Acceleration of Second and Fourth Metatarsal Fracture Healing with Recombinant Human Bone Morphogenetic Protein-2/Calcium Phosphate Cement in Horses

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Objective—To compare the efficacy of recombinant human bone morphogenetic protein-2 (rhBMP-2)/calcium phosphate (CP) to autogenous cancellous bone graft (CBG) and to no treatment on bone healing, in surgically induced osteotomies and ostectomies of the accessory metatarsal bones in an equine model.

Study Design—Experimental.

Animals—Adult horses (n = 9).

Methods—Segmental ostectomies of the second metatarsal bone (MT2) and osteotomies of the fourth metatarsal bone (MT4) were performed bilaterally in 9 horses. There were a total of 35 defects (1 MT4 was previously fractured) created and supplemented randomly either with no treatment (untreated control), rhBMP-2/CP cement, or matrix (CPC or CPM), or CBG. Radiography was performed every 2 weeks until study endpoint at 12 weeks. After euthanasia, bone healing was evaluated using radiography, mechanical testing, and histology. Data were analyzed with ANOVA followed by the Duncan's Multiple Range Test or nonparametric analyses.

Results—At 12 weeks, radiographic scores for union were significantly greater for the rhBMP-2 ($P < .0001$) and CBG ($P = .004$) groups compared with the untreated control group, for both MT2 ostectomies and MT4 osteotomies. The rhBMP-2 treated MT2 had greater maximum torque to failure in torsion than CBG and control limbs at 12 weeks ($P = .011$). Histologic analysis demonstrated increased bone formation and more mature bone at the ostectomy site for MT2 in the rhBMP-2 and CBG groups compared with the untreated control group.

Conclusion—Injection of rhBMP-2/CP into surgically induced ostectomies and osteotomies of the accessory metatarsal bones might accelerate early bone healing in the horse.

Clinical Relevance—RhBMP-2/CP may be as effective if not superior to CBG as an adjuvant treatment to accelerate healing of bone defects.

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INTRODUCTION

FRACTURES ACCOUNT for 71% of fatal musculoskeletal injuries in thoroughbred racehorses.[1] Forces involved in equine fractures are generally much greater than those acting on bones in humans or smaller animal species. As a result, fracture repair of long bones in the horse is often challenging and associated with unsatisfactory outcomes, with a higher incidence for complications, such as fixation failure or delayed healing, than in other species. Economic pressure in the horse-racing industry demands not only optimal fixation but

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faster healing to minimize economic losses because of prolonged convalescence. Thus, it is important to develop and evaluate new therapeutic modalities, designed to improve fracture treatment and accelerate healing.[1]

Over the past decade, several proteins and other growth factors with osteoinductive properties have been studied.[2] Among them, bone morphogenetic proteins (BMP), related to the family of differentiation factors, have been found to be responsible for the bone inductive activity of the bone matrix. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is produced in a biotechnology process offering unlimited supply and substantial control over purity and reproducible activity. Unlike other factors, rhBMP-2 is capable of initiating the entire cascade of de novo bone formation and is known to have similar osteoinductive activities in vivo.[2] rhBMPs result in increased bone formation by enhancing mesenchymal cell infiltration and differentiation of the cells into chondrocytes, removal of the cartilage, formation of bone, population of the bone with bone marrow elements and remodeling of the bone. When used at higher concentrations, rhBMP-2 can also result in direct (intramembranous) ossification.[3,4] Preclinical and clinical human and animal trials have demonstrated that the application of rhBMP-2 might be useful as an adjuvant treatment to accelerate the healing of bone defects.[5-15] In a canine model, significant acceleration of diaphyseal bone healing occurred after a single injection of rhBMP-2/CPM.[7]

Use of a carrier to deliver the protein helps maintain an adequate concentration of morphogenetic factor at the fracture site for a sufficient period of time allowing bone-forming cells to migrate to the area of injury where they proliferate and differentiate.[13,16] A rapidly resorbable calcium phosphate (CP) is suitable and biocompatible, having a high affinity for proteins such as osteogenic agents, being resorbed quickly and therefore minimally interfering with fracture healing and yet allowing direct deposit of bone by osteoblasts. Finally, being radio-opaque, it can be easily identified.[13]

