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The short-term therapeutic effect of recombinant human bone morphogenetic protein-2 on collagenase-induced lumbar facet joint osteoarthritis in rats[1]

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Summary

Objective: To determine whether an intra-articular injection of recombinant human bone morphogenetic protein-2 (rhBMP-2) alleviates cartilage degradation in a rat model of osteoarthritis (OA) of the lumbar facet joint.

Method: The right-side facet joint OA model was created by an intra-articular injection of collagenase (type II) 2 weeks before treatment. The OA rats were divided into four groups: (1) no treatment, or intra-articular injection of either (2) saline, (3) rhBMP-2 10 ng, or (4) rhBMP-2 100 ng. The left-side facet joint served as the normal control. At 3 and 6 weeks after treatment, histological analyses were performed on the cartilage, synovium, subchondral bone and bone marrow. The cartilage and synovium were graded using a modified Mankin score and a synovium score system. Extracellular type II collagen was evaluated by immunohistochemistry.

Results: Intra-articular injection of collagenase causes OA-like changes in the facet joint. OA rats treated with rhBMP-2 at both dosages tested showed reduced severity of their cartilage lesions compared with untreated and saline-treated groups. There was a statistically significant difference in the modified Mankin score compared to the untreated and saline-treated groups. However, some rhBMP-2-treated rats at the higher dose (100 ng) showed, as a side effect, joint space obliteration caused by cartilage overgrowth. Also OA rats treated with 100 ng of rhBMP-2 displayed a significant synovium reaction at 3 weeks compared with that in other groups. Immunohistochemical analysis showed that treatment with rhBMP-2 significantly increased the content of type II collagen.

Conclusion: This study demonstrates the potential efficacy of rhBMP-2 in the alleviation of arthritic changes in a rat model of OA of the lumbar facet joint. However, treatment with a high dosage of rhBMP-2 caused adverse side effects in some animals.

Key words: Bone morphogenetic protein-2, BMP-2, Facet joint, Zygapophysial joint, Collagenase, Osteoarthritis.

Introduction

The facet joint (zygapophysial joint) is a synovial joint, covered by hyaline cartilage and is enclosed by the synovium and the joint capsule[1,2]. In degenerative lumbar spinal disorders, osteoarthritis (OA) of the facet joint is one of the causes of low back pain[3]. Facet joints are clinically important sources of chronic pain even after spinal fusion or artificial intervertebral disc implantation[4,5]. Current treatment methods in facet joint arthritis include range of motion exercises, injection of steroid into the joint[6,7], or radiofrequency denervation[8–10]. However, none of these methods focuses directly on the degenerative process of articular cartilage.

Bone morphogenetic proteins (BMPs) are multi-functional growth factors that belong to the transforming growth factor beta superfamily. BMPs play important roles in embryonic development and skeletal growth[11–13]. BMP-2 is currently approved for multiple indications in the area of bone fracture repair and spinal fusion. The injection of the recombinant human BMP-2 (rhBMP-2) alone into muscle tissue is sufficient for the induction of ectopic bone formation[14]. On the other hand, BMP-2 has been also demonstrated to be a potent regulator of chondrocyte metabolism and differentiation[15–17]. BMP-2 is found to be upregulated in osteoarthritic chondrocytes and diseased cartilage[18]. These findings suggest that BMP-2 can act as a stimulus of

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anabolic activities in normal and osteoarthritic chondrocytes. BMP-2, in turn, increases the synthesis of chondrocyte extracellular molecules, aggrecan and type II collagen.

For the assessment of the osteoarthritic lesions of the lumbar facet joint, we developed an animal model by injection of collagenase into the facet joint in the rat. We showed that facet joints subjected to collagenase developed OA-like changes[19]. The purpose of this study was to examine the short-term therapeutic effect of rhBMP-2 on the osteoarthritic facet joint.

Methods

ANIMALS AND INDUCTION OF EXPERIMENTAL OA

This study was approved by the Institutional Animal Care and Use Committees of the participating institutions. Two-month-old Sprague Dawley rats, weighing between 250 and 270 g, were used in this study. Animals were maintained in accordance with the NIH 'Guide for the care and use of laboratory animals'. Collagenase type II (from *Clostridium histolyticum*, enzyme activity 321 U/mg) was obtained from Worthington Biochemical Corporation (Lakewood, NJ). The enzyme (1 mg) was dissolved in sterile phosphate-buffered saline (PBS) (320 μl) (pH 7.4) to provide a concentration of 1 U/μl. All the surgical procedures were performed under sterile operating conditions with the rats under intraperitoneal anesthesia (50 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride). A 6 cm midline incision was made on the back and the paraspinal muscle was dissected to expose bilateral lumbar facet joints. Five microliters (5 U, 1 U/μl) of collagenase was injected into the right lumbar facet joints with a 34 gage blunt NanoFil needle (WPI, Inc., FL) at a rate of 2 μl/min controlled by an infusion pump. The injection level of the facet joint was chosen randomly from L3/4 to L5/6. The fascial layers of the muscle and skin were then closed in routine fashion. After the surgery, the rats were housed in an animal room with a 12 h light/12 h dark cycle, and had free access to food and water. No restriction of movement was enforced during the test period.

The left-side lumbar facet joints were used as normal control.

EXPERIMENTAL GROUPS AND INTRA-ARTICULAR INJECTION OF rhBMP-2

The rhBMP-2 was provided by Medtronic Sofamor Danek USA, Inc. (Memphis, TN). The rhBMP-2 (1.2 mg) was dissolved in sterile distilled water (1 ml) and stored at −80°C until use. Then the rhBMP-2 (1.2 mg/ml) was diluted with sterile PBS (pH 7.4) to provide the indicated concentrations (2 ng/μl and 20 ng/μl) before intra-articular injection.

Two weeks after collagenase induction surgery, the rats were randomly divided into four groups and subjected to the second surgery ($n = 12$ in each group).

