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Recombinant Human Bone Morphogenetic Protein-2 and Collagen for Bone Regeneration

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Abstract: The study reported describes a combination of recombinant human bone morphogenetic protein-2 (rhBMP-2) and collagen (C) to regenerate bone. Unilateral critical-sized defects (CSDs) were prepared in radii of 32 skeletally mature New Zealand white rabbits. Rabbits were divided evenly among four treatments: autograft, absorbable C (Helistat®), 35 μg of rhBMP-2 combined with absorbable C (rhBMP-2/C), and untreated CSDs. The two euthanasia periods were 4 and 8 weeks. Radiographs were taken the day of surgery, every 2 weeks, and at term and the percent of radiopacity was measured. Data analysis revealed a time-dependent increase in the percent radiopacity with rhBMP-2/C. Histological examination revealed the rhBMP-2/C treatment regenerated osseous contour by 8 weeks. According to quantitative histomorphometry, the CSD and C groups had significantly less new bone than either autograft or rhBMP-2/C ($p \leq 0.05$). The results suggest that rhBMP-2/C could be an effective therapy to restore segmental bone defects.

Keywords: bone morphogenetic protein; bone regeneration

INTRODUCTION

The capacity for bone to regenerate is well known. However, the ability to restore form and function without scar formation is size limited and the concept of the critical-sized defect (CSD) reflects this physiologic phenomenon.[1] Augmentation of bone deficits with various therapies has become a common practice, and autogenous grafts and allogeneic bone bank preparations provide effective therapies. Yet, despite the effectiveness of these traditional therapies, recognized liabilities[2,3] have prompted a quest for alternatives.[4] A potential alternative has been developed that consists of recombinant human bone morphogenetic protein-2 (rhBMP-2) and collagen (C).

The efficacy and safety of rhBMPs were well documented in numerous preclinical trials in several animal models[5–13] and in a recent provocative clinical report.[14] A reoccurring theme in these studies was the emphasis on the delivery system for rhBMP. The delivery system fulfills important roles, such as allowing the surgical placement of the therapeutic agent, preventing soft tissue prolapse, and localizing rhBMP-2 at the wound site for interaction with cells. Several categories of materials were selected as delivery systems for BMPs, including calcium-phosphates, biodegradable polymers, bone derivatives, and C.[15] Selection criteria for delivery systems were based on expediency and physiological merit: expediency because some materials were available as clinical therapies acceptable to the Food and Drug Administration (FDA) and physiological merit because some materials fulfill biological, functional roles. We selected a C vehicle for rhBMP-2 based on the notion that a commercially available, FDA-approved C therapy for hemostasis could deliver rhBMP-2 and promote bone regeneration. This notion is not unfounded because demineralized bone matrix, principally type I collagen and soluble signaling factors (including BMPs), has a proven record for bone repair.[16]

In light of the bone inductive potency of rhBMP-2 and the conviction that a commercially available C therapeutic would fulfill an effective role as a delivery system, we proposed the hypothesis that the combination of a selected dose of rhBMP-2 plus Helistat® C (rhBMP-2/C) would promote an amount of bone equivalent to a traditional ''gold standard'' (i.e., autograft). Therefore, to test this hypothesis we designed a study using a CSD segmental

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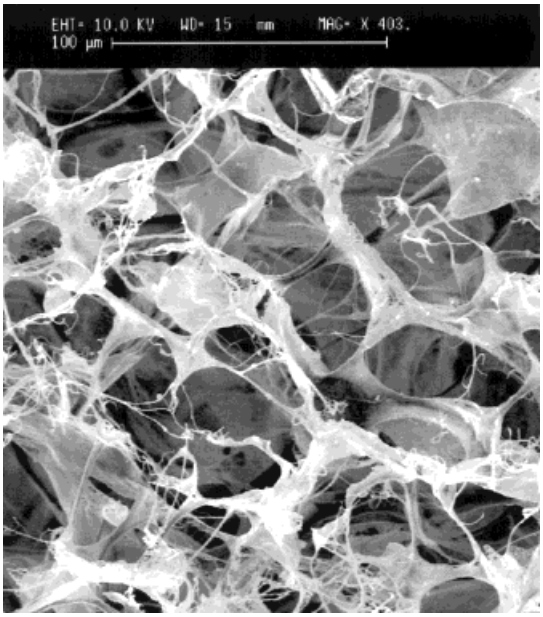


Figure 1. A scanning electron microscope micrograph reveals the highly fibrous and porous structure of the collagen delivery system.

wound model and four treatment groups: autograft, rhBMP-2/C, C, or untreated CSD.

Our goal is to develop a therapy that may be an alternative to either autografts or allogeneic preparations. Based on the evidence from this study, it appears rhBMP-2/C may be an effective therapy to restore segmental osseous defects.

