabilized by use of a craniomedial, unilateral, uniplanar, 6-pin, external skeletal fixator. Dogs allocated to the control group (group 1) underwent tibial osteotomy with no subsequent treatment. In dogs allocated to receive rhBMP-2 treatment, the bone at the osteotomy site was wrapped with an ACS (2.5 × 5.1 cm) that was loaded with 1.4 mL of rhBMP-2[a] at 1 of 2 concentrations: 0.2 mg/mL (total dose, 0.28 mg [group 2]) or 0.4 mg/mL (total dose, 0.56 mg [group 3]).

Every 2 weeks during an 8-week period after surgery, outcome measures were assessed via radiography and clinical lameness evaluation (which involved assignment of a lameness score by use of a VAS). Dogs were evaluated daily for development of complications or adverse effects. Mechanical and histologic assessments were performed on tibiae that were collected following euthanasia 8 weeks after surgery.

**Anesthetic and operative procedure**—For each dog, a fentanyl patch (5 µg/kg/h) was applied to the skin on the dorsal midline of the interscapular region 12 hours prior to surgery. The patch remained in place for 3 days after surgery. Thirty minutes prior to induction of anesthesia, dogs were administered hydromorphone (0.2 mg/kg, SC) and acepromazine (0.05 mg/kg, SC). Anesthesia was induced with thiopental sodium (7 mg/kg, IV) and maintained with halothane in 100% oxygen in a rebreathing circuit with spontaneous ventilation. Cefazolin (60 mg/kg, IV) was administered prophylactically at induction of anesthesia and again 6 hours later.

The surgical procedure was performed on the right hind limb of each dog by the same surgical team. A 5-cm skin incision that extended to the periosteum was made on the craniomedial aspect of the hind limb. A 1-mm transverse mid-diaphyseal osteotomy was performed by use of an oscillating saw[b] under copious irrigation with saline (0.9% NaCl) solution. The osteotomy gap was confirmed by use of a 1-mm-thick shim. A 1-cm section of the fibula was removed with rongeurs, both proximal and distal to the osteotomy. The tibia was stabilized by application of a craniomedially placed, unilateral, uniplanar, small external fixator[c] with 3 parallel, negatively threaded conical pins in each tibial segment, as previously described.[1-2] During the surgical procedure, the rhBMP-2/ACS implant was prepared at the assigned concentration and wrapped around the osteotomy site. Muscles were apposed, and the incision was routinely closed in 3 layers.

**Implant preparation**—The rhBMP-2 was reconstituted from a stock of lyophilized rhBMP-2 to concentrations of 0.2 and 0.4 mg/mL. No less than 10 minutes and no more than 30 minutes prior to implantation, 1.4 mL of the reconstituted rhBMP-2 solution was evenly applied to an ACS (2.5 × 5.1 cm) to provide a dose of 0.2 or 0.4 mg/mL. The sponge was carefully placed around the tibia, encompassing approximately 1.2 cm of each extremity of the bone fragments and the entire osteotomy site.

**Postoperative monitoring**—After surgery, staff who were unaware of treatment allocations examined each dog twice daily. Hydromorphone (0.2 mg/kg) was administered SC if signs consistent with excessive postoperative pain (eg, excess vocalization, signs of pain elicited during manipulation of the operated limb, or high heart or respiratory rates) were evident, as determined by a veterinarian who was also unaware of treatment. Any abnormality including discharge from the incision site, swelling at or discharge from a pin site, or swelling at the osteotomy site was noted in the medical record. If an orthopedic surgeon who was unaware of treatment considered that subjective evidence of infection (increasing redness, signs of pain, heat, swelling, discharge at the surgery site, or fever) was present, dogs were administered amoxicillin trihydrate–clavulanate potassium[d] (10 mg/kg, PO, q 12 h). Definitive diagnosis of infection via quantitative microbial culture was not performed.

**Radiography**—Craniocaudal and mediolateral radiographic views of each operated tibia were obtained immediately after surgery (week 0) and at 2, 4, 6, and 8 weeks following surgery. Radiographic signs of bone union were graded on a scale of 1 to 4 by 3 independent observers (who were unaware of treatment allocations) on the basis of bony union or bridging callus at bone cortices (grade 1 = no evidence of callus, union < 25%; grade 2 = union 25% to 49%; grade 3 = union 50% to 74%; and grade 4 = union ≥ 75%).