Group 1: Untreated control.
Group 2: PBS (pH 7.4) treatment.
Group 3: 10 ng of rhBMP-2 treatment (concentration: 2 ng/μl).
Group 4: 100 ng of rhBMP-2 treatment (concentration: 20 ng/μl).

Surgical procedures including sterile condition, anesthesia methods and surgical procedures to expose the lumbar facet joint were the same as described above. In the untreated group, sham operation was performed without injection of any material. In the saline-treated and rhBMP-2-treated groups, 5 μl of saline or rhBMP-2 was injected into the previously collagenase-injected lumbar facet joints, respectively. The injection was performed with a 34 gage blunt NanoFil needle and syringe. After surgery, the wound was irrigated with normal saline and closed in a routine fashion. The animals were euthanized at 3 or 6 weeks after the second surgery and the lumbar spines were removed upon sacrifice for histological and immunohistochemical study. The experimental protocol is diagrammed in Fig. 1(A).

HISTOLOGICAL AND HISTOCHEMICAL EXAMINATION

The lumbar spines were fixed with 10% neutral buffered formalin for 48 h, decalcified and then embedded in paraffin. Five micrometer sections from the facet joints were obtained for hematoxylin–eosin (H&E) and Safranin O staining. The articular cartilage in the superior articular

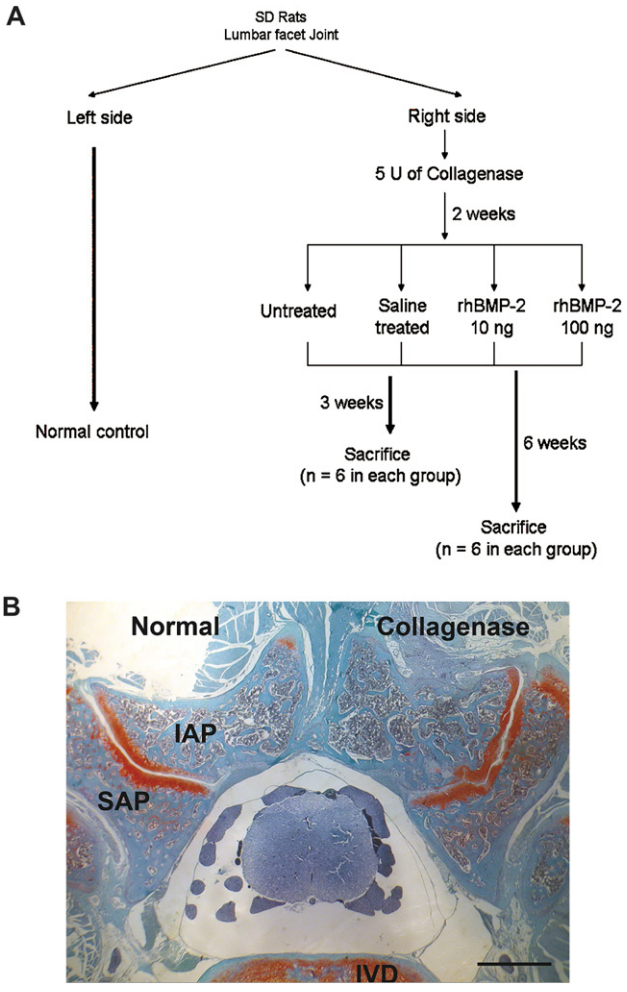


Fig. 1. Experimental protocol and representative section of collagenase-induced OA of the facet joint. (A) Experimental protocol. In the right-side facet joint, OA was induced by an intra-articular injection of 5 U of collagenase type II. After 2 weeks, four groups were studied according to the different treatments. (B) Photograph of facet joint stained with Safranin O. The left-side facet joint was used as normal control. The right-side facet joint showed spontaneous healing without treatment at 3 weeks. IAP = inferior articular process of vertebra. SAP = superior articular process of vertebra. IVD = intervertebral disc. Bar = 1 mm.

process was evaluated using a modified Mankin grading system (score range: 0–25, from normal to most severe reaction)[20–22] (Table I). The synovium was evaluated according to Yoshimi's histological grading (score range: 0–18, from normal to most severe reaction)[21]. Two independent examiners assessed histological findings obtained from these specimens in a blind manner.

IMMUNOHISTOCHEMICAL EXAMINATION

Sections taken from the 6-week treatment groups were subjected to immunohistochemical examination. Paraffin-embedded sections were deparaffinized with xylene and rehydrated with ethanol. These sections were incubated in 0.01 M citrate buffer (pH 6) for 8 min at 121°C. Endogenous peroxidase activity was blocked by incubation in 3% H_2O_2 for 30 min. These sections were washed with Tris-Tween buffered saline (TTBS) two times. Non-specific binding was blocked with normal goat serum [Vectastain rabbit IgG ABC kit (Vector Laboratories, Inc., Burlingame, CA)] for 30 min at room temperature. Sections were incubated overnight with primary antibodies [rabbit polyclonal antibody against rat type II collagen (Calbiochem, Germany)], diluted 1:100 in PBST buffer [phosphate buffered saline (pH 7.4), 0.3% Triton-X-100], at 4°C in a humidified chamber. After washing three times each for 3 min in TTBS, sections were incubated with the biotinylated secondary antibody (Vectastain rabbit IgG ABC kit) for 80 min at room temperature followed by three 3 min washes with TTBS. The horseradish peroxidase labeled avidin–biotin complexes (Vectastain rabbit IgG ABC kit) was applied for 30 min followed by two rinses in TTBS. The peroxidase was detected using 3,3′-Diaminobenzidine [Vector SK-4100 (Vector Laboratories, Burlingame, CA)], mixed with distilled water, buffer and H_2O_2 solution. Sections were then washed with tap water, dehydrated, cleared with xylene and mounted. For positive and negative control, normal rat facet joints were used. Negative control sections did not receive the primary antibody. Immunostaining for collagen type II was evaluated by using a semi-quantitative score. Scoring of the intensity of the staining was performed according to an arbitrary scale by two observers in a blinded manner as follows: 0, undetectable staining; 1, low staining; 2, moderate staining; and 3, strong staining.