MATERIALS AND METHODS

Unilateral, 20-mm length CSD ostectomies were prepared in the radial diaphyses of 32 skeletally mature New Zealand rabbits. Skeletal maturity was verified radiographically by closure of the epiphyseal plates. The 32 rabbits were divided evenly between two time periods (4 and 8 weeks) and among four treatment groups. Treatments consisted of autograft, untreated ostectomy, and Helistat® C either alone or with 35 μg of rhBMP-2.

The rhBMP-2 was produced by a Chinese hamster ovarian cell expression system. The protein was purified to >98%, placed in sterile glass vials containing a sodium glutamate buffer (5 m*M*, pH 4.5), and closed with rubber stoppers. The rhBMP-2 was provided by Genetics Institute (Andover, MA). Helistat® C (Fig. 1) was prepared in a proprietary manner by Colla-Tech, Inc. (a subsidiary of Integra Life Sciences Corporation, Plainsboro, NJ) from bovine tendon. Helistat® is a crosslinked atelopeptide product that is nonimmunogenic and composed of type I collagen. The ability of Helistat® plus rhBMP-2 to promote bone formation was confirmed by previous studies.[17,18]

Surgical Procedures and Treatment

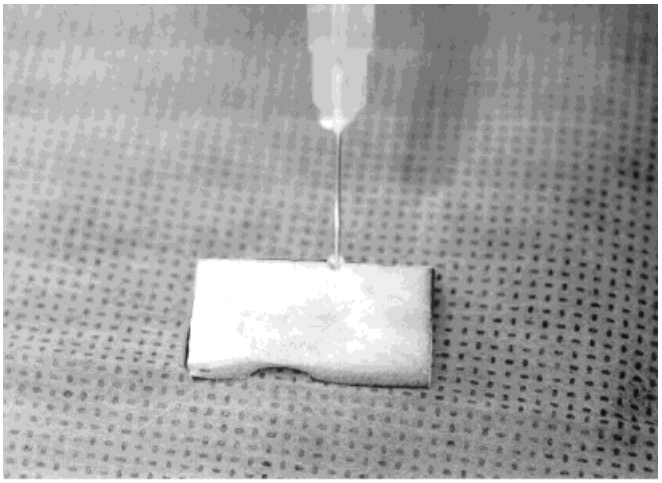
Surgery. The New Zealand white rabbits were anesthetized with 10 mL of ketamine hydrochloride, 100 mg/mL Ketaset (Fort Dodge Laboratories, Inc., Fort Dodge, IA), 5 mL of xylazine, and 20 mg/mL Gemini SA (Burns Veterinary Supply, Inc., Rockville Center, NY) at a dose of 0.65 mL/kg body weight administered intramuscularly (i.m.). Enrofloxacin (25 mg/kg, Baytril, Miles Inc., Shawnee Mission, KS) was given i.m. The operative site on either the left or right front limb was shaved, prepped, and draped for aseptic surgery with the rabbit in the supine position. A tourniquet was placed around the axillary region and the operative site was infiltrated with 0.5 mL of 2% lidocaine with epinephrine 1:100,000, 20 mg/mL xylocaine (Astra Pharmaceutical Products Inc., Westborough, MA). A 4-cm length superomedial incision was made and the tissues were dissected overlying the diaphysis of the radius. A 20-mm segmental defect was made in the radius with a surgical oscillating saw supplemented by copious 0.9% sterile saline irrigation, and the appropriately designated treatment was placed in the CSD. Fixation of the ostectomized bone was unnecessary due to the fibro-osseous union between the ulna and radius located distal and proximal to the surgical site. The soft tissues were approximated with interrupted 4-0 Vicryl sutures (Ethicon Inc., Somerville, NJ) and the skin was closed with surgical staples (Precise DS-25, 3M, St. Paul, MN). Immediately after surgery, standardized lateral radiographs were taken of the operated limb after which the animals were returned to their individual cages. Postoperative analgesia (Buprenex, 0.3 mg/mL, 0.3 mL i.m., Reckitt and Coleman Pharmaceuticals Inc., Richmond, VA) was given as needed. Water and food were supplied *ad libidum.*

Treatments. The autograft was prepared as a corticocancellous block from the operated site. For the C and rhBMP-2/C treatments, 0.17 mL of buffer containing either 0 or 35 μg of rhBMP-2 was transferred in a sterile fashion to the collagen by syringe and placed within the ostectomy gap [Fig. 2(A,B)]. Treatments were retained and stabilized by approximating flexor and extensor tendons and simple soft tissue closure. The fourth group consisted of untreated CSDs.