**Lameness score**—Before and at 2, 4, 6, and 8 weeks after surgery, dogs were evaluated for clinical evidence of lameness by an independent observer who was unaware of treatments. Dogs were assessed while trotting in a straight line and turning to the left and right. The observer assigned scores for the severity of lameness by use of a VAS (0 [sound] to 100 mm [could not be more lame]), as previously described.[12] Dogs that were more severely lame received higher VAS scores.

**Mechanical testing**—After euthanasia, both tibiae were collected from each dog, wrapped in towels soaked with saline solution, and tested within 6 hours. The central 7-cm span of each tibia (ie, 3.5 cm proximal and distal to the osteotomy site in the right tibia) was measured and marked. From the proximal and distal limits of the 7-cm central diaphysis, a distance of 4.5 cm was measured towards the proximal and distal epiphyses. Any bone exceeding these limits was trimmed, resulting in a specimen length of 16 cm. Two potting rods were placed horizontally in the proximal and distal limits of the 7-cm central diaphysis, and the ends of the specimen were potted in polyester and styrene by use of an alignment jig. The tibiae were tested in torsion by application of external rotation at 1.5°/s to a maximum of 45° or until failure occurred by use of a modified servohydraulic materials testing system.[e]

Load and deformation data were recorded continuously at 10 Hz by use of a data acquisition board, printed, and stored on a personal computer in a data file. Stiffness was calculated as the slope of the initial linear portion of each curve. Ultimate torque was also obtained from the data for each specimen. To control for individual variance in bone mechanical properties, all data were normalized to the contralateral tibia and results were expressed as a ratio of right (osteotomized) versus left (intact) tibia values. Radiographic views of the bone after failure were obtained and used to record failure location as either through the original osteotomy or outside the osteotomy.

**Histologic evaluation**—The mechanical testing apparatus returned the specimens to the original starting point and allowed alignment of the failed specimens for histologic processing. Immediately after the completion of mechanical testing, the specimens were fixed in 70% ethanol for processing without decalcification. Tissues were dehydrated by immersion in increasing concentrations of ethanol followed by immersion in acetone. Infiltration and embedding were performed with methylmethacrylate under vacuum. Blocks were cut coronally, and 200-µm-thick central sections encompassing the osteotomy region were made by use of a low-speed diamond saw.[f] These sections were subsequently ground to 100 µm by use of a speed-lapping machine[g] modified for undecalcified bone and stained for light microscopic examination. One section was stained by use of a modified toluidine blue method, and the other was stained by use of a modified Goldner trichrome method. By use of high-resolution radiographic plates,[h] fine-detail contact microradiography[i] (18 kVp; 2 minutes) was performed on one 100-µm section from each limb.

Sections were each evaluated histologically by 3 independent observers. At the medial and lateral cortices of the bone junctions, a score was assigned (scale of 1 to 4; **Appendix**) for the cortical gap and periosteal gap that accounted for evidence of bone union and the relative amounts of bone, cartilage, and fibrous connective tissue.

**Data analysis**—Continuous variables (VAS scores of lameness and mechanical data) are reported as mean ± SEM. For each dog, histologic scores and radiographic grades at each time point were determined on the basis of median values of the 3 scores or grades assigned. Histologic and radiographic data were then analyzed by group by use of the Wilcoxon rank sum test.[j] Repeated-measures analysis[k] was used to detect effect of treatment on lameness over all time points. Analysis of variance[l] was used to detect effect of treatment on mechanical testing results. The requirement for antimicrobial administration to treat presumptive pin tract infection and the failure location (through or outside the osteotomy site) during mechanical testing were analyzed by use of the Fisher exact test. Treatment effect was evaluated at the 5% level of significance (ie, values of $P < 0.05$ were considered significant).

## Results

At the initiation of the study, the mean ± SEM weight of the dogs was 25.6 ± 0.77 kg. There was no significant difference in weight among the 3 groups. Preparation and intra-operative use of the rhBMP-2/ACS implants were simple and proceeded without incident. There were no intra-operative complications, and all dogs recovered from anesthesia and surgery uneventfully.

