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Bone consolidation is enhanced by rhBMP-2 in a rabbit model of distraction osteogenesis

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Abstract

Recombinant human bone morphogenetic protein-2 (rhBMP-2) is a differentiation factor which has been shown to induce bone formation and heal bony defects in a variety of animal models. A possible application of rhBMP-2 is to accelerate bone regeneration during distraction osteogenesis, which clinically is a long procedure, often involving significant complications. In this study we tested the ability of rhBMP-2 to accelerate the consolidation phase of distraction osteogenesis in a rabbit model of leg lengthening. Tibiae were lengthened 2 cm over a period of ten days. rhBMP-2 was administered at the end of the lengthening phase. Two modes of rhBMP-2 application were tested: surgical implantation of rhBMP-2/ACS (absorbable collagen sponge) into the regenerate (50 μl of 1.5 mg/ml rhBMP-2, total dose = 75 μg rhBMP-2), and percutaneous injection of rhBMP-2/buffer (0.1 ml of 0.75 mg/ml rhBMP-2, total dose = 75 μg rhBMP-2) into three sites within the regenerate. Also, there were three groups of control animals: (1) no surgical intervention, (2) surgical implantation of buffer/ACS and (3) percutaneous injection of buffer. Rabbits were sacrificed at 5, 14 and 28 days after the interventions. Radiographic evaluation indicated a significant increase in bony union of the distraction regenerate in the rhBMP-2 treated groups compared with the untreated groups at 5 and 14 days. At 28 days, formation of a cortex and reestablishment of the medullary canal was evident only in the rhBMP-2 treated groups. The bone mineral content (BMC) of the regenerate was significantly higher in the rhBMP-2 treated groups at 5 and 14 days. However, at 28 days, BMC of the regenerate was similar in all groups. The average volumetric density of the regenerate was significantly higher in the rhBMP-2 injection group at day 14. In summary, both injection of rhBMP-2/buffer and implantation of rhBMP-2/ACS enhanced the consolidation stage of distraction osteogenesis in this rabbit model.

Keywords: rhBMP-2; ACS; Injection; Distraction osteogenesis; pQCT; BMC; Rabbit

Introduction

Induction of osteogenesis by means of an osteotomy, followed by fixation with an external fixator and subsequent controlled distraction of the callus, is a useful technique (distraction osteogenesis) with widespread clinical application in the treatment of bone defects, limb deformities and fracture non-unions [8,9,20,21]. However, the long duration of the bone consolidation phase of distraction osteogenesis treatment can be a cause of considerable morbidity for the patients, and in clinical practice, there is a burning need for augmentation of bone consolidation during distraction osteogenesis [20,21].

Bone formation in distraction osteogenesis resembles aspects of both normal fracture healing and embryonic limb developments [1,8,9,18,24,28]. Although many cytokines, growth factors, hormones and extracellular matrix components are capable of regulating specific aspects of bone regeneration and remodeling during bone growth and repair, BMPs are among the most potent of the osteoinductive factors [4,27,32,34,35]. Urist [32] made the key discovery that demineralized bone induces new bone formation when implanted intramuscularly. Subsequent purification of bone morphogenetic proteins (BMPs) has opened a new venue for

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skeletal tissue engineering. Among the BMPs, BMP-2 has been found to have pleiotropic functions that range from extraskeletal osteogenesis to bone regeneration [27, 35]. rhBMP-2 acts primarily as a differentiation factor for bone and cartilage precursor cells, and has been shown to induce bone formation and heal bony defects in a variety of preclinical models [2,12,29,33,39].

A possible application of rhBMP-2 is to enhance the bone regeneration during bone repair processes such as distraction osteogenesis. Thus, the goal of this study was to test the ability of rhBMP-2 to accelerate the consolidation phase of distraction osteogenesis in a rabbit model of leg lengthening. Data from a pilot feasibility study demonstrated that implantation of rhBMP-2 with ACS is surgically feasible and safe [25]. In addition, recent studies have shown that injection of BMP's, such as osteogenic protein-1 (BMP-7), into a fresh fracture may accelerate bone healing [5].

Materials and methods

Experimental animal model

Sixty-eight skeletally mature New Zealand white rabbits (male, 20–24 weeks, body weight 3.0–3.5 kg) were anaesthetized by intramuscular injection of Hypnorm (0.2 ml/kg, Janssen Animal Heath, High Wycombe, England), intravenous injection of Midazolam (Hypnovel, 1 mg/kg, Roche, Welwyn Garden City, England). Oxygen was supplied to the animal during surgery using a facemask. The left leg of the rabbit was shaved and prepared for sterile isolation. The skin and subcutaneous tissues over the surgical site were infiltrated with 0.25% Marcain (5 ml approximately, Janssen Animal Heath, High Wycombe, England). A 5-cm skin incision was made over the medial aspect of the left tibia. The periosteum was carefully preserved and retracted, and the tibia was exposed. A 1.8-mm Kirschner wire was used to drill both cortices of the most distal screw site using saline irrigation. The distal cortices were then overdrilled using a 2-mm drill. The distal screw (self-tapping tapered screw, Orthofix M300, total length 40 mm, thread length 15 mm, thread diameter 2/2.5 mm) was then inserted. An M100 fixator (Orthofix, Italy) was positioned and the most proximal screw applied. A metal sleeve was used to guide the Kirschner wire and the 2-mm drill to ensure the screw was centered in the clamp of the fixator. The remaining two screws were then inserted in a similar manner. A Macdonald's dissector was passed underneath the tibia and used to protect the soft tissues. An osteotomy was made in the tibia at the tibiofibular junction between the two inner screws using a hand saw. The blade of a small fret saw was passed underneath the tibia, and then connected to the saw handles. The tibia was cut transversely from deep to superficial with the fixator applied. Following the initial osteotomy, a 1-cm length of the tibial shaft was removed below the tibiofibular junction using a second osteotomy. The gap was immediately reduced by compressing the fixator. A 1-cm acute tibial shortening was therefore created in all of the animals. Penicillin powder was then applied to the region of each pin, and the deep tissue and skin were repositioned and closed using an absorbable suture. The operated leg was dressed and molded plastic was applied to protect the fixator. Each animal was housed in a separate cage with free access to food and water. After the operation, movement was unrestricted and the animals were free to bear weight upon the operated leg.