Two formulations of CP are available: CP matrix (CPM) and CP cement (CPC). CPM is an endothermically setting paste designed to form amorphous hydroxyapatite granules containing rhBMP-2 when fully reacted.[13,17] Prolonged release of rhBMP-2 is achieved through the resorption of CPM by osteoclasts and giant cells. In addition, CPM also promotes bone formation by osteoconduction.[13] CPC is an alloplastic material for osseous augmentation because of the unique combination of osteoconductivity, biocompatibility, and ease of molding. CPC can be molded and shaped to fill intricate bony defect sites, has excellent in vivo resorption, and self-hardens in situ. Several clinical studies have demonstrated that the CPC in putty form accelerated healing of bony defects.[18,19]

We evaluated the capacity of rhBMP-2/CPM and rhBMP-2/CPC to accelerate the healing of both ostectomies and osteotomies of the accessory metatarsal bones in an equine model.

MATERIALS AND METHODS

Experimental Design

Bilateral osteotomies of the fourth metatarsal bones (MT4) and ostectomies of the second metatarsal bones (MT2) were performed in 9 adult horses (mean [± SD] weight, 513 ± 49.1 kg; age range, 3–8 years). Thirty-five defects, created on 18 limbs (1 MT4 was excluded from the study because of previously healed fracture) were randomly (in a block design) assigned treatment with rhBMP-2, autogenous cancellous bone graft (CBG), or used as untreated control. MT2 and MT4 in 6 limbs were treated by the administration of rhBMP-2 (1 mg/mL) delivered in a rapidly resorbable CPC or CPM; 6 limbs were administered 10 mm^3 CBG harvested from the tibial crest; and 6 limbs served as untreated controls. Approximately 2 mg rhBMP-2/CPC was used at MT2 ostectomy site and ∼ 0.5 mg of rhBMP-2/CPM at the MT4 osteotomy site (Table 1).

Surgical Procedure

Ampicillin (10 mg/kg), gentamicin (6.6 mg/kg), and phenylbutazone (4.4 mg/kg) were administered intravenously (IV) 30 minutes before induction of anesthesia with xylazine (1 mg/kg IV), ketamine (2 mg/kg IV) and diazepam (0.1 mg/kg IV). Horses were positioned in dorsal recumbency and anesthesia maintained with isoflurane in oxygen. Both hind limbs were clipped circumferentially from the coronary band to the stifle and aseptically prepared for surgery with 0.05% chlorhexidine. After draping, a 20 g needle was inserted laterally into the tarsometatarsal joint. A sterile measuring device and pen were used to circumferentially mark 10 cm distal to the needle. Medial and lateral 5-cm skin incisions centered on the marked line were made on both hind limbs, over the palpable surface of the accessory metatarsal bones, which were isolated by cutting their attachments to the suspensory ligament and the third metatarsal bone. A curved spatula was placed under the accessory metatarsal bone and a nitrogen-driven oscillating bone saw (Maxi Driver 3M, St. Paul, MN) was used to transect MT4 (saw blade thickness 1 mm) or to remove a 5 mm fragment of the MT2. Incisions were flushed copiously with sterile saline to remove bone dust and debris.

Table 1. Treatment Assignment within Metatarsal Bones 2 (MT2) and 4 (MT4) in 9 Horses

	Control	Cancellous bone graft (CBG)	Bone morphogenetic protein-2 (BMP-2)	Total
MT2 defect	6	6	6 (calcium phosphate cement)	18
MT4 defect	6	5	6 (calcium phosphate matrix)	17
Total (9 horses)	12	11	12	35

When needed, CBG was collected by making a 1.5-cm incision over the proximal medial aspect of the tibia and using a 5.5-mm hollow drill bit to harvest the graft[20]; $\sim 10\,mm^3$ of CBG was harvested and implanted in selected MT4 (osteotomy) and MT2 (ostectomy) sites.