Postoperative abnormalities that were typical for this type of surgery and method of fixation developed in all groups; these abnormalities included swelling at the osteotomy site and swelling and discharge at the pin sites. Swellings at the osteotomy sites matured to hard areas of bony proliferation by the end of the study in all dogs (regardless of treatment) except for 3 control dogs. One control dog developed a fever and became listless, and another developed a small abrasion on 1 carpus; both dogs were administered antimicrobial treatment prophylactically. Not including those 2 dogs, 7 dogs in the control group received antimicrobial treatment because of subjective assessment of clinical signs associated with the pin tracts, whereas only 2 dogs each in groups 2 and 3 were administered an antimicrobial.

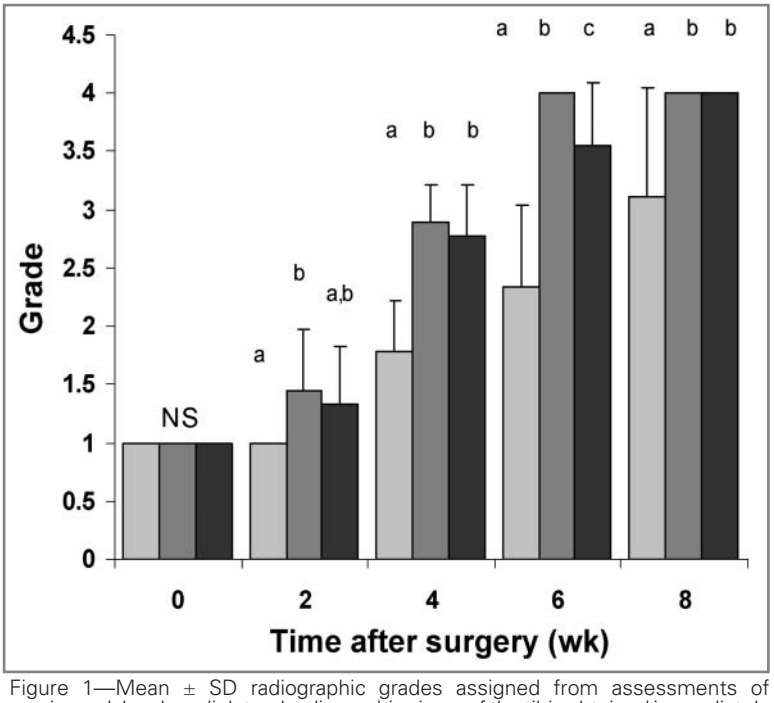


Figure 1—Mean ± SD radiographic grades assigned from assessments of craniocaudal and mediolateral radiographic views of the tibia obtained immediately after surgery (week 0) and at 2, 4, 6, and 8 weeks after surgery in dogs undergoing tibial osteotomy (right hind limb) that received no intra-operative treatment (control group 1; light gray bars), rhBMP-2/ACS (0.2 mg/mL) treatment (group 2; dark gray bars), or rhBMP-2/ACS (0.4 mg/mL) treatment (group 3; black bars). There were 9 dogs/group, and radiographic signs of bone union were graded on a scale of 1 to 4 by 3 independent observers (who were unaware of treatment allocations) on the basis of bony union or bridging callus at bone cortices (grade 1 = no evidence of callus, union < 25%; grade 2 = union 25% to 49%; grade 3 = union 50% to 74%; and grade 4 = union ≥ 75%). [a-c]At each time point, different letters indicate significant ($P < 0.05$) differences among groups. NS = No significant difference among groups at this time point.

Significantly ($P < 0.05$) more dogs in the control group were treated with an antimicrobial for the presumptive diagnosis of pin tract infection than in the 2 rhBMP-2–treated groups.