After a seven-day latency period following ostectomy, distraction was carried out at a rate of 2.0 mm/day for 10 days. In all animals the distraction was accomplished in equal steps (6 quarter turns twice a day). All animal experiment procedures were approved and licensed by the Home Office (UK), in line with Animals (Scientific Procedures) Act 1986.

Experimental groups

Among the 68 experimental animals, eight rabbits died or were euthanized before or during the experimental period because of various reasons, including anaesthetic accident ($n = 2$), pin hole fracture ($n = 4$) and soft tissue complications ($n = 2$). These animals were excluded from the experimental groups. The remaining 60 rabbits were randomly divided into 15 experimental groups, each group consisting of 4 rabbits. rhBMP-2 or control interventions were administered immediately at the end of lengthening period. In addition, another control group underwent lengthening and had no further intervention.

rhBMP-2 was delivered to the regenerate either by surgical implantation of rhBMP-2 soak-loaded onto an absorbable collagen sponge (ACS) or by percutaneous injection of rhBMP-2 in buffer. Controls groups included (1) surgical implantation of buffer/ACS into the regenerate, (2) percutaneous injection of buffer into the regenerate and (3) no intervention. For the rhBMP-2/ACS group, 50 μl of a 1.5 mg/ml solution of rhBMP-2 (75 μg total dose of rhBMP-2) was dripped on a piece of ACS ($1 \times 0.5 \times 0.3$ cm^3), placed in a sterilized plastic tube, and allowed to soak for 30 min prior to use. For the buffer/ACS group, a similar volume of buffer was placed in an identical fashion on the same size of ACS. A 1-cm cut was made into the center of the regenerate and the rhBMP-2/ACS or buffer/ACS was inserted into the regenerate. Following insertion, the incision was sutured. For the rhBMP-2/buffer injections, 0.1 ml of 0.75 mg/ml rhBMP-2 solution (75 μg total dose of rhBMP-2) was injected into the regenerate. Injections were done at the center of the regenerate (400 μl), and 0.5 cm distal (300 μl) and proximal (300 μl) from the central point. The injection control group was received with similar volume injections of buffer at the same locations. The final control group had no interventions.

Rabbits ($n = 4$/group per timepoint) were sacrificed at 5, 14 and 28 days after the intervention. The rabbits were euthanized with an intravenous injection of barbiturate (2 mg/kg) at the end of the experiment. Immediately after sacrifice, the regenerate plus 5 mm of the cortical bone proximal and distal to the regenerate was excised and fixed in 95% ethanol for 2 weeks.

Radiographic examination

Radiographs were taken weekly or otherwise on a specific study time point using an Atomscope HF100 X-ray machine (Seer Green, Buckinghamshire, England) at 46 kV, 20 mA, and 0.06 s. The anteroposterior (AP) and lateromedial (LM) radiographs included the distraction regenerate as well as the knee and ankle joints. The initial tibia length and the length of the distraction gap were measured from the radiographs. The length of the tibia was measured from the lateral condyle of the proximal tibia to the lateral portion of the articular surface of the distal tibia.

The percentage area of the distraction gap occupied by new bone was scored by two independent and blinded observers according to methods reported by Yasko et al. [38] and Kirker-Head et al. [12]. The percentage area of the distraction gap occupied by new bone was graded from 0 to 5 (Table 1). The scores were made using the AP and LM radiographs from post treatment days 5, 14 and 28. The distraction gap was considered united when bony continuity was restored across >75% of its cross-sectional area. An average score from the two observers was taken for each set of radiographs. After decoding the animal groups, the means of the scores of each group were calculated and recorded.

Table 1
Bone formation grading scores for radiographic analyses

Grade	Percent of total distraction gap filled by new bone
0	No formation
1	<25%
2	>25%
3	>50%
4	>75%
5	100% formation

Dual-energy X-ray absorptiometry

The bone mineral content (BMC) of the regenerate region was determined using dual-energy X-ray absorptiometry (DXA; QDR 2000+, Hologic, Inc., Bedford, MA). The excised tibiae were scanned using the small animal, regional high-resolution protocol. The entire tibial section was scanned, including both the regenerate and surrounding cortical bone. The scans were analyzed using the subregion lumbar analysis mode. A region of interest was drawn to include only the mineralized tissue in the regenerate, and the BMC of this region was computed.