Incisions were closed in layers (subcutaneous tissue and skin) and a sterile bandage applied. Bandages were maintained for 2 weeks until suture removal. Postoperatively, potassium penicillin (10,000 U/kg IV every 6 hours for 5 administrations), gentamicin (6.6 mg/kg IV single administration), and phenylbutazone (2 mg/kg orally twice daily for 2 days, then once daily for 3 days) were administered.

rhBMP-2 Formulation

rhBMP-2/CPC was reconstituted by adding 3 mL of a 1.66 mg/mL rhBMP-2 solution to 7.5 g of CP powder. The CPM formulation was obtained by adding 0.8 mL of a 1.75 mg/mL rhBMP-2 solution to 1.125 g of CP powder. For the ostectomy site, rhBMP-2/CPC was shaped and inserted into the 5 mm defect. For the osteotomy site, the appropriate volume of CPM was transferred to 3 mL syringes. Injection of rhBMP-2/CPM was performed through an 18 g needle within 5–10 minutes after preparation. Time for complete hardening (CPC, CPM) at body temperature was <1 hour. Both formulations were used at a concentration of 1 mg/mL when injected at the surgical site; ~ 0.5 mL was injected at the MT4 osteotomy site and ~ 2 mL at the MT2 ostectomy site.

Clinical Assessment

Horses were monitored daily. Bandages were changed on day 1 and then every other day until suture removal. Surgical sites were monitored for drainage and excessive swelling by assistants unaware of treatment assignment. Swelling was measured daily for 2 weeks but not scored.

Horses were euthanatized at 12 weeks by an overdose of IV pentobarbital solution after sedation with xylazine HCl. The distal aspects of the limbs were harvested.

Radiography

Dorsolateral and dorsomedial oblique radiographic projections were taken immediately before and after surgery and then at week 1, week 2, and every other week until 12 weeks. Radiographs were scored for fracture gap healing using a scale (1–4) where 1 = no evidence of callus, 2 = 25–50% union, 3 = union $\geq$ 50–75%, and 4 = fusion or union $\geq$ 75% at the surgical site.[7]

Mechanical Testing

Mechanical testing was performed on a modified servohydraulic materials testing system (Model 858, MTS Systems Corporation, Eden Prairie, MN) as previously reported.[9] Before testing, MT2 and MT4 were trimmed to an 8-cm-long span centered on the ostectomy/osteotomy site, labeled axial, abaxial, cranial, and caudal, and then embedded in potting rods (2.0 mm wide, 25 mm long) using polyester/styrene potting plaster.

Equipment was calibrated for torque and rotation before the first bone and after the last bone were tested, using 3 certified 5 lb test weights and a goniometer. A 50 Nm load cell was used. MT2 and MT4 were tested to failure in counterclockwise torsion at 1.5°/s to a maximum of 90° or until failure, using displacement control. Load and deformation data were recorded continuously at 10 Hz with an analog/digital board and stored on a read only floppy disk. Torque rotation data was used to compute maximum torque to failure and torsional stiffness. Stiffness was calculated as the slope of the initial linear portion of each curve. Six pairs of intact MT2 and MT4 harvested from 6 adult horses (comparable in sizes to those used but not related to the current study) were also tested following the same protocol and the results obtained classified as normal intact bone control data for statistical analysis.

Failure Mode

High-detail radiography (Faxitron; Faxitron Radiographs, Buffalo Grove, IL) was performed on failed accessory metatarsal bones. Mechanical failure was scored as 1 (failure at the osteotomy/ostectomy), 2 (partly through the osteotomy/ostectomy and partly through intact bone), and 3 (failure outside of the osteotomy/ostectomy).

Histology

The same accessory metatarsal bones that were used for mechanical testing were correctly oriented, aligned together, and then embedded undecalcified in polymethylmethacrylate (PMMA), sectioned (100 μm) in the longitudinal frontal plane (EXAKT system; EXAKT Technologies Inc., Oklahoma City, OK), stained with Goldner's Trichrome and scored for callus composition, maturity, cortical continuity, and osteotomy bridging. Both the cortical and the periosteal gap were evaluated in an axial and abaxial position. Gap filling/bridging was scored using a scale of 0–4, with 0: minimal to no tissue/no bridging, 1 = predominance of fibrous tissue, 2 = predominance of cartilage, 3 = predominance of woven bone, and 4 = predominance of lamellar bone/cortical union.[9]

Clinical assessment, radiographic, and histologic evaluations were determined by 3 surgeons (P.M., L.Y., M.M.) unaware of group assignments.