There were four rabbits for each experimental cohort at each time period. This quantity was determined by power analysis from previous data,[19,20] indicating that the α risk would be 0.05 and the β risk would be 0.10.[21]

Radiography and Radiomorphometry

Standardized radiographs were taken immediately postoperatively, every 2 weeks in life, and at the time of euthanasia. Briefly, the surgical sites were radiographed in a standard manner in a Minishot X-ray cabinet (AXR Corporation, East Haven, CT) at 35 kvp, 3 mA, and 25 s using a constant object to film to X-ray cone distance and ultrahigh



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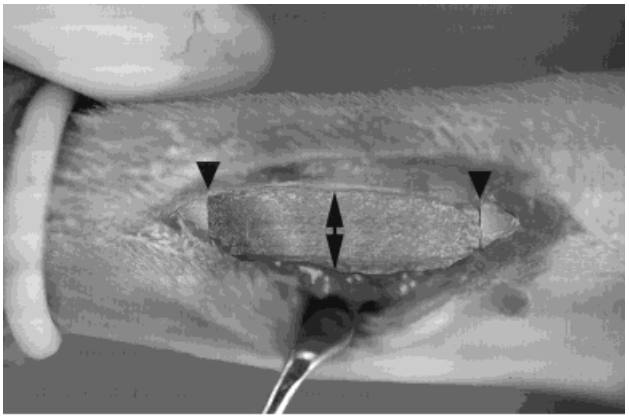

B

Figure 2. (A) At the time of surgery, 0.17 mL of buffer containing either 0 or 35 μg of rhBMP-2 was transferred in a sterile fashion by syringe to the collagen delivery vehicle. (B) The rhBMP-2/C within the ostectomy gap. (▲) The edges of the resected radius.

contrast mammography film (X-OMATL, Kodak, Eastman Kodak, Rochester, NY). The X-ray films were assessed by gray level densities and were reported as ''percent radiopacity'' across the ostectomy as previously described.[20,22] A standard-sized computer-generated reference frame was used for each experimental site; if radiopacity completely filled the site, a value of 100% radiopacity was recorded.

Histology and Histomorphometry

Following euthanasia, experimental sites were recovered as a block and placed immediately into 70% ethanol, taken to 100% ethanol, embedded in poly(methylmethacrylate), sectioned to a 5-μm thickness, and consecutive serial sections stained either with a modification of the Goldner–Masson trichrome stain or von Kossa stain. Using bright field light microscopy, the Goldner–Masson trichrome-stained sections were examined for cell type, morphology, and stromal detail using a Zeiss Axiophot Microscope (Zeiss Instruments Co., Inc., New York). New bone was detected and measured in square millimeters from the von Kossa stained specimens using a Leica 970 Image Analysis System (Leica Instruments Ltd., Cambridge, U.K.) interfaced with a Zeiss Axiophot microscope as previously described.[20,22]

Statistical Analyses

Histomorphometry and radiomorphometry data were analyzed by multiple ANOVA to determine if there was an overall difference in the percent of radiopacity and area of new bone among treatments over time. Individual differences between treatments at each time period were determined by Fisher's protected least significant difference test for multiple comparisons. Statistical significance was established at $p < 0.05$.

RESULTS

The clinical course for all treatments was uneventful. There were no indications of adverse sequelae.

Radiography

Throughout the 8-week study, the CSDs did not achieve a radiopaque union. Autograft-treated recipient sites displayed callus formation at 4 and 8 weeks, and consolidation was more evident at 8 weeks than at 4 weeks. Minimal radiopacity was observed at the bone margins for the C cohort at 4 and 8 weeks; by 8 weeks the recipient sites treated with rhBMP-2/C had radiopaque bridging (Fig. 3).

Figure 4 depicts the radiographic progression for the CSD and 35 μg rhBMP-2/C treatments immediately following surgery and until necropsy. Radiolucency immediately following surgery (postop day 0) is evident in the 35-μg rhBMP-2/C group, and from 2 through 4 weeks the radiopacity gradually increased. At 6 weeks a bonelike pattern could be noted, and by 8 weeks radiopaque bridging with a contour reminiscent of the preostectomized segment was apparent. (Verification that radiopacity is new bone was made by histology.)

Radiomorphometry

The 35-μg rhBMP-2/C treatment promoted a significant increase in the percent of radiopacity from 2 to 4 weeks, after which time there was no difference (Fig. 5). Furthermore, 35-μg rhBMP-2/C treated sites displayed significantly more radiopacity than either the CSD or C-treated defects throughout the experiment's duration. In contrast, the autograft recipient beds had a relatively consistent pattern of radiopacity.