Table I
Grading of articular cartilage lesion for histological evaluation

Parameter	Grade
(1) Structure	
a. Normal	0
b. Surface irregularities	1
c. 1–3 superficial clefts	2
d. >3 superficial clefts	3
e. 1–3 clefts extending into the middle zone	4
f. >3 clefts extending into the middle zone	5
g. 1–3 clefts extending into the deep zone	6
h. >3 clefts extending into the deep zone	7
i. Clefts extending to calcified cartilage	8
j. Complete disorganization or joint space obliteration	9
(2) Chondrocytes	
a. Normal	0
b. Slight hypercellularity	1
c. Moderate hypercellularity	2
d. Severe hypercellularity	3
e. Slight cloning (≦4)	4
f. Moderate cloning (>4 but ≦8)	5
g. Severe cloning (>8)	6
h. Slight hypocellularity	7
i. Moderate hypocellularity	8
j. Severe hypocellularity	9
k. Disappearance of cells	10
(3) Safranin O staining	
a. Normal	0
b. Loss of staining in the superficial zone for <50% of the length of the articular surface	1
c. Loss of staining in the superficial zone for ≧50% of the length of the articular surface	2
d. Loss of staining in the superficial and middle zones for <50% of the length of the articular surface	3
e. Loss of staining in the superficial and middle zones for ≧50% of the length of the articular surface	4
f. Loss of staining in all three zones for <50% of the length of the articular surface	5
g. Loss of staining in all three zones for ≧50% of the length of the articular surface	6

DATA AND STATISTICAL ANALYSIS

All data are presented as mean ± standard deviation. The Kruskal–Wallis and Mann–Whitney *U* tests were used to evaluate the statistical significance of differences between groups. The data were analyzed using SPSS (release 10.0 SPSS Inc., Chicago, IL). *P* values less than 0.05 were considered significant.

Results

DESCRIPTION OF THE MODEL

An axial section of the facet joint from L3/4 stained with Safranin O is shown in Fig. 1(B). The left-side facet joint was the normal control (no collagenase injection). The right-side facet joint showed collagenase-induced OA without treatment. Intra-articular injection of collagenase caused OA-like changes in the facet joint. These findings included the articular matrix lesions (surface irregularity, fissures, and erosion of articular surface with loss of Safranin O staining), changes in chondrocytes (cluster or diminishment of chondrocytes), inflammatory reaction of the synovium, and changes in subchondral bone and bone marrow.

HISTOLOGY OF ARTICULAR CARTILAGE IN THE UNTREATED OR SALINE-TREATED GROUP

Compared with the normal control [Fig. 2(A and B)], the histological changes observed in the cartilage of the collagenase-exposed joints, untreated or treated with saline, included deep fissures into the middle and deep zones, chondrocyte hypercellularity and cloning, and loss of Safranin O staining at 3 weeks [Fig. 2(C and E)]. At 6 weeks, the degeneration of cartilage was greater. Erosion or denudation of the cartilage surface with loss of Safranin O staining and hypocellularity of chondrocyte was observed [Fig. 2(D and F)].

HISTOLOGY OF ARTICULAR CARTILAGE IN THE rhBMP-2-TREATED GROUP

In both rhBMP-2-treated groups, a reduction in the severity of the cartilage lesion was observed after 3 weeks [Fig. 2(G–J)]. These findings include enhanced chondrocyte proliferation, decrease in the severity of structure damage, and attenuation of the loss of Safranin O staining.

In the rhBMP-2 100 ng treated group, adverse side effects were observed in two of the six rats after 3 weeks of treatment. Cartilage overgrowth and severe synovial fibroblast