Data Analysis

Multivariate ANOVA was performed blocked on horse and limb using a randomized block design with a general linear models (GLM) procedure. The ANOVA was used to compare age and mechanical properties of MT2 and MT4 among rhBMP-2, CBG, no treatment, and intact bone groups. When ANOVA revealed significant differences among groups, a Duncan's Multiple Range Test was performed to separate these differences. Comparison of the radiographic subjective scores at different time intervals and histologic scores among treatment groups was performed by the Kruskal–Wallis test.
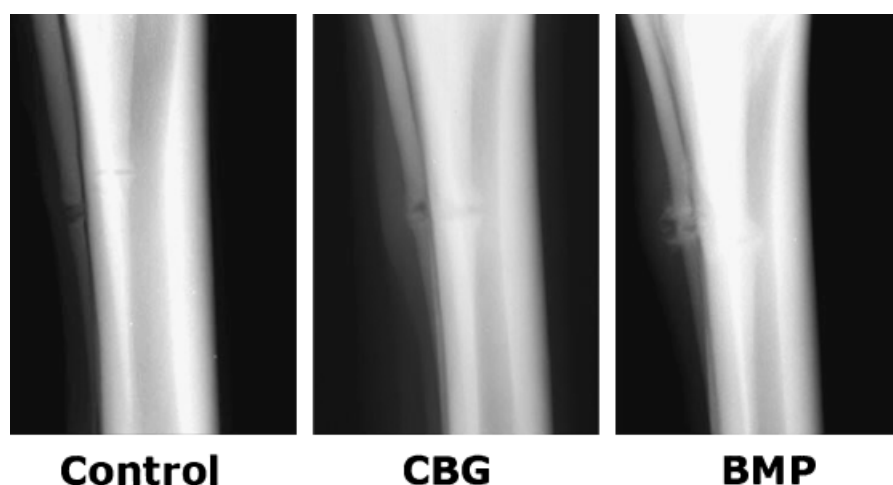
Fig 1. Radiographic images of the second metatarsal bone (MT2) ostectomy for untreated control (score 2), cancellous bone graft (CBG) (score 4), and bone morphogenetic proteins (BMP) (score 4) groups at 12 weeks.

A Fisher's Exact test was used to compare osteotomy failure patterns among the groups and to determine any gender differences among groups. Differences were considered significant at $P < .05$. Statistical analyses were performed with a commercially available software program (SAS Version 8e; SAS Institute Inc., Cary, NC). When comparisons were not significant, the difference between populations necessary to detect a significant difference with a power of 0.8 and $\alpha = 0.05$ was calculated.

RESULTS

Age, Gender

Mean ($\pm$ SEM) ages for groups (rhBMP-2 = 6.2 $\pm$ 0.7 years; CBG = 5.0 $\pm$ 0.7 years; untreated control = 5.5 $\pm$ 1.0 years) were not significantly different ($P = .59$, power = 0.8 to detect a difference of 36%). There was no age effect on any outcome variable. Group gender distri-

bution was BMP (3 females, 3 geldings), CBG (3 females, 3 geldings), and untreated control (4 females, 2 geldings) and was not significantly different among groups ($P = .14$).

Complications

No complications were associated with the surgical procedure. Horses were not lame postoperatively. Mild swelling occurred in the rhBMP-2 group. Subjectively, this appeared to be more than what occurred at other surgery sites.