Peripheral quantitative computed tomography

To assess the volumetric density and morphology of the regenerate, the excised bone was scanned using peripheral quantitative computed tomography (XCT3000; Stratec, Pforzheim, Germany) using the software version 5.20 (Norland Stratec Medizintechinik GmbH, Birkenfeld, Germany). Prior to scanning, the center of the regenerate was identified using a fluoroscope. At least ten contiguous slices were obtained. The slices were centered at the middle of the regenerate, as identified by the scout view of the peripheral quantitative computed tomography (pQCT) system. The CT slices were perpendicular to the diaphyseal axis and encompassed the regenerate and included at least one slice of intact bone from each end. The slices were 2.5 mm thick, 2 mm apart, with an in-plane voxel size of 0.2 mm. To analyze each pQCT slice, a region of interest was drawn around the regenerate or host bone. A density threshold of 275 mg/cm^2 was selected, based on sampling of all scans, to include mineralized tissue and exclude soft tissue. The average densities of the regenerates from three slices per rabbit were compared. The three slices included the center of the regenerate and the two slices 4 mm proximal and distal to the center. In addition to the quantitative evaluation, high-resolution images from all slices were evaluated qualitatively to assess the organization of the regenerate and re-establishment of the marrow cavity.

Histological examination

After the DXA and pQCT examinations, the specimens were processed and embedded in methyl-methacrylate (MMA). The infiltration process was carried out at 4 °C, by placing the bone specimens into a solution of MMA and dibutylpthalate (3:1) for 48 h, followed by another 48 h in MMA. Embedding of the infiltrated specimens was done in fresh MMA, dibutylpthalate (3:1), and 2.5% benzoyl peroxide solution at 20 °C. Polymerization was completed within 48 h. Attempts were made to standardize the sectioning at a mid-sagittal plane of each specimen by cutting the embedded specimen in half (longitudinally in a sagittal plane) using a low-speed diamond saw. Thin sections (5 μm) were cut on a Reichert Jung microtome (Germany) with a carbide steel knife. Sections were mounted on the 1% gelatin coated slides, covered with a piece of plastic film, and gently flattened using a small metal roll. For histology examination, MMA resin was removed by immersing the slides in methoxyethyl acetate (BDH, UK), two changes of 20 min at room temperature. Slides were then taken through graded ethanols and distilled water, then stained with Goldner trichrome, adjacent sections were stained by Von Kossa staining for detection of mineralization.

Statistical analysis

The study was based on a two-factor design: treatment and time. The five levels of the factor "treatment" were no treatment, buffer/ACS, rhBMP-2/ACS, buffer injection, and rhBMP-2/buffer injection. The three levels of the factor "time" were 5, 14 and 28 days after treatment. There were equal numbers of animals ($n = 4$) in each group, and the animals were randomly distributed into each group. Continuous outcome variables, such as BMC from DXA and bone density from pQCT, were assessed using a two-factor analysis of variance. If the interaction between treatment and time was significant, then each timepoint was analyzed separately by a one-way analysis of variance. Post-hoc testing was performed using Fischer's protected least significant differences (PLSD) test. Comparisons were considered significant at $p < 0.05$. For variables with a non-normal distribution, the nonparametric Kruskal–Wallis test was used instead.

Results

Radiographic analysis

The average (±SD) lengthening of all the animals was 2.2 ± 0.4 cm. The radiographic score was higher in the rhBMP-2 treated groups (grade 2 and 3) compared with the untreated groups (grade 0.5–1.5) at 5 days post treatment (Table 2). At 14 days, partial bony union was seen in all groups (Fig. 1), but the union was complete only in the rhBMP-2 treated groups. Radiographic bone formation scores were also higher in the rhBMP-2 treated groups at this timepoint (Table 2). At 14 days, the radiographic signs of cortical continuity were observed in 2/4 rabbits in rhBMP-2/ACS group, in 3/4 rabbits in rhBMP-2/buffer injection group, and in none of the control rabbits (Table 2). At 28 days after treatment, although bony unions were seen in all groups, formation of a new cortex was evident only in the rhBMP-2 treated groups (Fig. 1). The radiographic signs of cortical continuity were observed in *all* of the rhBMP-2 treated rabbits, compared to only 3/12 of the control rabbits (Table 2).

DXA results

BMC of the regenerate was similar in all groups after 5 days of treatment (Fig. 2). After 14 days of treatment, the buffer/ACS, buffer injection, and the two rhBMP-2 treated groups had significantly higher BMC (+148–172%) than no treatment group ($p < 0.02$, ANOVA with Fisher's PLSD test for pair-wise comparisons). Furthermore, BMC in the rhBMP-2 buffer injection group was significantly greater than that in the rhBMP-2/ACS group ($p < 0.02$), buffer injection group ($p < 0.001$) and saline/ACS group ($p < 0.02$, Fig. 2). After 28 days of treatment, there were not differences in BMC among the groups (Fig. 2).

Table 2
Summary of radiographic evaluation

Experiment groups	Days after lengthening				
	Radiographic grade			Cortical continuity	
	5 d	14 d	28 d	14 d	28 d
No treatment	0.5	3.25	4.25	0/4 (0%)	1/4 (25%)
Buffer/ACS	1.25	4	5	0/4 (0%)	1/4 (25%)
Buffer injection	1.5	4	5	0/4 (0%)	1/4 (25%)
rhBMP-2/ACS	2	5	5	2/4 (50%)	4/4 (100%)
rhBMP-2/ buffer injection	3	5	5	3/4 (75%)	4/4 (100%)