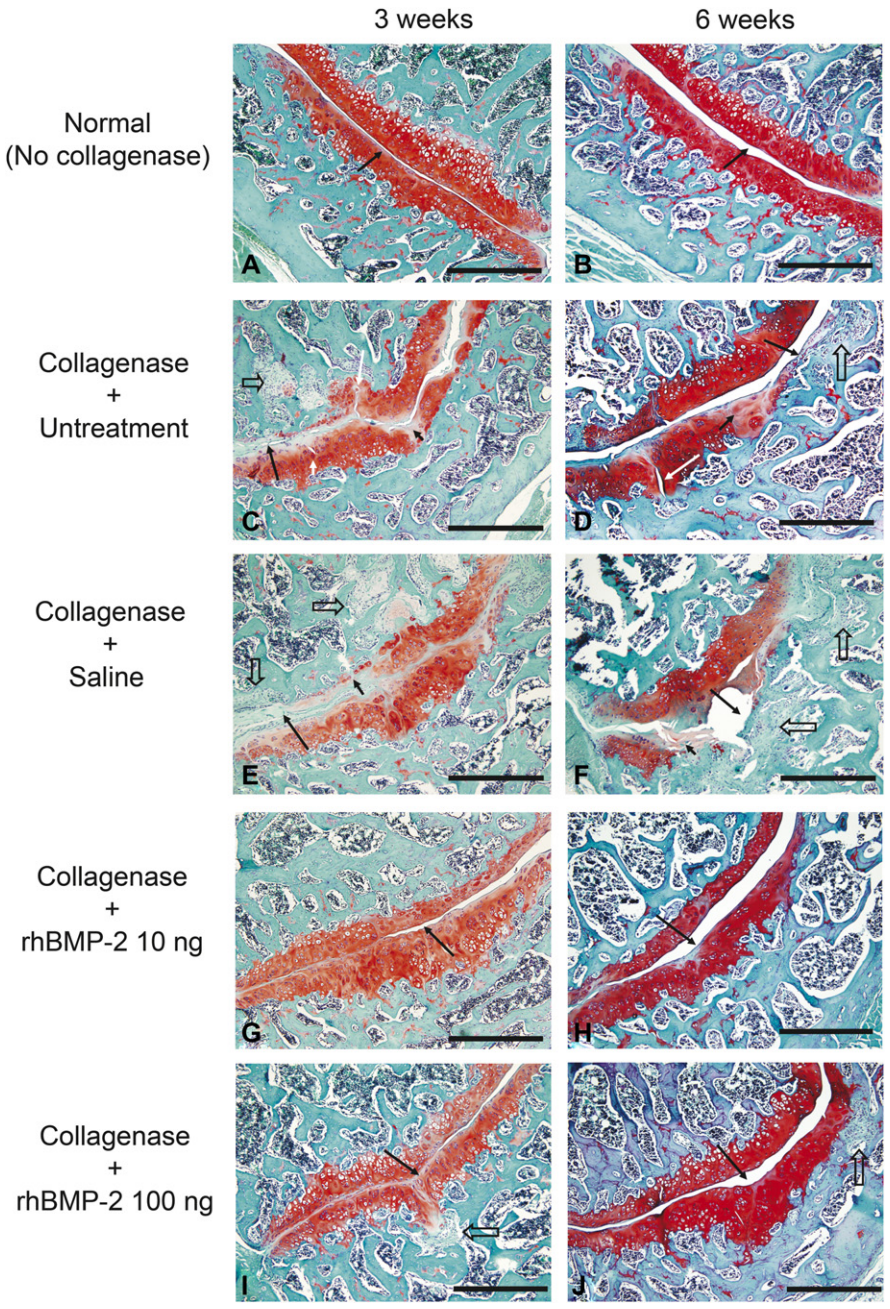


Fig. 2. Photographs of facet joint cartilage stained with Safranin O in the normal control group (A,B), collagenase-exposed joint (C–J), the untreated group (C,D), the saline-treated group (E,F), the rhBMP-2 10 ng treated group (G,H), and the rhBMP-2 100 ng treated group (I,J) at 3 and 6 weeks after treatment. In the normal control group (A,B), the articular surface was intact (black arrow). The cartilage matrix stained strongly with Safranin O. In the untreated group (C,D) and the saline-treated group (E,F), fissures extending into the middle and deep zones (long white arrow) and cartilage erosion (long black arrow) were observed. The cartilage matrix with loss of Safranin O staining was noted. Chondrocyte cloning (short white arrow) and hypocellularity of chondrocyte (short black arrow) were observed. Inter-trabecular fibrous tissue proliferation including young fibroblasts and new blood vessels, and focal new bone formation were observed (open arrow). In the rhBMP-2-treated groups (G–J), the severity of structure damage was decreased (arrow). Chondrocyte proliferation was observed. Most of the cartilage matrix was stained well with Safranin O. Focal changes in subchondral bone were observed (open arrow). Bar = 500 μm.

proliferation were induced. Both of these caused obliteration of the facet joint space [Fig. 3(B)]. It also induced the formation of a cartilage-like proteoglycan containing tissue at the dorsal aspect of the inferior articular process of vertebra [Fig. 3(B)]. These structures are referred to as chondrophytes. At 6 weeks, these chondrophytes changed into bone-like structures (osteophytes) by endochondral ossification [Fig. 3(C and D)].

THE MODIFIED MANKIN SCORE AND THE SCORES OF SEPARATE HISTOLOGIC PARAMETERS FOR CARTILAGE ASSOCIATED WITH COLLAGENASE-INDUCED DEGENERATION

The untreated and saline-treated groups showed significantly higher mean values for the modified Mankin score ($P < 0.01$) [Fig. 4(A)] and scores of separate histologic parameters (structure, chondrocyte, and Safranin O