Radiographic Union

CBG and rhBMP-2 groups had better radiographic union scores than untreated controls at 12 weeks, for both MT2 and MT4 ($P < .0001$ and $P = .0035$, respectively; Figs 1 and 2; Table 2). At 10 weeks,
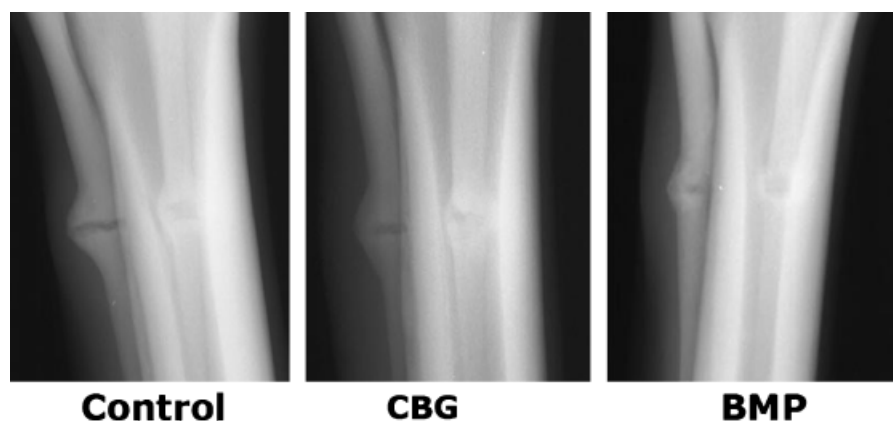
Fig 2. Radiographic images of the fourth metatarsal bone (MT4) osteotomy for untreated control (score 2), cancellous bone graft (CBG) (score 3), and bone morphogenetic proteins (BMP) (score 4) groups at 12 weeks.

Table 2. Mean (± SD) Radiographic Scores for Metatarsal Bones 2 (MT2) and 4 (MT4)

	Control	Cancellous Bone Graft (CBG)	Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2)
MT2			
Week 1	1.0 ± 0.0[A]	1.0 ± 0.0[A]	1.0 ± 0.0[A]
Week 2	1.0 ± 0.0[A]	1.0 ± 0.0[A]	1.0 ± 0.0[A]
Week 4	1.7 ± 0.4[A]	1.7 ± 0.3[A]	1.5 ± 0.4[A]
Week 6	1.7 ± 0.4[A]	2.0 ± 0.4[A]	1.8 ± 0.3[A]
Week 8	1.9 ± 0.5[A]	2.4 ± 0.4[A]	2.2 ± 0.3[A]
Week 10	2.0 ± 0.5[A]	3.0 ± 0.6[B]	2.9 ± 0.2[B]
Week 12	2.2 ± 0.6[A]	3.6 ± 0.5[B]	3.7 ± 0.1[B]
MT4			
Week 1	1.0 ± 0.0[A]	1.0 ± 0.0[A]	1.0 ± 0.0[A]
Week 2	1.0 ± 0.0[A]	1.0 ± 0.0[A]	1.0 ± 0.0[A]
Week 4	1.8 ± 0.2[A]	1.8 ± 0.2[A]	1.7 ± 0.4[A]
Week 6	1.8 ± 0.2[A]	2.1 ± 0.4[A]	2.1 ± 0.4[A]
Week 8	2.1 ± 0.5[A]	2.5 ± 0.7[A]	2.4 ± 0.5[A]
Week 10	2.3 ± 0.7[A]	3.0 ± 0.6[A]	2.9 ± 0.4[A]
Week 12	2.3 ± 0.6[A]	3.1 ± 0.8[B]	3.7 ± 0.3[B]

Means within a row with different superscripts are significantly different from each other in the same time period ($P < .05$).

both CBG and rhBMP-2 groups had better radiographic union scores than untreated controls for MT2 ostectomies ($P = .004$), but not for MT4 osteotomies ($P = .102$, power = 0.8 to detect a difference of 23%).

Mechanical Testing

MT2. No significant difference in maximum torque was detected between intact MT2 and the rhBMP-2 group ($P = .061$, power = 0.8 to detect a difference of 44%). Maximum torque of MT2 for the rhBMP-2 group was greater than CBG and untreated control groups ($P = .011$). There was no significant difference in torsional stiffness of MT2 among groups ($P = .156$, power = 0.8 to detect a difference of 51%); however, they were significantly less than the stiffness of intact bone ($P < .05$).