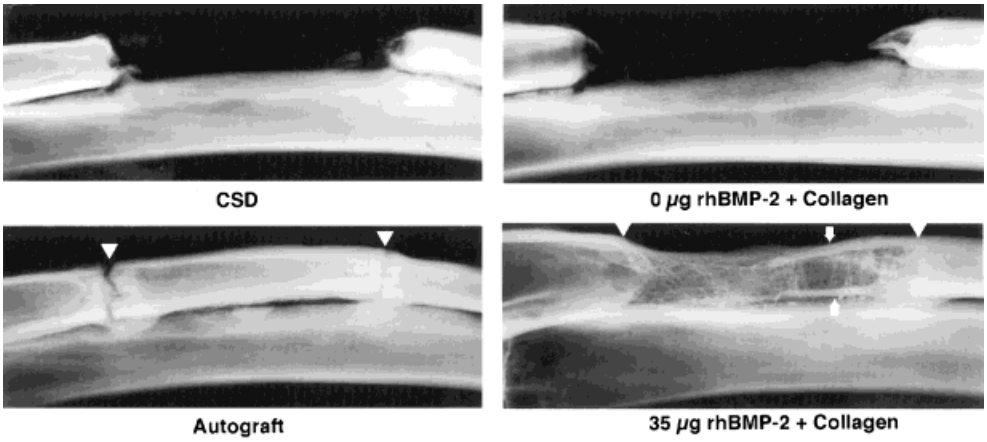

Figure 3. Representative radiographs of the four treatments at 8 weeks. The radiolucent CSD validates that the 20-mm ostectomy does not heal by bone formation. A radiolucent junction may be seen between the autograft and host bone (▽). The 0 μg of rhBMP-2 delivered in C is radiolucent. The 35 μg of rhBMP-2 and collagen treatment promoted new cortical bone ⇩. The host bone is identified by (▲).

Histology and Histomorphometry

A series of light micrographs for each treatment at 4 and 8 weeks depicts the appearance wound beds (Fig. 6). At 4 weeks the recipient beds administered 35 μg rhBMP-2/C had numerous bony trabeculae. By 8 weeks the trabeculae were consolidating and cortices were reforming. In contrast, experimental sites treated with either C or left as untreated CSDs gradually went on to a fibrotic union. The autografts integrated with the host bone through callus formation.

Residual C was detected microscopically with polarized light at 4 weeks; none could be detected by 8 weeks (Fig. 7). Moreover, there was no evidence of an adverse cellular reaction to the C at either 4 or 8 weeks.

Histomorphometric data indicated the CSD and C groups had significantly less bone than the other two groups, whereas no difference was detected between the 35-μg rhBMP-2/C and autograft groups (Fig. 8).

DISCUSSION

The results comply with our purpose to regenerate CSDs in rabbits' radii with rhBMP-2 and C. Furthermore, we proved the stated hypothesis that a combination of rhBMP-2 and C would regenerate as much bone as the gold standard autograft. The autograft formulation has been applied in previous rabbit studies in our laboratory. Moreover, the bone formation response evoked by the segmental autograft could be measured and compared to the bone formation response promoted by the other experimental treatments.

We selected a dose of 35 μg of rhBMP-2 combined with C for comparison to the autograft. This decision was based on previous investigations using the same wound model in rabbits.[19,20] Reports on long bone regeneration with either partially purified BMP or rhBMP and various delivery systems used a range of doses, different animal models, and assorted time periods for assessments. Therefore, it was problematic to extrapolate BMP doses from these studies. However, some key features from these investigations provided valuable guidance and should be emphasized to underscore crucial issues of reproducibility and accuracy in animal wound modeling, experimental design, BMP dosing, and BMP delivery that impact on a therapy for patients.

Stevenson and colleagues prepared bilateral diaphyseal defects (8 mm in length) in rat femora and reconstructed them with combinations of hydroxyapatite and tricalcium phosphate (HA/TCP) amended with 100 mg of partially purified bovine osteogenin (also known as BMP-3).[23] This dose combined with the HA/TCP cylinder promoted more bone formation in the HA/TCP devices than HA/TCP without osteogenin. However, Stevenson et al. determined there was no biomechanical difference between the two treatment cohorts (i.e., HA/TCP vs. 100 mg BMP-3/HA/TCP).[23] Another long bone study in rat femora involved unilateral defects that were 4 mm in length.[24] The authors reported that at 4 and 8 weeks only the 10 mg of extract from Saos-2 cells with bovine collagen promoted bone union.[24]

Yasko et al. used a combination of rhBMP-2 (either 1.4 or 11 μg) and guanidine-hydrochloride extracted demineralized rat bone matrix to regenerate ostectomies in rat femora.[25] Their evidence suggested that the 11-μg rhBMP-2 dose caused radiographic bridging of the 5-mm ostectomy gaps. The authors reported that by week 9 there was a cortical bridge across the defect with reconstitution of the intramedullary canal.

Marden and coworkers administered either 2.2 or 6.5 μg of rhBMP-2 with guanidine-hydrochloride extracted demineralized rat bone matrix in craniotomies in rats and