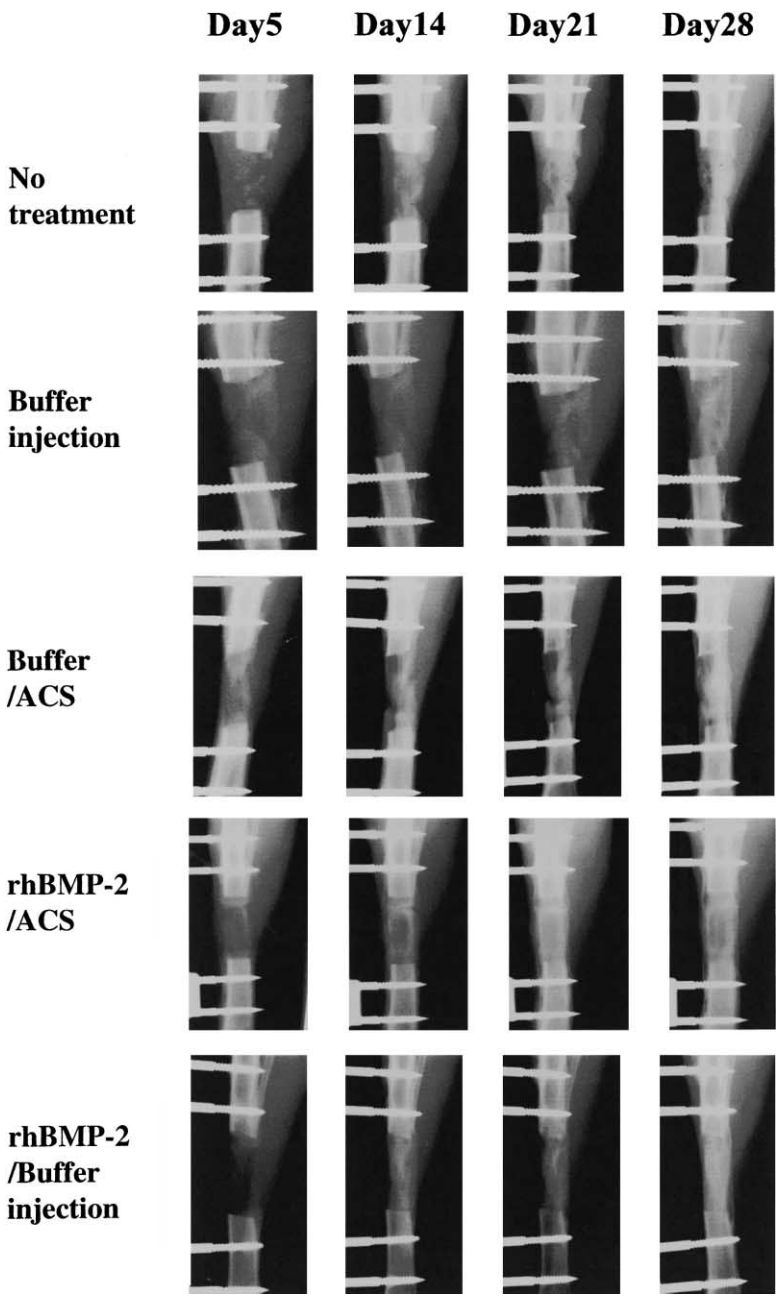

Fig. 1. Representative radiographs of all experimental groups at days 5, 14, 21 and 28. There is a significant increase in callus formation in the rhBMP-2 treated groups compared with the untreated groups at days 5 and 14. At 28 days, although bony unions were seen in all groups, formation of a new cortex was evident only in the rhBMP-2 treated groups.

pQCT results

pQCT images revealed that five days after treatment, bone formation was sparse in all groups. There was no sign of bone formation in any of the rabbits in the no treatment group, and only 1/4 rabbits had signs of bone formation in the buffer/ACS and buffer injection groups. In contrast, 3/4 rabbits in the rhBMP-2/ACS group had signs of bone formation, as did 4/4 of the rabbits in the buffer/rhBMP-2 injection group (Table 3).

At 14 days after treatment, images of the central regenerates showed newly formed cortices in 3/4 rabbits in both rhBMP-2 treated groups. In addition, formation of a bone marrow cavity was seen in 3/4 rabbits in the rhBMP-2/ACS group and 2/4 rabbits in the rhBMP-2/buffer injection group (Table 3 and Fig. 3). There was only sparse bone formation in all of the control groups, and there were no signs of formation of a new cortex or re-establishment of a bone marrow cavity formation in any of the rabbits that received no treatment (Fig. 3). At

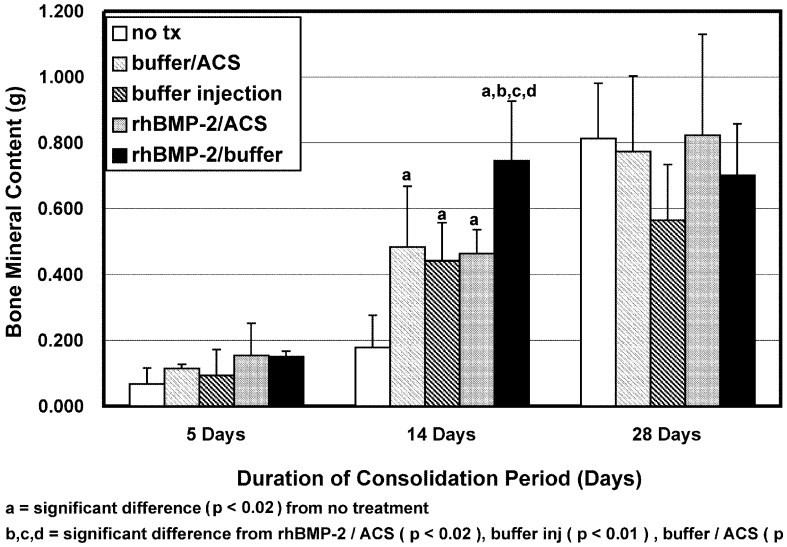

Fig. 2. BMC in the regenerate of all experimental groups at days 5, 14 and 28. At day 5, there were no differences among all the groups. At day 14, both the control groups (buffer/ACS and buffer injection) and the rhBMP-2 treated groups had significantly higher BMC than the no-treatment group (a, $p < 0.02$, two-factor ANOVA). In the rhBMP-2/buffer injection group, the BMC of the regenerate was significantly greater than that in the rhBMP-2/ACS group (b, $p < 0.02$); buffer injection group (c, $p < 0.01$) and buffer/ACS group (d, $p < 0.02$). At 28 day, there were no differences in BMC among groups. Error bars represent 1 standard deviation.