MT4. There was no significant difference in torsional stiffness for MT4 among rhBMP-2 and CBG groups compared with untreated control ($P = .403$, power = 0.8 to detect a difference of 51%). Although maximum torque and stiffness were greater in intact MT4 compared with untreated control, there was no significant differences in the maximum torque and stiffness among intact MT4, rhBMP-2, and CBG groups ($P = .151$, power = 0.8 to detect a difference of 22% and $P = .119$, power = 0.8 to detect a difference of 35%; Fig 3).

Failure Mode

Control. Four of six untreated MT2 and 3 of 6 untreated MT4 bones failed through the ostectomy and osteotomy site in the untreated control group whereas all intact bones failed in a spiral pattern, which is the most
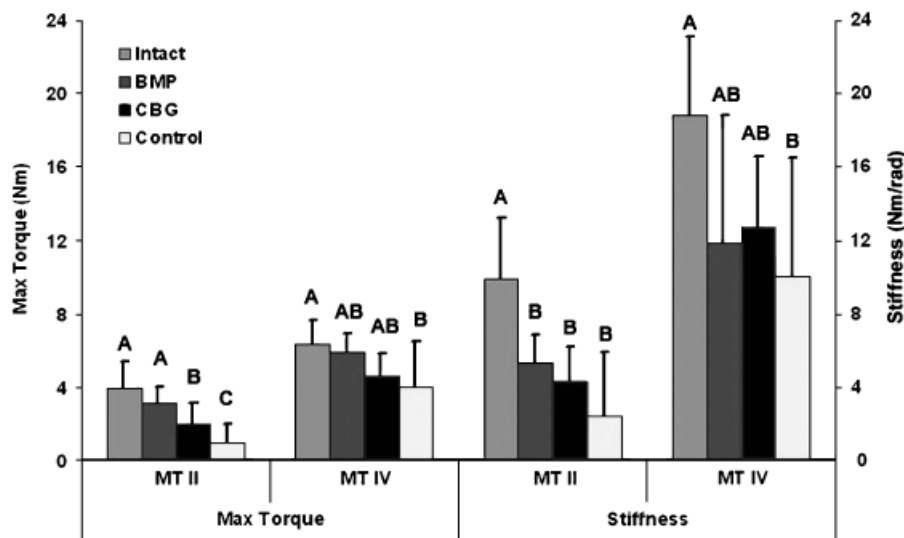

Fig 3. Bar chart of mechanical testing of the second metatarsal bone (MT2) and the fourth metatarsal bone (MT4). Different letters indicate significant difference ($P < .05$). Maximum torque of MT2 for the bone morphogenetic protein-2/calcium phosphate cement (BMP-2/CPC) group was greater than the cancellous bone graft (CBG) and untreated control groups. Although maximum torque and stiffness were greater in intact MT4 compared with no treatment, differences between these 2 groups and the BMP-2 and cancellous bone graft (CBG) groups did not reach significance ($P > .05$).
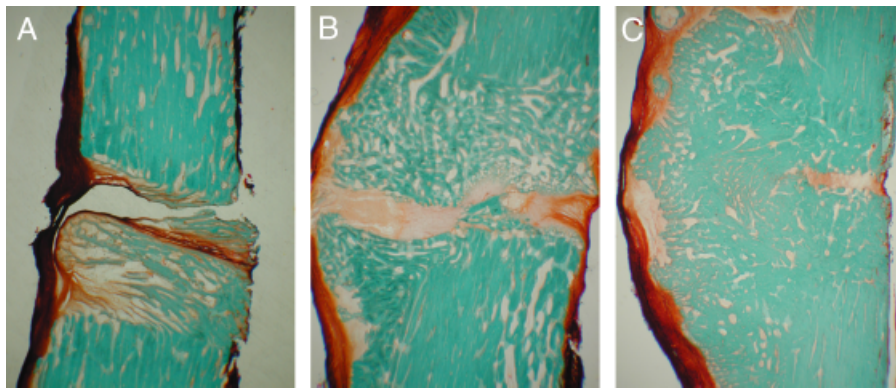
Fig 4. Histologic analysis of the second metatarsal bone (MT2) at 12 weeks. A, untreated control group (grade 0); B, cancellous bone graft group (grade 1); C, recombinant human bone morphogenetic protein-2 (rhBMP-2)/calcium phosphate (CP) group (grade 3).

commonly seen pattern of failure of normal bones under similar testing conditions.

rhBMP-2. Of 6 MT2 bones treated with rhBMP-2/CPC, 1 failed through the ostectomy whereas 5 had spiral fractures through the diaphysis during torsional testing. Of 6 MT4 bones treated with rhBMP-2/CPM, none failed through the osteotomy.