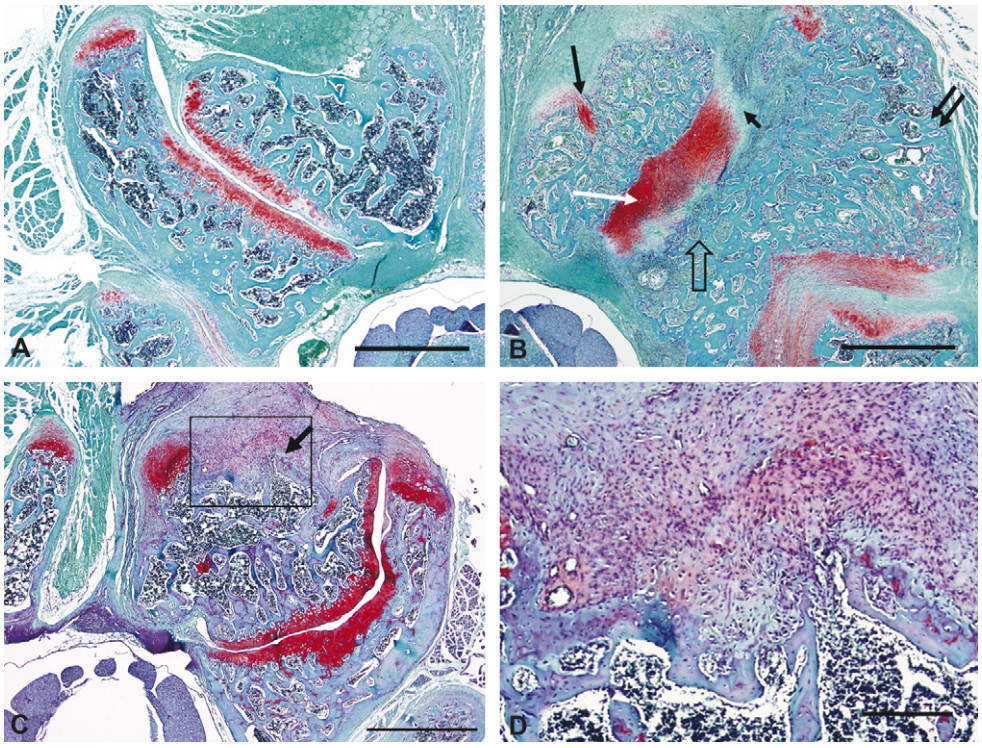


Fig. 3. Safranin O stained sections showed adverse side effects of high dosage rhBMP-2 treatment. (A) Normal control of facet joint. (B) Three weeks after rhBMP-2 100 ng injection, overgrowth of cartilage (long white arrow) and severe proliferation of synovial fibroblast (short black arrow) caused an obliteration of joint space. Chondrophyte formation was observed (long black arrow). Multifocal fragmentation of bony trabeculae surrounded by osteoclast and fibrous tissue was noted (open arrow). Bone marrow elements were replaced by loosely arranged spindle cells in a fibrous stroma. Hypertrophy of the superior articular process of vertebra was noted (double black arrows). The representative photographs in A and B were obtained from the same section. (C) Six weeks after rhBMP-2 100 ng injection, newly formed osteophyte (black arrow) was observed (A–C: bar = 1 mm). (D) A higher magnification of the rectangular outlines in C demonstrates the process of endochondral ossification. Bar = 200 μm.

staining) compared with the normal control group at 3 and 6 weeks after treatment (all $P < 0.01$) [Fig. 4(B)]. There were no differences in these histologic parameters between the untreated and saline-treated group at week 3 or 6.

The modified Mankin scores were significantly lower in both rhBMP-2-treated groups than the untreated or saline-treated groups at 3 and 6 weeks ($P < 0.01$ for rhBMP-2 10 ng at 3 and 6 weeks, $P < 0.01$ for rhBMP-2 100 ng at week 3, and $P < 0.05$ for rhBMP-2 100 ng at week 6) [Fig. 4(A)]. No significant differences were detected in the modified Mankin score between the rhBMP-2 10 ng and 100 ng treated groups [Fig. 4(A)].

The separate histological parameter scores are shown in Fig. 4(B). When compared with the untreated or saline-treated groups, the rhBMP-2 10 ng treated group showed significantly lower mean values in the structure score ($P < 0.01$ at weeks 3 and 6), the chondrocyte score ($P < 0.01$ at week 3, $P < 0.05$ at week 6), and the Safranin O staining score ($P < 0.05$ vs the untreated group at week 3; $P < 0.01$ vs the saline-treated group at week 3; $P < 0.01$ at week 6). When the rhBMP-2 10 ng treated group was compared with normal control, only the Safranin O staining score at 6 weeks showed no statistically significant differences.

In the rhBMP-2 100 ng treated group, the structure score at 3 weeks increased significantly compared to the rhBMP-2 10 ng treated group ($P < 0.05$) and there were no statistically significant differences compared to the untreated or saline-treated groups. But the structure score in the rhBMP-2 100 ng treated group decreased at 6 weeks and no significant differences were observed compared to the rhBMP-2 10 ng treated group. In the other histologic parameters, when compared to the untreated or saline-treated groups, the rhBMP-2 100 ng treated group showed significantly lower mean scores in chondrocyte score ($P < 0.01$ at week 3, $P < 0.05$ at week 6), and the Safranin O staining score ($P < 0.05$ at week 3, $P < 0.01$ at week 6). There were no statistically significant differences in the Safranin O staining score at 6 weeks between the rhBMP-2 100 ng treated group and those in the normal control.

HISTOLOGY AND SCORING OF SYNOVIUM

Compared to the normal control [Fig. 5(A and B)], synovial tissue in all groups showed hypertrophy and hyperplasia of the synovial lining cells from 3 weeks after the second surgery [Fig. 5(C–J)]. Subsynovial tissue showed proliferation of granulation tissue, vascularization, and the infiltration of inflammatory cells compared to the normal control.

The synovium scores are shown in Fig. 5(K). The differences in the synovium scores at weeks 3 and 6 were statistically significant between the normal control and other four groups ($P < 0.01$). At week 3, the synovium score in the 100 ng of rhBMP-2-treated group increased significantly compared to the saline-treated group ($P < 0.01$) or the 10 ng of rhBMP-2-treated group ($P < 0.05$). However, at week 6, there were no differences in scores between the rhBMP-2-treated groups and the saline-treated group.

A

Modified Mankin Score

Weeks after treatment	Normal control	Untreated group	Saline treated	rhBMP-2 10 ng	rhBMP-2 100 ng
3	1.8 ± 1.6	18.7 ± 1.6 †	18.0 ± 1.5 †	10.1 ± 2.3 † # ¶	12.3 ± 3.9 † # ¶
6	1.7 ± 0.8	18.5 ± 1.4 †	20.3 ± 3.1 †	9.8 ± 5.2 † # ¶	10.5 ± 5.2 † § ‡

† $P < 0.01$, versus normal control group value.
# $P < 0.01$, § $P < 0.05$, versus untreated group value.
¶ $P < 0.01$, ‡ $P < 0.05$, versus saline treated group value.