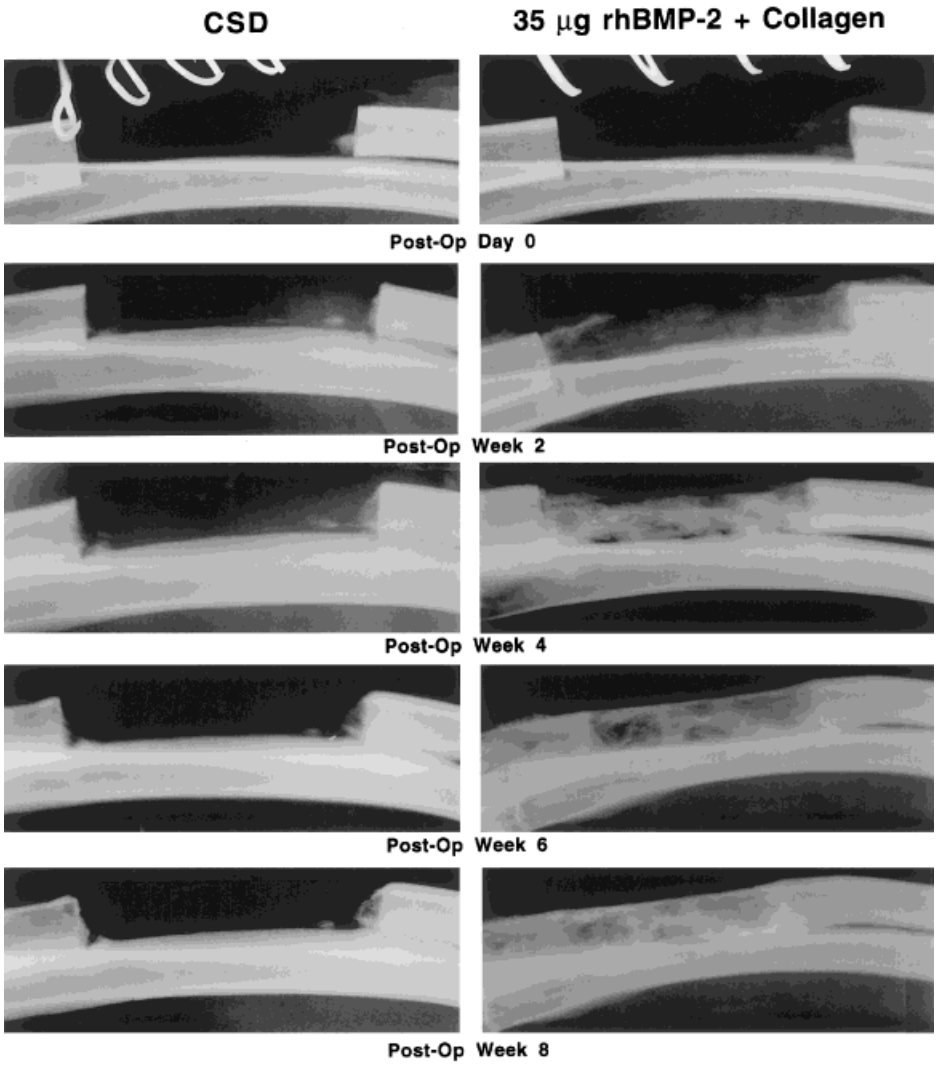

Figure 4. The radiographic progression CSDs and 35 μg of rhBMP-2 and collagen treatment from the day of surgery until euthanasia (8 weeks). Both treatments at postop day 0 are radiolucent immediately after ostectomy. The 35 μg of rhBMP-2 and collagen became slightly radiopaque by 2 weeks. Radiopacity for this treatment increased from 4 through 8 weeks, at which time a dense radiopaque bridging with a contour reminiscent of the preostectomized segment was apparent.

determined that by 14 days, 6.5 μg of rhBMP-2 completely regenerated the craniotomy.[7] In contrast, with a dose of 11 μg rhBMP-2 Yasko and associates observed radiographic bridging by 4.5 weeks and restoration of the cortex and medullary canal by 9 weeks.[25] These two rat studies are highlighted to underscore the variability in doses between two experiments exploiting the same animal and delivery system for rhBMP-2 at different recipient sites. Therefore, it is noteworthy to review several key observations. In rats, irrespective of embryogenesis, the intramembranous skull CSD and the endochondral femur CSD will regenerate in response to rhBMP-2. The rate of bone healing appears to be site specific; for example, the skull CSD regenerated in 2 weeks (with 6.5 μg rhBMP-2) whereas the femur (with 11 μg rhBMP-2) required 9 weeks. This observation may be due to enriched vascularity and cellularity of the craniofacial complex versus an extremity. Moreover, recombinant BMP appears to be 10 000 times more potent than partially purified BMP for regenerating bone in rats.[23,25]

A rabbit model used by Bostrom and coworkers consisted of bilateral, 20-mm long ulnar defects and one of several treatments of polylactic glycolic acid, autogenous blood, and four rhBMP-2 doses (27–300 μg) were administered.[11] By 8 weeks the X-ray films showed the two higher rhBMP-2 doses promoted union. For histology slides a bone score was used to compare responses at the wound sites; however, data reported were difficult to interpret.[11] The authors observed neither polylactic glycolic acid remnants nor multinucleated giant cells at the recipient sites. This observation is contrary to reports in other studies where a polylactic polyglycolic acid delivery system either with or without BMP incited a multinucleated giant cell reaction.[9,19,20,26]