Table 3
Summary of quantitative evaluation of pQCT images from the central slice of the regenerate

Experiment group	Days after lengthening								
	New bone formation (number of rabbits)			New cortex formation (number of rabbits)			Bone marrow cavity formation (number of rabbits)		
	5 d	14 d	28 d	5 d	14 d	28 d	5 d	14 d	28 d
No treatment	0	4	4	0	0	2	0	0	1
Buffer/ACS	1	4	4	0	0	0	0	0	0
Buffer injection	1	4	4	0	0	1	0	0	0
rhBMP-2/ACS	3	4	4	0	3	3	0	3	3
rhBMP-2/buffer injection	4	4	4	1	3	4	1	2	4

28 days after treatment, remodeling of the regenerate region was minimal in the control groups. For example, only 1/12 rabbits in the control groups showed signs of re-formation of the cortex (Table 3). In addition, focal bone defects were still observed in the regenerate, indicating mechanical weakness of the callus (Fig. 4). In contrast, the rabbits treated with rhBMP-2/ACS implantation or rhBMP-2/buffer injection demonstrated advanced bony union and remodeling of the regenerate. Formation of a new cortex and re-establishment of a marrow cavity was clearly seen in 3/4 rabbits in the rhBMP-2/ACS group and in all rabbits in the rhBMP-2/buffer injection group (Fig. 4), indicating advanced bone maturation in these rabbits.

The volumetric density of the regenerate was similar in all groups at 5 and 28 days after treatment (Fig. 5). However, at 14 days after treatment, the volumetric densities of the regenerate was significantly higher in the rhBMP-2 injection group compared with the other groups ($p < 0.01$), which were not different from each other (Fig. 5).

Histology results

At 5 days after treatment, bone formation was sparse in all groups. Although there was no bone formation in the center of the regenerate in any of the control rabbits, a few rabbits from the two rhBMP-2 treated groups showed signs of bone formation (Fig. 6). At 14 days after treatment, bone formation was sparse in the non-treated groups and focal defects were frequently seen in the regenerate (Fig. 6). Bone formation was slightly greater in the buffer injection and buffer/ACS groups compared with the no-treatment group. In comparison, bone formation and bone remodeling was more advanced in the two rhBMP-2 treated groups. The

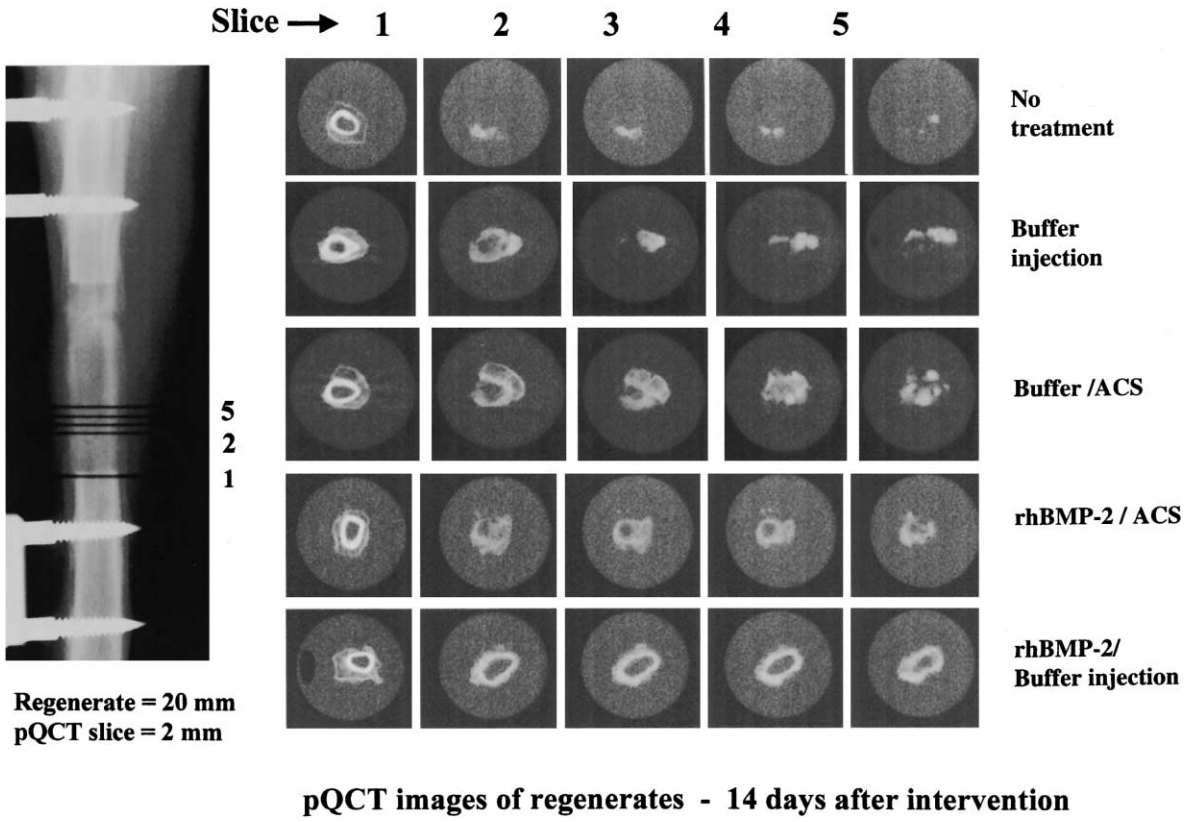

Fig. 3. Representative pQCT images of the regenerate at 14 days post-lengthening. In the two rhBMP-2 treated groups, there is evidence of newly formed cortices and re-establishment of the bone marrow cavity. In contrast, there was only sparse bone formation and no sign of cortex or bone marrow cavity formation in any of the control groups.