For dogs in each group, radiographic grades were assigned at 2, 4, 6, and 8 weeks after surgery (**Figure 1**). With the exception of radiographic views obtained immediately after surgery (week 0), group 2 (ie, dogs treated with rhBMP-2/ACS [0.2 mg/mL]) had significantly higher radiographic grades (ie, more extensive union), compared with the control group at all time points. Similarly, with the exception of radiographic views obtained immediately after surgery, group 3 (ie, dogs treated with rhBMP-2/ACS [0.4 mg/mL]) had higher radiographic grades than those of the control group at weeks 4, 6, and 8. At 6 weeks, dogs in group 2 also had significantly higher radiographic grades, compared with dogs in group 3. Despite the higher rhBMP-2 dose, dogs in group 3 did not achieve significantly higher radiographic grades than did dogs in group 2 at any time point. The number of dogs receiving the maximal radiographic grade of 4 on the basis of the median grade from the 3 evaluators at 6 and 8 weeks after surgery was calculated. On the basis of median scores assigned by the 3 evaluators, all 9 dogs treated with rhBMP-2/ACS (0.2 mg/mL) achieved maximal radiographic healing grade by week 6 (grade 4 was assigned in 20/27 [74%] evaluations), whereas only 5 of 9 dogs in group 3 and none of the 9 control dogs received a median radiographic grade of 4 (grade 4 was assigned in 14/27 [52%] of group 3 evaluations and in 2/27 [7%] of control group evaluations). At 8 weeks, all dogs in groups 2 and 3 had achieved the maximal radiographic grade (grade 4 was assigned in 27/27 [100%] evaluations and in 25/27 [93%] evaluations, respectively), whereas only 3 of 9 dogs in the control group achieved the maximal grade (grade 4 was assigned in 9/27 [33%] evaluations).

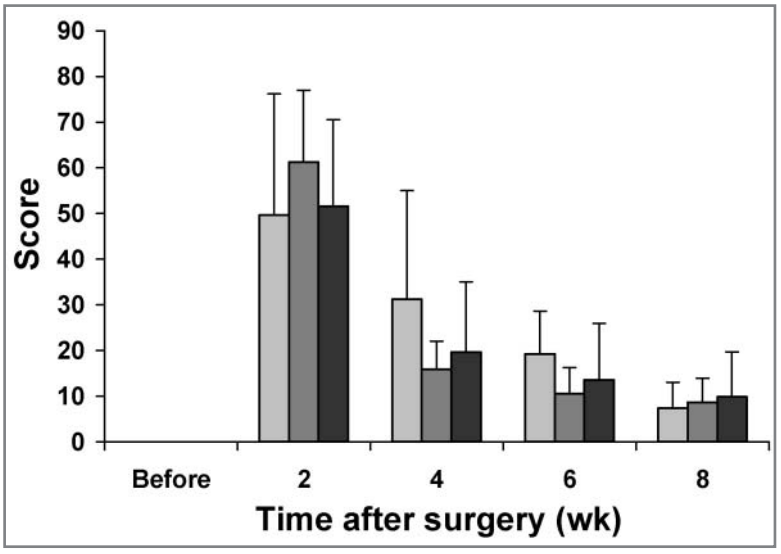


Figure 2—Mean ± SEM lameness scores assigned by an independent observer (who was unaware of treatment) on the basis of clinical evidence before and at 2, 4, 6, and 8 weeks after surgery in dogs undergoing tibial osteotomy (right hind limb) that received no intra-operative treatment (control group 1; light gray bars), rhBMP-2/ACS (0.2 mg/mL) treatment (group 2; dark gray bars), or rhBMP-2/ACS (0.4 mg/mL) treatment (group 3; black bars). There were 9 dogs/group. Dogs were assessed while trotting in a straight line and turning to the left and right; the observer assigned scores for the severity of lameness by use of a VAS (0 [sound] to 100 mm [could not be more lame]). There are no significant differences among groups within any time point.

Lameness VAS scores were assigned to each dog before and at 2, 4, 6, and 8 weeks after surgery (**Figure 2**). Before surgery, all dogs were assigned a score of 0, indicating no observable lameness at that time. In general, after an initial postoperative increase, VAS scores decreased (ie, lameness severity decreased) in all groups over time. There was no significant difference among groups at any time point, although the mean scores for dogs in group 2 were actually lower than scores for dogs in groups 1 or 3 at 4 and 6 weeks.