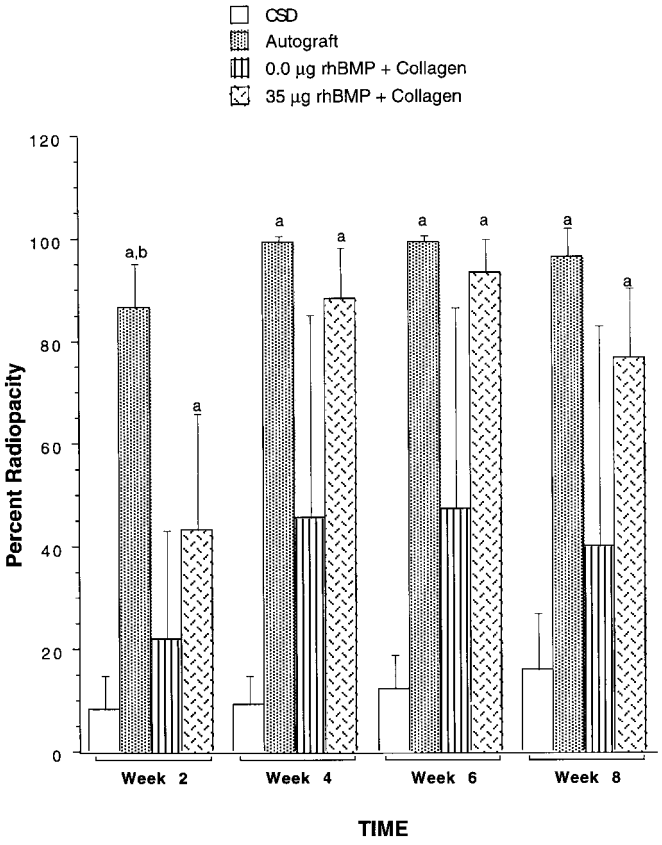

Figure 5. At week 2, the radiomorphometry data revealed that CSD and 0 μg of rhBMP-2 and collagen treatments had equivalent radiopacity (indicated by a) but were different than other treatments. Also at week 2, the radiopacity for the autograft group was greater than the other cohorts (indicated by b) ($p \leq 0.05$). By 4 and 6 weeks the CSD group had less radiopacity than contemporary treatments (a), except for the 0 μg of rhBMP-2 and the collagen group. This relationship among groups was consistent for weeks 6 and 8.

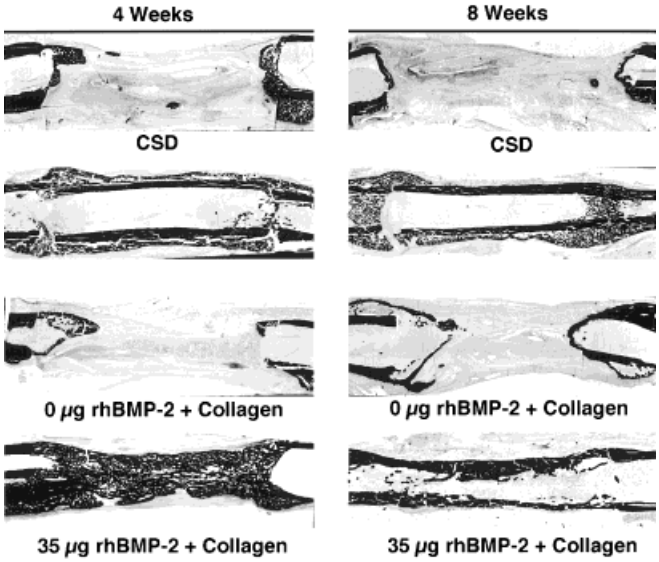

Figure 6. Representative micrographs for each treatment at 4 and 8 weeks depict the appearance of healing bone at the wound sites. At 4 weeks the 35 μg of rhBMP-2 and collagen treatment produced numerous trabeculae and by 8 weeks trabeculae consolidated and cortices developed. (Bone is stained black). The CSD and 0 μg of rhBMP-2 and collagen treatment did not promote new bone formation. The autograft-treated defects healed by callus formation. von Kossa stain; original magnification $\times$1.

Two other important issues concern rhBMP-2 dosing and wound model selection. We report a 35-μg dose of rhBMP-2. The elevated rhBMP-2 dose (e.g., 300 μg) selected by the Bostrom group may reflect the need either to offset liabilities in the delivery system or the instability caused by a bilateral wound model. We determined in a pilot study that the unilateral, 20-mm length radius resection was a stable critical-sized defect and did not compromise function for the experimental animal. We also determined bilateral 20-mm ostectomies were neither stable nor permitted the rabbit to favor one limb and resulted in pseudoarthrosis in many bilateral wounds treated with 35 μg rhBMP-2. Therefore, in a critical-sized unilateral rabbit radius model, 35 μg rhBMP-2 promotes bone regeneration whereas 300 μg rhBMP-2 promotes bone regeneration in bilateral ulnar defects.