distraction gap was completely united with woven bone, and cortex and bone marrow cavity formation were evident. At 28 days, bone formation continued in all groups. Focal bone defects and partial discontinuities of the cortices were still evident in all of the three non-rhBMP-2 treated groups, but were not visible in the two rhBMP-2 treated groups (Fig. 6). In the two rhBMP-2 treated groups, bone remodeling was evident, and new cortex and bone marrow cavity formation were almost complete (Fig. 6).

Discussion

Distraction osteogenesis is a unique process in which bone formation occurs in parallel with elongation of the surrounding soft tissue envelope [1,8,9]. This technique is widely used clinically for the treatment of many challenging orthopedic conditions, such as correction of congenital deformities, limb reconstruction following tumor resection, and treatment of severe skeletal and soft tissue damage due to trauma [20,21]. Despite the promising results, a prolonged period of external fixation is usually required to allow sufficient bone consolidation before the fixator is removed. Complications, such as refracture, cause tremendous pain and morbidity. In this study we have demonstrated that a single application of rhBMP-2, either by implantation or injection, significantly enhances bone consolidation and overall maturation of the regenerate.

The rabbit model of leg lengthening is a well-established model of distraction osteogenesis and has been used extensively to study various aspects of bone regeneration during distraction osteogenesis [6,7,11,13–16,23,30]. Previous reports using this model have shown that the optimal rate of lengthening is between 0.7 and 1.3 mm, twice daily lengthening [14,16]. At these slower lengthening rates there is variable consolidation of the regenerate that is not seen in human clinical cases. However, when lengthened at a higher rate (>1.3 mm/day), the rate of consolidation is decreased, the quality of bone formation is poor and there is a high incidence of soft tissue complications [14,16,30]. In this study, we deliberately chose a fast rate of lengthening (2 mm/twice a day) to mimic clinical situations of poor bone formation during distraction osteogenesis. An initial 1-cm tibial shortening was introduced to avoid soft tissue complications during lengthening and to reduce the limb length discrepancy. The total lengthening was 20% of the original tibial length, which was well tolerated by the

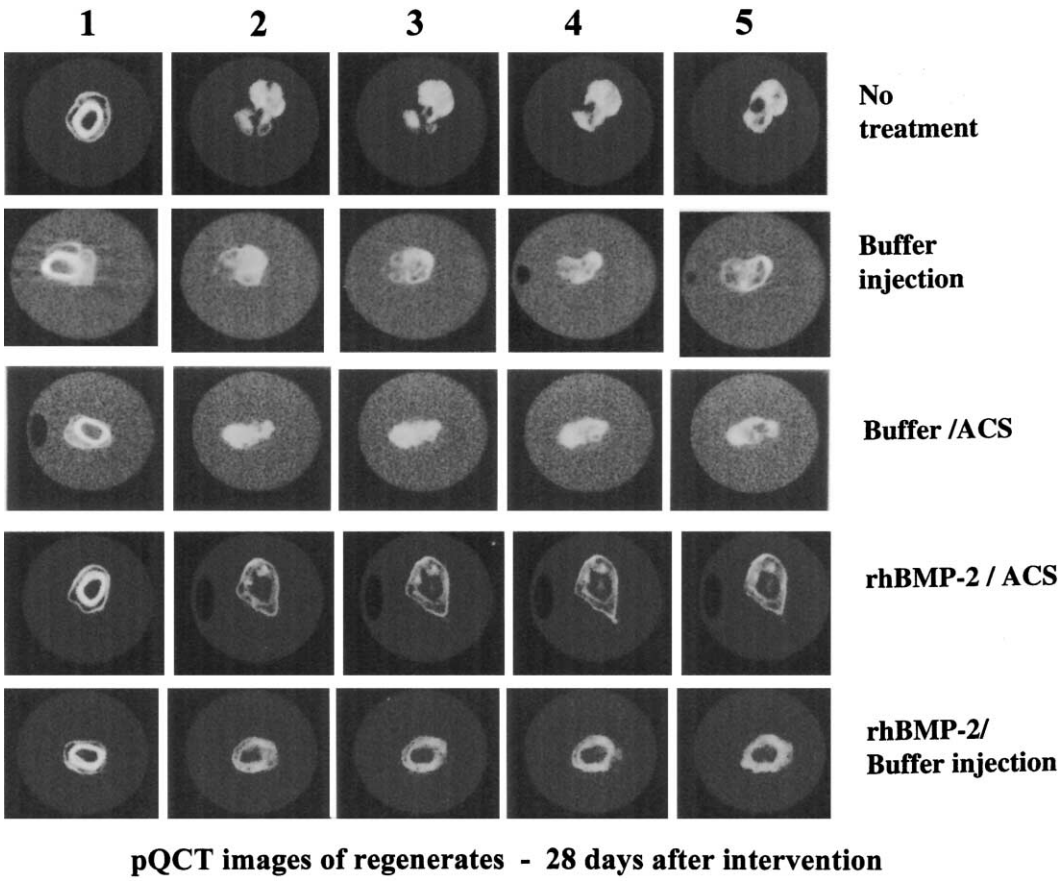

Fig. 4. Representative pQCT images of the central regenerates at 28 days after treatment. Partial bridging and focal bone defects were still seen in all the control groups. In contrast, the specimens treated with rhBMP-2 implantation or injection demonstrated an advanced bony union, and formation of new cortices and a marrow cavity.

rabbits. Our previous experience had indicated rapid lengthening without prior shortening was not well tolerated by the rabbits [30].