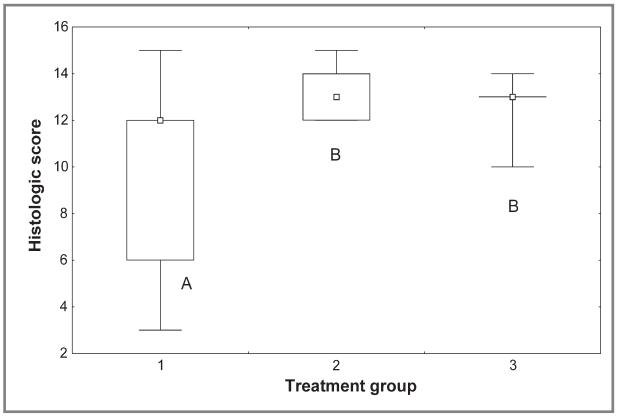


Figure 3—Box-and-whisker plot of histologic scores assigned during microscopic examination of sections of tibiae obtained 8 weeks after surgery in dogs undergoing tibial osteotomy (right hind limb) that received no intra-operative treatment (control group; group 1), rhBMP-2/ACS (0.2 mg/mL) treatment (group 2), or rhBMP-2/ACS (0.4 mg/mL) treatment (group 3). Sections were evaluated by 3 independent observers according to a qualitative scale (Appendix) that accounted for evidence of bone union and the relative amounts of bone, cartilage, and fibrous connective tissue at the medial and lateral cortices of the bone junctions. Small squares represent the median value; boxes represent the 25% to 75% interquartile range; and bars represent the minimum and maximum scores. [A,B]Different letters indicate significant ($P < 0.05$) differences among groups (Wilcoxon rank sum test).

Eight weeks after surgery, there was no significant difference in mechanical variables (torque to failure and stiffness) among groups. Mean maximum torque of the osteotomized tibia (as a percentage of the torque of the contralateral intact tibia) was 81.2 ± 9.0%, 85.2 ± 5.0%, and 85.9 ± 3.0% in groups 1, 2, and 3, respectively. The mean maximum stiffness of the osteotomized tibia (as a percentage of the torque of the contralateral intact tibia) was 88.5 ± 5.0%, 79.7 ± 4.0%, and 87.0 ± 6.0% in groups 1, 2, and 3, respectively. During mechanical testing, significantly ($P < 0.05$; 1-sided test) more specimens mechanically failed through the osteotomy site in the control group (5/9 tibiae), compared with specimens from the 2 rhBMP-2–treated groups (1/9 tibiae in group 2 and 2/9 tibiae in group 3).

Compared with findings in control dog tibiae, histologic scores were significantly ($P < 0.05$) higher (ie, indicative of greater healing) for dogs in both rhBMP-

2–treated groups (**Figure 3**). The mean ± SEM and median histologic scores for control tibiae were 9.44 ± 1.33 and 12 (95% confidence interval, 6.37 to 12.52), respectively. For tibiae treated with rhBMP-2/ACS (0.2 mg/mL), mean and median histologic scores were 13.22 ± 0.36 and 13 (95% confidence interval, 12.38 to 14.06), respectively. For tibiae treated with rhBMP-2/ACS (0.4 mg/mL), mean and median histologic scores were 12.78 ± 0.4 and 13 (95% confidence interval, 11.85 to 13.70), respectively. Histologic scores did not differ between groups 2 and 3.

## Discussion

Compared with the control treatment, treatment of tibial osteotomy sites in dogs with rhBMP-2/ACS (0.2 mg/mL) or rhBMP-2/ACS (0.4 mg/mL) resulted in significantly better radiographic grades of healing at 4, 6, and 8 weeks after surgery and at week 2 after surgery among dogs treated with rhBMP-2/ACS (0.2 mg/mL). More dogs receiving the 0.2 mg/mL dose achieved radiographic union at 6 weeks, compared with those treated with the 0.4 mg/mL dose or those receiving no treatment (control dogs). Furthermore, dogs treated with rhBMP-2/ACS (0.2 mg/mL) appeared to be less lame than control dogs at 4 and 6 weeks after surgery, although this difference was not significant. Significantly fewer rhBMP-2–treated tibiae failed through the osteotomy site during mechanical testing, compared with control bones. Finally, dogs treated with either dose of rhBMP-2/ACS were less likely to be administered an antimicrobial agent during their convalescence than control dogs in the present study.

Previous studies[1-5,8] have revealed a positive effect of rhBMP-2 treatment on bone healing in dogs. This effect is thought to result from osteogenic induction of mesenchymal stem cells.[10] The data obtained in the present study along with the results of other investigations[1,8] appear to illustrate a clinically important dose-dependent effect of rhBMP-2 on bone healing in dogs. A minimum threshold dose of rhBMP-2 is necessary for a beneficial effect to occur,[8] but a higher dose does not necessarily result in a better outcome, compared with administration of a moderate dose.