Cook et al. assessed 12 treatment combinations that included seven doses of rhBMP-2 combined with guanidine extracted, rabbit derived, demineralized matrix to restore a rabbit ulnar defect model (15 mm in length).[27] Conclusions about treatment outcomes were ambiguous because of the limited and variable number of replicates per treatment cohort and lack of statistics, except for a Student's *t* test to analyze the biomechanical data, which indicated no differences among treatments.

Either partially purified BMP or rhBMP-7 has been applied to restore long bone segmental defects in dogs.[5,28,29]

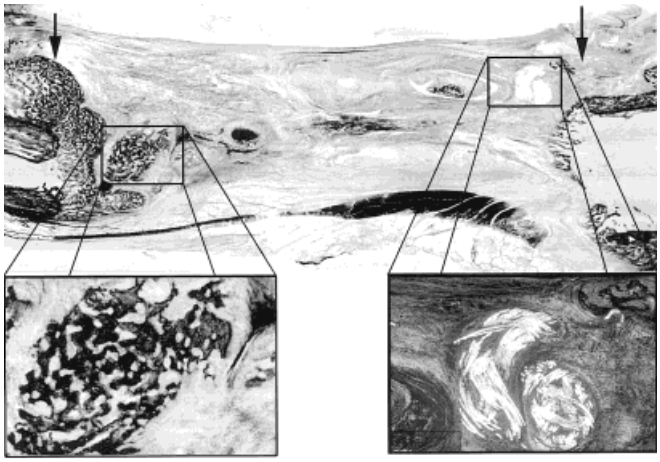

Figure 7. At 4 weeks the upper panel of a defect implanted with 0 μg of rhBMP-2 and collagen (Goldner trichrome stain, original magnification $\times$3.2). Polarized light reveals residual collagen, which can be seen as swirls of polarized fibers (insert to reader's right, original magnification $\times$25). (↓) New bone trabeculae are seen adjacent to the edge of the defect (insert to reader's left, original magnification $\times$25).

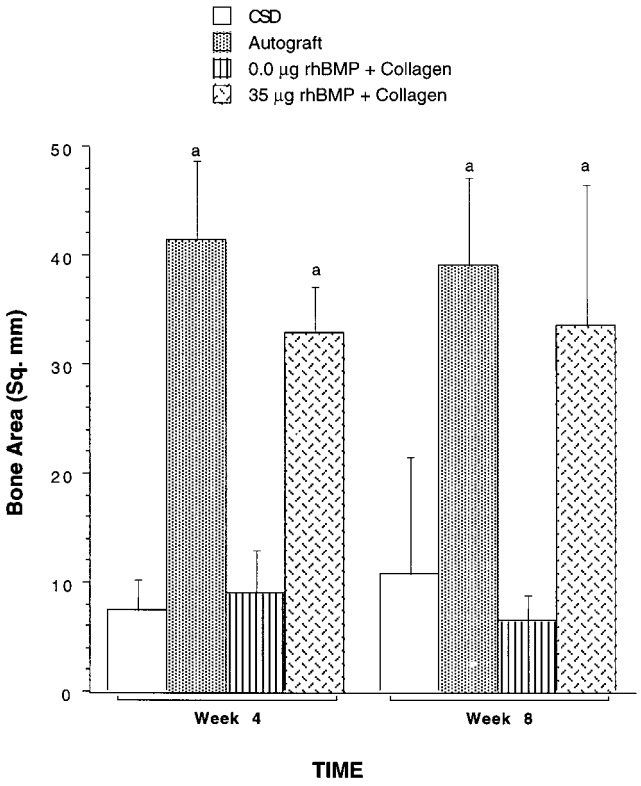

Figure 8. At 4 and 8 weeks the histomorphometry revealed that the quantity of bone at CSDs and 0 μg of rhBMP-2 and collagen-treated groups did not differ but was significantly less than other treatments ($p \leq 0.05$). The quantity of bone at defects treated with either 35 μg of rhBMP-2 and collagen or autograft did not differ (indicated by a).

Nilsson et al. used 100 mg of bovine derived, partially purified BMP delivered by an aggregate of γ-carboxyglutamic acid, histone 2-B, and calmodulin to restore 25-mm long ulnae ostectomies in dogs and reported osseous union by 12 weeks.[28] Heckman and colleagues prepared 3-mm long ostectomies in the diaphyses of dogs' radii.[29] Treatments included combinations of polylactic acid, guanidine-inactivated bovine bone matrix, and partially purified BMP from either bovine or dog sources. The bovine derived BMP was administered at a dose of 15 mg and the dog derived partially purified BMP was administered at 1.5 mg per defect. Results from the Heckman et al. study are difficult to interpret for several reasons: the inequity in sample sizes of two to six among seven treatment groups; ambiguities in statistical analyses; a xenogeneic (bovine) bone matrix carrier for allogeneic BMP (which is contradictory to reports advocating an allogeneic matrix for partially purified BMP[30–32]); and internal fixation on some dogs and no fixation on others.