The best carrier for a given BMP may vary depending on the specific clinical indication and skeletal site being treated. Considerations include biodegradability, structural integrity, absence of immunogenicity and rate of release of BMP [26]. Recent studies of rhBMP-2 pharmacokinetics from a variety of biomaterial carriers in a rat ectopic model have shown that after 3 h, retention of rhBMP-2 at the healing varied among the carriers. The collagenous sponges retained the highest fraction of implanted dose [31]. Collagenous carriers released rhBMP-2 gradually from the implant site. In contrast, some of the mineral-based carriers retained a fraction of the implanted rhBMP-2 within the implants. In this study, we tested two modes of rhBMP-2 delivery: surgical implantation with ACS or percutaneous injection with a buffer. Biodistribution studies in a rabbit fracture model indicated that ≈70% of the administrated dose of rhBMP-2 was initially retained at the fracture site following implantation of rhBMP-2/ACS, and that ≈10% remained two weeks after implantation [3].

One disadvantage of using a collagen sponge to deliver rhBMP-2 is that it requires a second surgery for implantation. Some positive effects of the secondary operation on bone consolidation were noticed in the surgical control group. For instance, at day 5, implantation of buffer/ACS in the regenerates appeared to increase new bone formation slightly on radiographs (Fig. 1) and histological sections (Fig. 6). At day 14, the bone formation and BMC in the buffer/ACS group was greater than that in the no-treatment control group (Fig. 2). In contrast, no difference of bone formation was seen at day 5 in the buffer injection group compared with the non-treatment group; however, there was a slight increase of bone formation in the buffer injection group at day 14. Taken together, these data indicate that surgical interventions (implantation and injection) at early stages of bone consolidation may induce some moderate positive effects on bone formation. These effects may be due to trauma-induced inflammation which releases certain growth factors and cytokines, such as platelet derived growth factor, TGF-βs and interleukins, that may themselves promote bone formation. Nevertheless, the surgical interventions did not induce any better

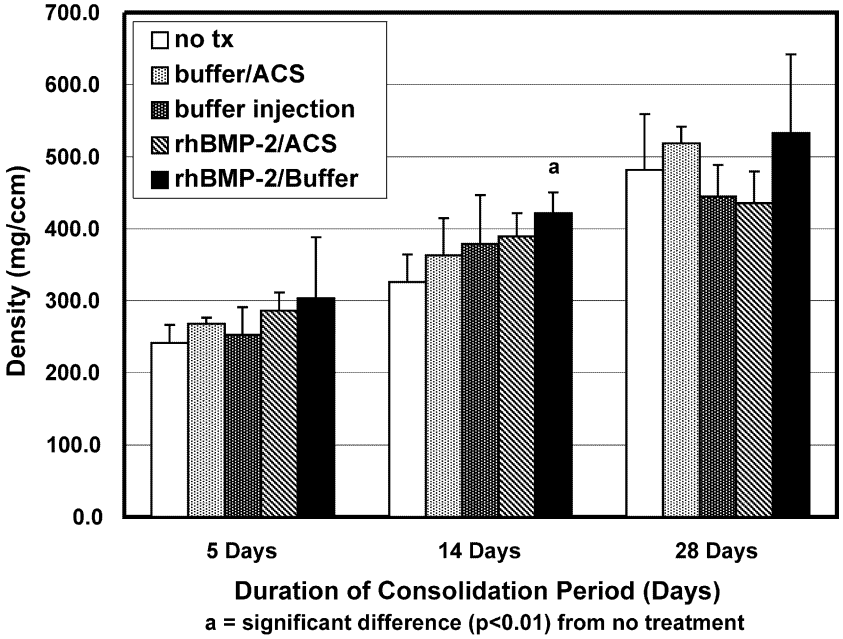

Fig. 5. Volumetric densities of the regenerates estimated by the pQCT. At 14 days after treatment, density of the regenerate in the rhBMP-2/buffer injection group was significantly greater than the density of the regenerate in the no-treatment group ($p < 0.01$).

quality of bone formation, not any positive effects on the late stages of bone remodeling. Moreover, rhBMP-2 induced more bone formation and enhanced remodeling to a greater extent than any of the control interventions.

Injection of rhBMP-2 is an appealing method of delivery. Injection allows a precise dose of the protein to be delivered and makes repeated deliveries possible. In the present study injection of rhBMP-2 in buffer appeared to be superior to implantation of rhBMP-2/ACS. There were few differences between rhBMP-2/ACS and rhBMP-2/buffer groups in the radiographic, pQCT and histology examinations of the regenerates at 5 and 14. However, at 28 days, bone remodeling was more advanced in the rhBMP-2/buffer injection group compared to the rhBMP-2/ACS implantation group as assessed by pQCT and histology (Figs. 4 and 6). Although the present study did not demonstrate any dramatic differences in bone consolidation and maturation between the rhBMP-2/ACS implantation and rhBMP-2/buffer injection groups, delivery of rhBMP-2 to the regenerate via percutaneous injection may be preferable, as it is less invasive and appears as effective as rhBMP-2/ACS implantation.