Treatment with rhBMP-2 at various doses has improved biomechanical characteristics of healing bone in dogs other studies.[1,2,8,9] However, increasing the rhBMP-2 dose does not consistently result in a mechanically stronger bone. In a study by Sciadini and Johnson,[8] dogs underwent radial osteotomy with the creation of a 2.5-cm diaphyseal defect; administration of higher doses of rhBMP-2 resulted in increased cyst-like bone voids that were detectable radiographically and histologically, and there was a positive correlation between radiographic detection of bone voids and reduced biomechanical values. Cyst-like bone voids were not detected after use of the higher rhBMP dose in the present study.

The lack of biomechanical difference among all groups in our study may more likely be an artifact resulting from the design of the study, specifically as a consequence of the time point at which the tibiae were evaluated. The amount of periosteal callus has a direct effect on the maximum torque and torsional stiffness.[13] As bone heals, the callus (and therefore the maximum torque) increasingly develops until a maximal amount is formed; the development then plateaus as periosteal and endosteal callus is resorbed and remodeled into cortical bone. At 8 weeks after surgery in the present study, histologic and radiographic data indicated that a maximum amount of callus had already developed (at approx 6 weeks after surgery) and that periosteal callus was undergoing remodeling and active resorption in the rhBMP-2–treated tibiae, whereas callus formation was just reaching maximal amounts in control tibiae. This may be a source of error in study design; if maximum torque and stiffness had been measured earlier in the course of healing (eg, at 6 weeks after surgery), treatment of tibiae with rhBMP-2 may have resulted in significantly greater mechanical properties, compared with untreated control bones.

On the basis of subjective criteria used to guide treatment in the present study, dogs treated with rhBMP-2/ACS required antimicrobial administration less frequently than did control dogs. In a large clinical trial in humans,[11] a decreased rate of infection of open tibial fractures treated with rhBMP-2 in an ACS (compared with fractures that were not treated with BMP) was identified; however, that study involved humans, and the findings are therefore difficult to compare with those of the study reported here. In that human study,[11] increased stability of the fracture fragments was proposed as a potential mechanism of decreased infection rate because increased stability results in increased blood supply and decreased cyclic trauma at the pin-bone interface.

In dogs of the present study, infection of the osteotomy or pin site was not confirmed via microbial culture; thus, definitive statements cannot be made regarding the effect of rhBMP-2 on postoperative infection rates. However, treatment with an antimicrobial was initiated only if drainage from the surgery site was deemed severe enough to warrant intervention; fever developed; or, as for 1 dog, there was a need for prophylactic administration of an antimicrobial to prevent infection in a nonosteotomized limb. Subjective assessment of pin tract infection has been compared with results of quantitative microbial culture in goats with experimentally induced pin tract infection.[14] Investigators in that study determined that use of a clinical criterion alone had 100% sensitivity and 58% specificity for diagnosis of pin tract infection. To further explore this phenomenon in future studies, quantitative microbial culture of appropriate samples would yield more objective information.

There are similarities and differences in findings between the present study and a previous study[1] of rhBMP-2/ACS performed in dogs undergoing tibial osteotomy in our laboratory. Previously, dogs treated with rhBMP-2/ACS (0.2 mg/mL) were less lame (as determined via VAS scoring) at 2 and 4 weeks after surgery and when data for each treatment was pooled to include all postoperative time points, compared with control dogs. Although the data obtained in the present study did not reveal a significant difference in lameness between dogs treated with rhBMP-2/ACS (0.2 mg/mL) and control dogs, the treated dogs did appear to have less lameness at 4 and 6 weeks after surgery. Also, the

tibiae treated with rhBMP-2/ACS (0.2 mg/mL) in the previous study were stronger and stiffer than control tibiae after 8 weeks. This finding differs from that of the present study, in which no significant difference in mechanical strength was detected between those 2 groups after 8 weeks. However, in both studies, there were statistically greater radiographic grades and histologic bone healing scores in dogs treated with rhBMP-2/ACS (0.2 mg/mL), compared with control dogs.