CBG. One MT2 and 1 MT4 failed through the ostectomy and osteotomy sites, 5 of 6 MT2 and 4 of 5 MT4 bones failed in spiral fracture outside of the ostectomy and osteotomy sites.

There were no significant differences in failure patterns for both MT2 (CBG versus control: $P = .12$; BMP versus control: $P = .12$; CBG versus BMP: $P = .77$) and MT4 (CBG versus control: $P = .35$; BMP versus control: $P = .09$; CBG versus BMP: $P = .45$) among the BMP-2, CBG, and untreated control groups.

Histology

MT2 had increased bone formation and more mature bone at the ostectomy site of the rhBMP-2 and CBG groups compared with untreated control (Fig 4). In all groups, no significant quantity of cartilage was present at the osteotomy/ostectomy sites. For MT4, there were no significant differences in the total histologic scores among rhBMP-2, CBG, and untreated control groups ($P = .11$, power $= 0.8$ to detect a difference of 48%). rhBMP-2 and CBG groups had better histologic scores compared with untreated control at the periosteal site for both MT2 and MT4 ($P = .002$ and .003; Fig 5).

DISCUSSION

We chose ostectomy and osteotomy of MT2 and MT4 because they are easily reproducible with minimal complications. Southwood et al reported that nonweight bearing bone may be considered a good model for evaluating fracture healing in horses[21] and Ishihara et al demonstrated greater relative potency of rhBMP-2 in accelerating equine metatarsal osteotomy healing.[22] We found that rhBMP-2 treatment of accessory metatarsal bone defects in horses accelerated early bone healing compared with untreated control defects particularly for larger MT2 defects, and was either equal or better than CBG in all variables evaluated.

As reported previously, use of CP as a carrier allowed adequate delivery of the rhBMP-2; it was biocompatible, rapidly resorbed, and allowed bone formation. rhBMP-2/CP was easy to use and no complications occurred. Use of the cement formulation was easier to manipulate resulting in less product dispersion at the surgery site. The lack of viscous flow is a serious limitation for CPC for ease of injection; however, injection of CPC had been adopted for treating calcaneal bone cyst and tibial plateau fracture.[23,24] Compared with CPC, CPM is typically in a liquid flow condition and easily injected by syringe and needle; however, leakage of the CPM formulation at the fracture site was observed repeatedly at injection and could have resulted in decreased area of contact, reduced osteoclastic resorption, and osteogenic factor release.[2]

In vivo efficacy studies of rhBMP-2 on bone healing have been conducted in rats, rabbits, and dogs,[5–12,14,15] but to our knowledge, this is the first study conducted in a horse model. Edwards et al[7] used a bilateral tibial osteotomy model in dogs treated with a single percutaneous injection of rhBMP-2/CPC. The callus area in the rhBMP-2/CPC treated limbs was significantly greater than for any other limbs. The rhBMP-2/CPC treated limbs were also stiffer in bending and torsion, and histologic analysis demonstrated increased bone formation and more mature bone.[7]