BMP-induced bone formation in vivo is clearly a complex multistage process and likely involves the activities of multiple locally produced growth factors and systemically available hormones [27,34,35]. Osteoblastic or osteoprogenitor cells in general respond to treatment with the BMPs by increasing cell proliferation [35]. BMP-2 has also been shown to induce differentiation of osteoprogenitor cells to an osteoblastic cells [10,19,36, 37]. The BMPs have chemotactic effects on mesenchymal cells, osteoblastic cells [22] and endothelial cells [17], suggesting the enhancement of bone formation by rhBMP-2 may be related to an increase in recruitment of bone-forming cells and enhancement of neovascularization. It is unlikely that the implanted or injected rhBMP-2 remains at the site long enough to direct all of these processes in vivo, although it may be that a single administration of rhBMP-2 induces a cascade of the expression of multiple BMPs and growth factors that regulate the bone formation processes. In the setting of distraction osteogenesis, the regenerate provides a rich source of osteoprogenitor cells. The primary effect of rhBMP-2 in this setting is likely therefore to be more rapid differentiation of the cells into mature osteoblasts, resulting in the more rapid bone formation, consistent with what was observed in this study.

In conclusion, we have clearly demonstrated that both injection of rhBMP-2 in buffer and implantation of rhBMP-2 in an ACS significantly enhance the consolidation stage of distraction osteogenesis in the rabbit model. The rhBMPs (BMP-2 and BMP-7) are now been tested for clinical use in many orthopaedic applications, and early clinical trails indicate that they may provide an important adjunct to the treatment of non-unions and segmental bone loss [5]. Using rhBMP-2 to accelerate the consolidation phase of distraction osteogenesis may have potential clinical application in reducing the treatment time and lowering the number of complications associated with this procedure.

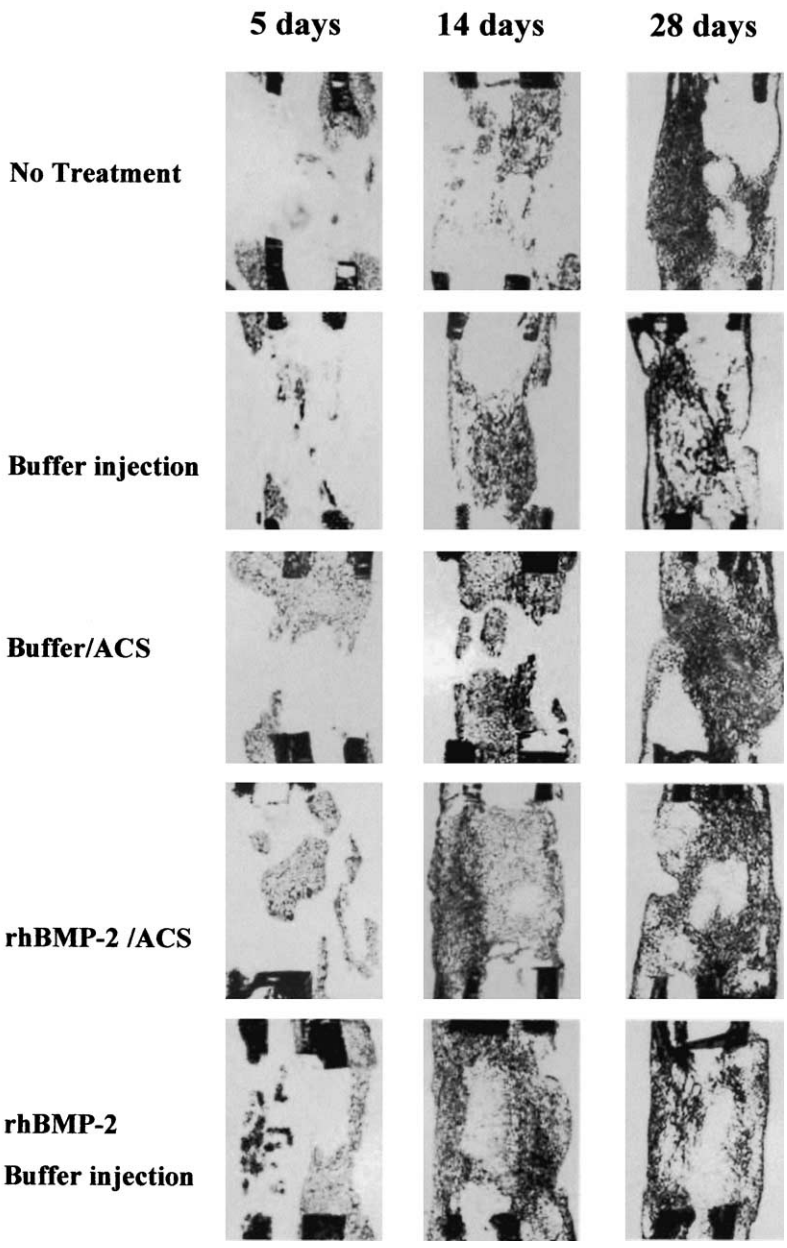

Fig. 6. Representative histologic images at days 5, 14 and 28 after treatment. At 5 days, callus formation was sparse in all groups and bone formation was only seen in the two rhBMP-2 treated groups. At 14 days, the amount of bone formation was greater in the buffer injection and buffer/ACS groups compared with the non-treatment group: cortex and bone marrow cavity formation was evident only in the two rhBMP-2 treated groups. At 28 days, focal bone defects and partial discontinuities of cortices were still evident in all three control groups, but formation of a new cortex and bone marrow cavity were almost complete in the two rhBMP-2 treated groups. Von Kossa stain, magnification ×1.

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