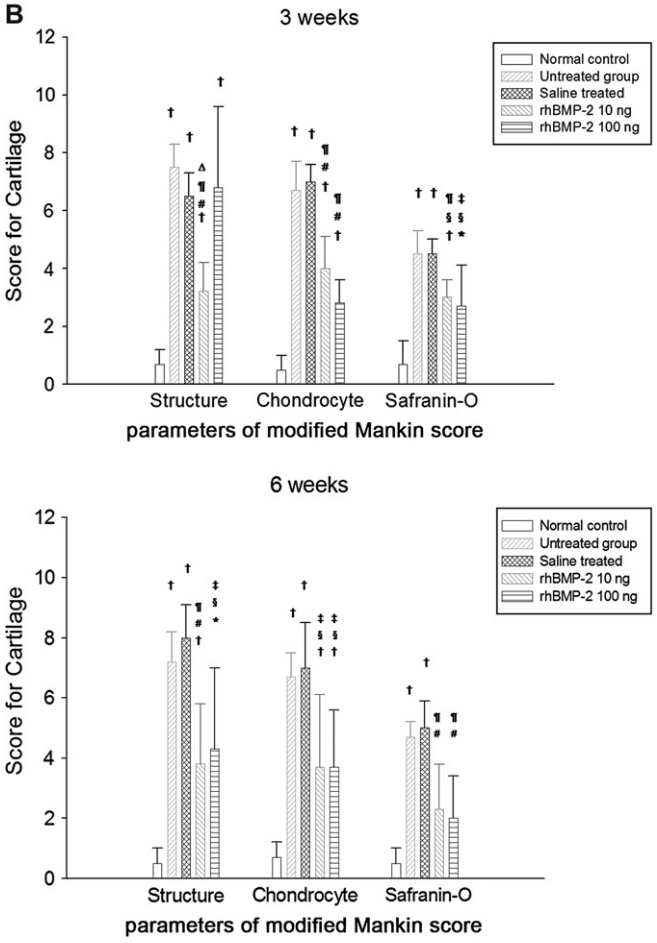
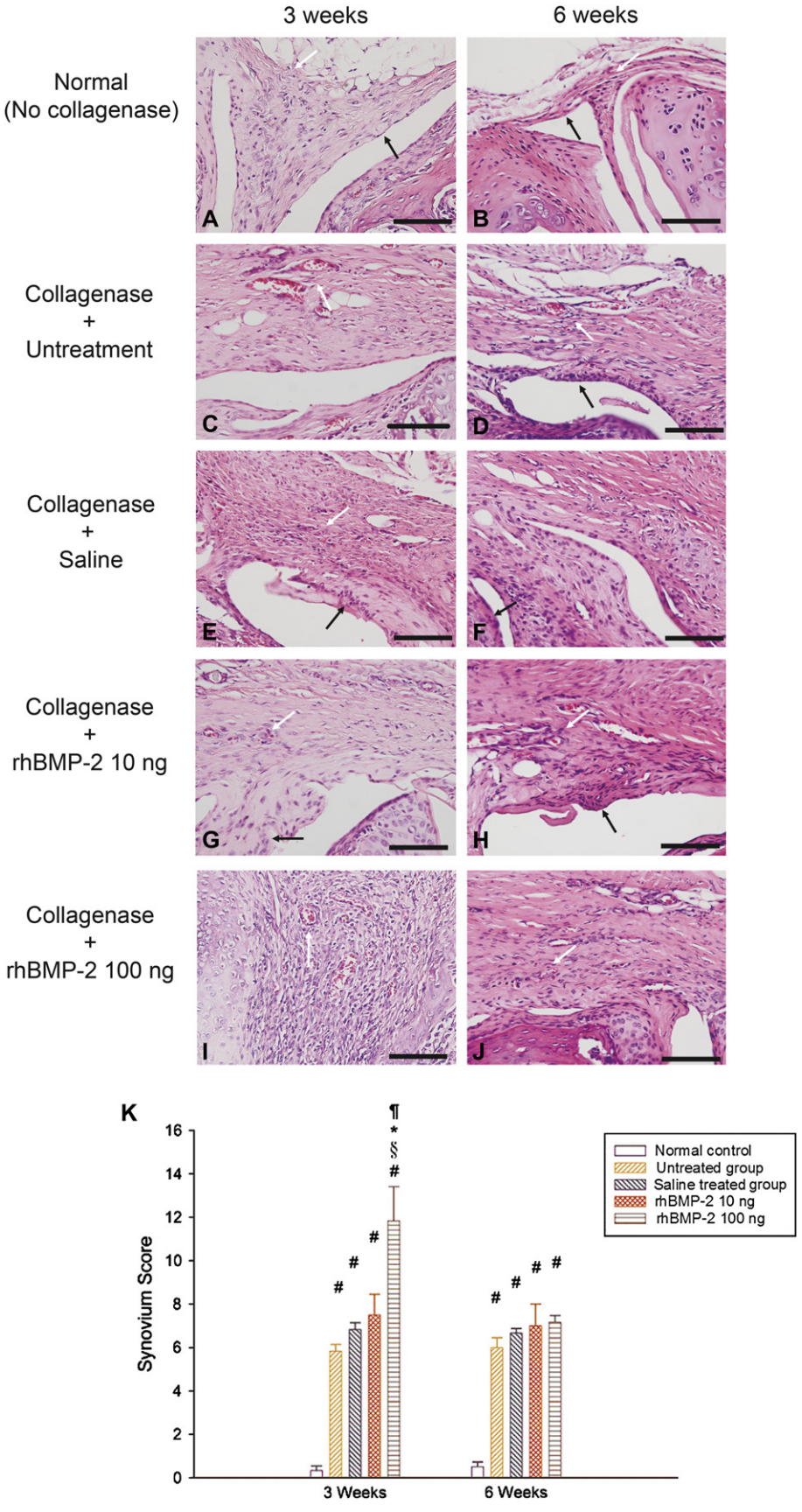


Fig. 4. Histological scores for cartilage in facet joint for the normal control, the untreated group, the saline-treated group, the rhBMP-2 10 ng treated group, and the rhBMP-2 100 ng treated group at 3 and 6 weeks post-treatment. The values presented are the means and standard deviation for six animals. (A) Shown are values of the modified Mankin scores with comparisons between groups. (B) Shown are scores for the histologic parameters (structure, chondrocyte, and Safranin O staining) with comparisons between groups. † $P < 0.01$, * $P < 0.05$: significantly greater than the normal control group; # $P < 0.01$, § $P < 0.05$: significantly less than the untreated group; ¶ $P < 0.01$, ‡ $P < 0.05$: significantly less than the saline-treated group; Δ $P < 0.05$: significantly less than the rhBMP-2 100 ng treated group, by the Mann–Whitney *U* test.