We found that rhBMP-2 treated MT2 had greater maximum torque to failure than CBG or untreated control
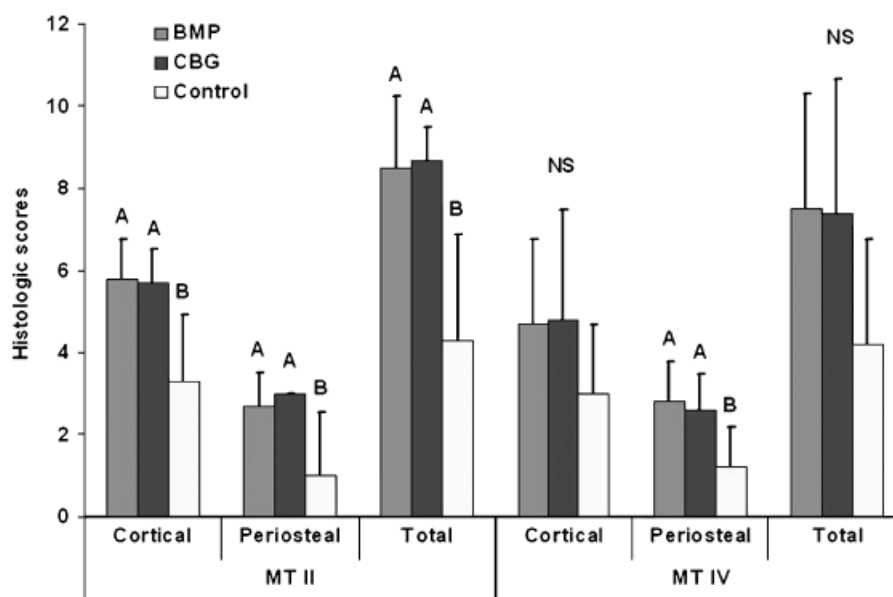
Fig 5. Bar chart of histologic scores of the second metatarsal bone (MT2) and the second metatarsal bone (MT4). Different letters indicate significant difference ($P < .05$). NS, no significant difference. Bone morphogenetic proteins (BMP-2) and cancellous bone graft (CBG) groups had better histologic scores compared with control at the periosteal site ($P < .05$).

limbs. This did not occur with MT4, which might be explained by the smaller defect created (osteotomy: 1 mm gap, approximately the thickness of the saw blade), and more advanced healing at 12 weeks in the untreated control bones for the MT4 1 mm gap versus the 5 mm gap in MT2, minimizing the effect of either CBG or BMP on bone healing. Therefore, it can be assumed that small defects such as an osteotomy might heal well without BMP or CBG, thus explaining the absence of significant differences in mechanical testing results between groups. Significant difference in stiffness among groups was not detected.

Healing MT2 had increased bone formation and more mature bone at the ostectomy site in the rhBMP-2 and CBG groups compared with untreated controls. No cartilage filling was observed, which may be related to the concentration of BMP-2 used which resulted in direct ossification at the surgery site.[2] We hypothesized that it could also have been a result of lack of motion resulting in intramembranous bone formation. For MT4, differences in the total histologic scores between the BMP-2 and CBG groups compared with untreated control did not reach the level of significance probably again because of the simple fracture with small gap resulting in more natural bone healing even without any treatment.

Limitations

The accessory metatarsal bones are not primary weight-bearing bones and may not reflect healing condi-

tions that occur after long bone fracture. Studies using a different fracture model will be necessary to fully evaluate the effects of rhBMP-2 on fracture healing of major weight-bearing bones. Use of radio-opaque CP cement made the radiographic evaluation of early mineralization challenging; however, healing could still be evaluated based on the amount of callus around the osteotomy/ostectomy sites. Although we have not specifically evaluated the difference in healing between a 1-mm and 5-mm defect of an equine splint bone, previous studies that we have conducted in other species, specifically the tibia in dogs, have shown progressively longer healing times when the fracture gap increases from 1 to 2 to 5 to 15 to 25 mm, even though none of these are critical-sized defects.[7,9,25,26] In this study, the control bones for the 1 mm gap had greater progression toward union than the 5 mm gap bones, realizing that this evaluation was conducted in 2 different splint bones. The 1 mm gap was intended to mimic a fracture within close to perfect anatomic reduction, whereas the 5 mm gap was intended to mimic a bone with a defect.

Results based on histological scores may be subjective. Quantitative measurement of percentage bone formation in the defect should be used in future studies. CPC or CPM only control groups should be used to make a complete comparison in future studies. Finally, the cost of rhBMP-2/CPC or CPM was not determined for clinical use in equine patients.

Overall, we found that the use of rhBMP-2/CPM at the ostectomy site of MT2 improved bone healing

compared with controls based on radiographic and histologic analysis; rhBMP-2 proved that it was as efficient in accelerating bone healing as CBG. Future studies using rhBMP-2 are needed to evaluate efficacy in treatment of long bone fractures and other bone defects in horses.

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