In our laboratory, we commonly use the external skeletal fixator system[c] with negatively threaded conical pins to stabilize a midtibial transverse osteotomy in our research projects. This system provides rigid stabilization of bone fragments and allows repeatable results during evaluation of bone healing in dogs. We have thoroughly investigated the clinical, radiographic, densitometric, mechanical, and histologic effects of the use of this fixator system on canine tibiae.[1,2,15–17] A 1-mm-gap osteotomy was performed in dogs of the present study because this reflects a common clinical situation after fragment reduction and rigid fixation. More importantly, to compare data from the present study with previously published findings,[1] use of this system in this manner was required and variation in technique would have considerably diminished the value of these data. However, this fixator system does not reflect the current state-of-the-art apparatus in veterinary external skeletal fixation, and although we do not believe that use of a different form of rigid fixation would have dramatically altered the results of our study, it should be emphasized the conditions in which the fixator system was used may not entirely reflect clinical conditions. A prospective trial in dogs is needed to evaluate whether bone healing is improved and postoperative complications are reduced with rhBMP-2 treatment in clinical situations.

Fentanyl patches were used in our study to provide analgesia after surgery because they have a proven analgesic effect in dogs,[18] are commonly used in postoperative settings, and are logistically feasible to use in a research environment. Other delivery methods could be considered to augment analgesia, including continuous rate IV infusion of opioid analgesics, but that procedure is more logistically difficult in a research setting. Epidural analgesia may have been advantageous to augment short-term postoperative analgesia in the dogs in the present study.

Overall, results of the present study have indicated that treatment of tibial osteotomy in dogs with rhBMP-2/ACS (0.2 mg/mL) accelerates bone healing and reduces lameness after surgery and appears to be superior to treatment with rhBMP-2/ACS (0.4 mg/mL) for clinical augmentation of bone healing in this experimental situation. The use of either dose improved histologic evidence of healing bone after surgery. Intraoperative use of the product was rapid and undertaken without complication. Clinical trials are needed to evaluate the efficacy of rhBMP-2/ACS for enhancing healing of different bones, for use in various fracture and defect configurations, and for use with different stabilization techniques.

a. Recombinant human bone morphogenetic protein-2, Fort Dodge Animal Health, Princeton, NJ.
b. Oscillating saw, Model 1370, Stryker Corp, Kalamazoo, Mich.
c. Upper extremity fixator, Orthofix Inc, Richardson, Tex.
d. Clavamox, Pfizer, New York, NY.
e. Servohydraulic materials testing system, Model 858, MTS Systems Corp, Eden Prairie, Minn.
f. Isomet Plus, Buehler Ltd, Lake Bluff, Ill.
g. ML-521D, Maruto Instrument, Tokyo, Japan.
h. Kodak high-resolution plates, Kodak, New Haven, Conn.
i. Faxitron X-ray System, Model 43855A, Hewlett-Packard, McMinnville, Ore.
j. PROC NPAR1WAY, version 8.2, SAS Institute Inc, Cary, NC.
k. SAS PROC MIXED, version 8.2, SAS Institute Inc, Cary, NC.
l. ANOVA, SAS PROC MIXED, version 8.2, SAS Institute Inc, Cary, NC.

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## Appendix

Scoring system used to assign bone healing values on the basis of on light microscopic analysis of 100-µm-thick undecalicified coronal sections of tibiae collected 8 weeks after surgery from dogs that underwent tibial osteotomy (right hind limb) and received no intra-operative treatment (control group), rhBMP-2/ACS (0.2 mg/mL) treatment, or rhBMP-2/ACS (0.4 mg/mL) treatment.

| Region of the section | Histologic appearance | Medial cortex score | Lateral cortex score |
|---|---|---|---|
| Cortical gap | Filled with minimum or no tissue | 0 | 0 |
| | Filled predominantly with fibrous tissue | 1 | 1 |
| | Filled predominantly with cartilage | 2 | 2 |
| | Filled predominantly with woven bone | 3 | 3 |
| | Filled predominantly with lamellar bone | 4 | 4 |
| Periosteal gap | No bridging | 0 | 0 |
| | Predominantly fibrous bridge at osteotomy site | 1 | 1 |
| | Predominantly cartilaginous bridge at osteotomy site | 2 | 2 |
| | Predominantly woven bone bridge at osteotomy site | 3 | 3 |
| | Predominantly lamellar bone bridge at osteotomy site | 4 | 4 |