Fig. 5. (A–J) Histological demonstration of synovium stained with H&E, the normal control group (A,B), collagenase-induced OA (C–J), the untreated group (C,D), the saline-treated group (E,F), the rhBMP-2 10 ng treated group (G,H), and the rhBMP-2 100 ng treated group (I,J) at 3 and 6 weeks after treatment. In the normal control (A,B), the synovium consists of a thin layer of synovial cells (black arrow) on top of a loose connective tissue subintimal layer (white arrow). In the collagenase-exposed joint (C–J), synovial reaction included hypertrophy or hyperplasia of synovial lining cells (long black arrow), proliferation of granulation tissue (long white arrow), and infiltration of inflammatory cells. Bar = 100 μm. (K) Synovium score with comparisons between groups at 3 and 6 weeks. # Significantly greater than the normal control group ($P < 0.01$); § significantly greater than the untreated group ($P < 0.01$); * significantly greater than the saline-treated group ($P < 0.01$); ¶ significantly greater than the 10 ng of rhBMP-2-treated group ($P < 0.05$), by the Mann–Whitney *U* test.


3 weeks
6 weeks
Normal
(No collagenase)
Collagenase
+
Untreatment
Collagenase
+
Saline
Collagenase
+
rhBMP-2 10 ng
Collagenase
+
rhBMP-2 100 ng
A
B
C
D
E
F
G
H
I
J
K
Synovium Score
0
2
4
6
8
10
12
14
16
3 Weeks
6 Weeks
Normal control
Untreated group
Saline treated group
rhBMP-2 10 ng
rhBMP-2 100 ng

HISTOLOGY OF SUBCHONDRAL BONE AND BONE MARROW

In the untreated and saline-treated groups, some changes in subchondral bone region immediately adjacent to the damaged cartilage were observed at 3 weeks. These changes included proliferation of loose fibrous tissue and new small vessels in the inter-trabecular space and focal new bone formation [Fig. 2(C and E)]. At week 6, the severity of these changes was increased [Fig. 2(D and F)]. In the rhBMP-2-treated groups, these changes were present only in small areas [Fig. 2(G–J)], but more extensive changes in bone marrow and subchondral bone were observed in two rats complicated with joint space obliteration in the rhBMP-2 100 ng treated group. Multifocal fragmentation of bony trabeculae surrounded by osteoclast and fibrous tissue was noted. Bone marrow elements were replaced by loosely arranged spindle cells in a fibrous stroma. Compared to the normal control, hypertrophy of the superior articular process of vertebra was observed (Fig. 3B).

IMMUNOHISTOCHEMICAL FINDINGS

In normal cartilage, the extracellular matrix strongly stains for type II collagen. Compared with normal control specimens, untreated specimens or saline-treated specimens showed a statistically significant decrease in stain intensity at 6 weeks (all $P < 0.01$). However, both rhBMP-2-treated specimens showed no significant differences in stain intensity compared with normal control specimens at 6 weeks (Fig. 6).

Discussion

In the present study, we demonstrated that BMP-2 reduced the severity of OA in the cartilage of lumbar facet joints and increased the presence of type II collagen, the major extracellular matrix component of articular cartilage. These results support the concept that, in OA, the anabolic agent BMP-2 is involved in the repair of cartilage lesions.

Exogenous BMP-2 has potent anabolic activities on adult chondrocyte *in vitro*[18] and *in vivo*[23,24], increasing proteoglycan and type II collagen production[25–27] and maintaining the chondrocyte phenotype[28]. Some studies have shown that rhBMP-2 can improve the healing of full-thickness defects of articular cartilage in a rabbit model[29,30]. But until now, no *in vivo* data concerning the effects of rhBMP-2 on OA cartilage in facet joints were available.

In the present study, the time point for treatment was chosen to be 2 weeks after collagenase injection. Our previously established animal model showed that OA in the facet joint was induced after this time period[19]. In this study, treatment with BMP-2 effectively alleviated the OA-like changes in the