Cook et al. reported rhBMP-7 restored 25-mm long, unilateral, ulnar ostectomies in six adult mongrel dogs.[5] The delivery system was 500 mg of guanidine inactivated bovine bone matrix. Responses were assessed radiographically and biomechanically. However, the radiographic ''grading'' was not analyzed statistically and biomechanical data did not indicate a significant difference between the two treatments (i.e., 500 mg of bovine carrier and 2.5 mg of rhBMP-7 vs. the unoperated limb).

Recombinant human BMP-2 was used to regenerate CSDs in dogs' mandibles and osseous continuity of alveolar clefts in dogs.[12] Toriumi and colleagues observed that 250 μg of rhBMP-2 plus inactivated, bone derived (dog) matrix restored mandibular continuity by 6 months[33]; Mayer et al. noted 200 μg of rhBMP-2 delivered by poly(lactide-*co*-glycolide) regenerated the bony contour of alveolar clefts in 4 months.[12]

A study by Cook and colleagues involved 20-mm length ostectomies in 28 African green monkeys evenly divided according to wound sites in either diaphyses of tibiae or ulnae.[8] Treatments at the 14 ulnar sites included 1 mg rhBMP-7 delivered in 400 mg of bovine bone matrix, matrix alone, and autogenous grafts. There were five treatments for the 14 tibiae, including four dose combinations of rhBMP-7. Results from this study were ambiguous because of the experimental design with unequal replicates. Several groups had only one or two animals per treatment. Moreover, it was unclear what statistical analyses were used.

A number of reports detailed rhBMPs for long bone regeneration in sheep,[34,35] spine fusion in rabbits and dogs,[9,10,13,36,37] and mandibles in rhesus nonhuman primates.[18] Various doses of BMPs and delivery systems (including polylactic polyglycolic acid, bone derivatives, and C) were used. Moreover, in a recent clinical report by Boyne and colleagues, doses of 1.77 to 3.4 mg of rhBMP-2 were administered to 12 patients in a collagen delivery system.[14] The authors claimed this combination was a useful therapy for augmenting the floor of the maxillary sinus.

For our study we chose a commercially available collagen formulation (Helistat®) to deliver rhBMP-2. The rhBMP-2 dose was derived from previous laboratory work indicating three doses of rhBMP-2 (17, 35, and 70 μg) delivered with a porous rod of poly(D,L-lactide) promoted an equivalent quantity of new bone at 4 and 8 weeks within 20-mm length, unilateral CSDs in rabbits' radii.[19,20] The area of new bone measured in this study (Fig. 8) was consistent with the quantity previously reported.[20] The difference between the two experiments was the carrier for the rhBMP-2: collagen versus poly(D,L-lactide). It appears that irrespective of the carrier in a unilateral rabbit radius model, the same dose of rhBMP-2 (i.e., 35 μg) promotes nearly identical bone healing. The consistency of these results helps to fortify the dosing relevancy in bone healing studies with polymers, such as poly(D,L-lactide) and C. Moreover, the efficacy of Helistat® and rhBMP-2 was reported for maxillofacial reconstruction in monkey mandibles and a canine spinal fusion model.[17,18]

Helistat® is manufactured as an absorbable collagen sponge for hemostasis control. The Helistat® C is derived from bovine Achilles tendon treated in a proprietary manner to render the product nonimmunogenic. Although no adverse sequelae (clinical or histologic) were observed

with the Helistat® C used in our model, there are limited reports of an immune response to bovine C.[38,39] The immune reaction may be difficult to predict across species and in varying tissues. Despite the apparent biocompatibility of the Helistat® and its capacity as a BMP delivery system, a laboratory-produced carrier should be developed that is not derived from animal sources. The laboratory-produced delivery system may be a useful alternative in situations where a collagenous delivery system may elicit an immune response. A laboratory-produced delivery system could be tailor-made to provide sustained delivery of signaling factors and to degrade in a time-dependent fashion.

The goal of our study was to restore osseous structure across CSDs in rabbits' radii with C and a selected dose of rhBMP-2 and to prove our hypothesis that rhBMP-2 would produce a quantity of bone in the CSD that is equivalent to that promoted by the autograft. We were successful in fulfilling our goal and proving our hypothesis. Furthermore, the reproducible and clearly defined animal wound model that is amenable to quantitative morphological assessments confirms this as a test site to validate the efficacy of segmental bone regenerative therapies. This study and previous studies exploiting the same wound model underscore the clinical relevance of a well-defined wound model that can be used to develop a judicious dosing regimen for the BMPs.

CONCLUSION

Efficacy and biocompatibility was validated for the combination of rhBMP-2 and C. By 8 weeks the combination was more effective than other treatments in promoting bone formation at CSDs. The favorable response from rhBMP-2 and C suggests that it could be an effective therapy to regenerate segmental bone defects.

The rhBMP-2 and collagen were generously supplied by the Genetics Institute (Andover, MA).

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