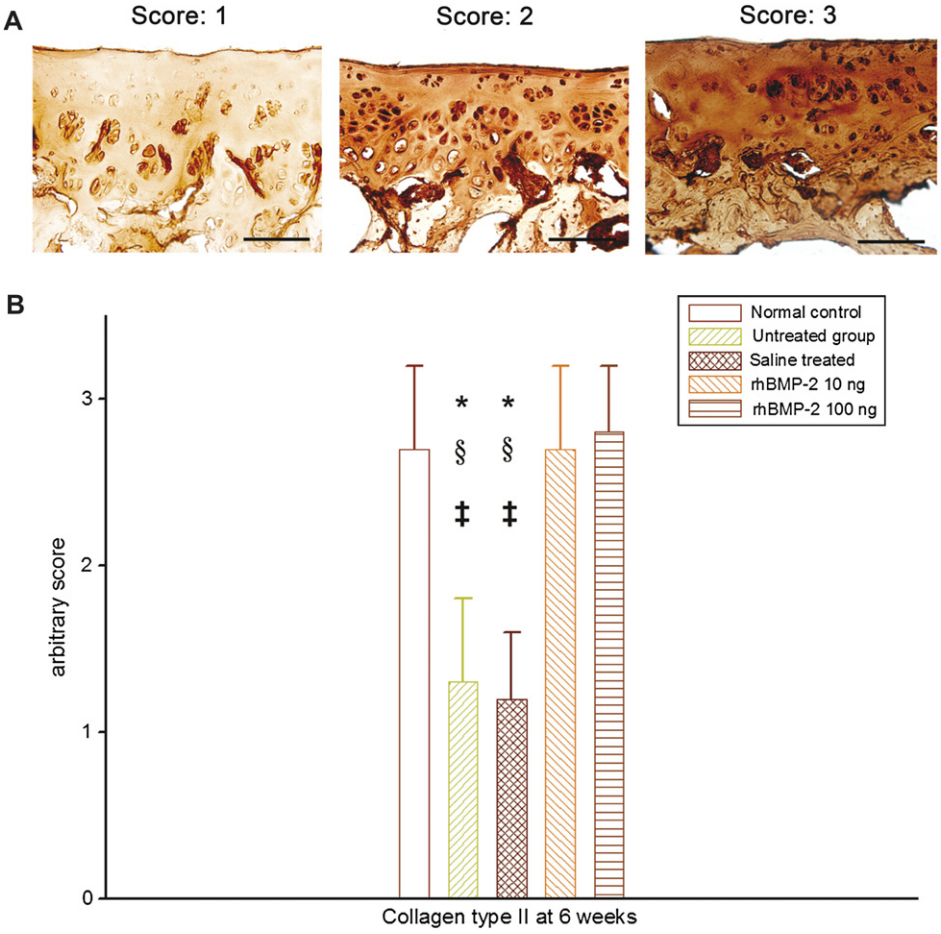


Fig. 6. (A) Representative immunohistochemical stain score for collagen type II. Bar = 100 μm. (B) Collagen type II was analyzed based upon the staining intensity using an arbitrary score. The values presented are the means and standard deviation for six animals. ‡ Significantly reduced compared to the normal control group; § significantly reduced compared to the rhBMP-2 10 ng treated group; * significantly reduced compared to the rhBMP-2 100 ng treated group (all $P < 0.01$), by the Mann–Whitney U test.

facet joint during the 6-week period of observation. Either 10 ng or 100 ng of BMP-2 treatment can reduce the progression of OA, but the injection of the high dosage BMP-2 caused adverse side effects (obliteration of joint space) in some rats at 3 weeks. This led to poorer results in the structure score compared with the low dosage BMP-2 treatment at this time. Nevertheless the whole modified Mankin score showed no significant differences between both BMP-2-treated groups. That was due to the fact that treatment with the higher dosage of BMP-2 also caused improvement in chondrocyte growth and reduction of proteoglycan depletion detected by Safranin O staining. Some authors reported that intra-articular injection of BMP-2 stimulates proteoglycan synthesis only in normal knees but not in a model of destructive arthritis[31]. The proteoglycan synthesis was detected by ^{35}S-sulfate incorporation method in that study[31]. Because loss of Safranin O staining is indicative of cartilage matrix proteoglycan depletion, proteoglycan synthesis cannot be demonstrated by this staining method. Further studies are required to establish with certainty the nature of the changes in proteoglycan synthesis in the osteoarthritic facet joints after BMP-2 treatment.

In our study, high dosage BMP-2 injections into the facet joints not only affected articular cartilage, but also induced the periosteum to form a proteoglycan-rich cartilaginous structure (chondrophytes). The structure was located in the dorsal side of inferior articular process of vertebra. The newly formed cartilaginous structures lost their proteoglycan content and developed into osteophytes at a later time point. Injections of low dosage BMP-2 had little effect in this respect. In OA, the process of osteophyte formation at the joint margins is often considered a defense mechanism to increase joint stability. It seldom contributes to the symptom[32]. In this study, the newly formed osteophyte was located in the extra-articular region. This change did not affect the articular surface structure. Similar chondrogenic activity after injections with BMP-2 was reported in the murine knee joint[23,24], but the location appeared to be restricted to the regions in the growth plate in contact with the patellofemoral joint.

Some authors reported that an injection of BMP-2 into the murine knee joint had an effect of inducing fibrosis[24]. In this study, high dose of rhBMP-2 induced a severe synovium reaction (lining cell hypertrophy, hyperplasia and subsynovial granulation tissue formation) 3 weeks after treatment. But the reaction decreased after 6 weeks. The inflamed synovium caused overproduction of the proinflammatory cytokines (such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha)) that may contribute to the progression of OA[33,34]. On the other hand, some *in vitro* studies showed that BMP-2 induced the expression of mRNA for Sox-9, type II collagen and aggrecan (chondrocyte-specific genes) in bovine synovium-derived mesenchymal progenitor cells[35]. Our recent observation showed that an intra-articular injection of rhBMP-2/rhBMP-7 heterodimer (more potent than rhBMP-2[36]) into the facet joint caused chondrometaplasia of the synovium (unpublished observation). Further studies are required to evaluate the balance between the chondrogenic effect associated with the synovial mesenchymal cells and the catabolic effect of the proinflammatory cytokines related to the synovitis and then the response to BMP-2 treatment.

In our study, subchondral bone involvement was observed after an intra-articular collagenase injection. These changes developed in the trabecular bone immediately situated beneath the region of cartilage damage. The changes are consistent with the initiation of bone remodeling. Although BMP-2 is used for spinal fusion, our results showed that an intra-articular administration of low dosage of BMP-2 did not cause overgrowth of subchondral bone.

Previous studies revealed that the therapeutic potential of BMP-2 by an intra-articular injection into knee joint was limited, because of the adverse side effects (osteophyte formation and fibrosis)[23,24,37]. The complexity of BMP signaling demonstrates that fine-tuning of this signaling pathway is likely to be a challenge in the development of regenerative therapeutic strategies[32]. Our results show that low dosage of BMP-2 administration to the osteoarthritic lumbar facet joint may be able to block or suppress cartilage lesion progression and avoid some of the adverse side effects of excessive or prolonged exposure to BMP-2. Although BMP-2 is not able to overcome all the aspects of the severe damage induced by an intra-articular collagenase injection, it becomes apparent that key parameters reflecting cartilage metabolism (type II collagen and proteoglycan localization, chondrocyte proliferation, preservation of joint space) have been very significantly improved.

In summary, the present study demonstrated that a low dose of rhBMP-2 reduces the progression of structural changes of the cartilage in a rat lumbar facet joint model of OA. However, at a high dose of rhBMP-2 it causes adverse side effects.

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