Original Article

Bone morphogenetic protein-7 accelerates fracture healing in osteoporotic rats

Ashish D Diwan[1,2], Anthony Leong[2], Richard Appleyard[1], Divya Bhargav[1], Zhi Ming Fang[1], Aiqun Wei[1]

Abstract

Background: Osteoporosis is characterized by low bone mass, bone fragility and increased susceptibility to fracture. Fracture healing in osteoporosis is delayed and rates of implant failure are high with few biological treatment options available. This study aimed to determine whether a single dose of bone morphogenetic protein-7 (BMP-7) in a collagen/carboxy-methyl cellulose (CMC) composite enhanced fracture healing in an osteoporotic rat model.

Materials and Methods: An open femoral midshaft osteotomy was performed in female rats 3 months post-ovarectomy. Rats were randomized to receive either BMP-7 composite (n = 30) or composite alone (n = 30) at the fracture site during surgery. Thereafter calluses were collected on days 12, 20 and 31. Callus cross-sectional area, bone mineral density, biomechanical stiffness and maximum torque, radiographic bony union and histological callus maturity were evaluated at each time point.

Results: There were statistically significant increases in bone mineral density and callus cross-section area at all time points in the BMP-7 group as compared to controls and biomechanical readings showed stronger bones at day 31 in the BMP-7 group. Histological and radiographic evaluation indicated significant acceleration of bony union in the BMP-7 group as compared to controls.

Conclusion: This study demonstrated that BMP-7 accelerates fracture healing in an oestrogen-deficient environment in a rat femoral fracture healing model to scientific relevance level I. The use of BMP-7 composite could offer orthopedic surgeons an advantage over oestrogen therapy, enhancing osteoporotic fracture healing with a single, locally applied dose at the time of surgery, potentially overcoming delays in healing caused by the osteoporotic state.

Key words: Bone morphogenetic protein-7, carboxy-methyl cellulose, oestrogen, osteoporotic fracture healing, rat femur

Introduction

Osteoporosis is a significant health problem, particularly in postmenopausal women and the geriatric population, in whom oestrogen withdrawal results in further decreases in bone density. Low bone mass and micro-architectural deterioration of osteoporotic bone structure leads to bone fragility and increased susceptibility to fractures, the healing of which is often biomechanically impaired.[1] Indeed, a lack of oestrogen has been shown to retard bone formation in studies of spinal fusion or fracture in an osteoporotic rat model.[2,3] The ability to enhance the healing process in osteoporotic fractures is desirable, particularly in view of the frequent failure of metallic instrumentation in fragile osteoporotic bones.[4,5]

Bone morphogenetic proteins (BMPs) play a critical role in new bone formation.[6] Initially discovered as components of demineralized bone extracts that promote bone growth,[7] BMPs form a family of related proteins with wide ranging effects on developing and regenerating tissues,[8] including stimulation of osteoblast differentiation and function.[6,9,10] Furthermore, BMPs appear to mediate cell migration,[11] and influence the expression of multiple target genes through receptor-mediated triggering of complex intracellular signaling pathways.[12]

Studies have shown recombinant human BMP-7 (rhBMP-7) promotes fracture healing in animal models[13-16] and in humans.[17-19] Distal tibial fractures, which are difficult to treat, heal slowly and often lead to complications; when treated with BMP-7, they showed significantly faster healing rates and required fewer secondary interventions as compared to controls.[19] BMP-7 also proved beneficial in complex and persistent cases of pelvic ring instability, where the

[1]Orthopaedic Research Institute and [2]Department of Orthopaedic Surgery, St. George Hospital Clinical School, University of New South Wales, Kogarah, New South Wales, Australia

Address for correspondence: Dr. Ashish D. Diwan, Orthopaedic Research Institute and Department of Orthopaedic Surgery, St. George Hospital Sydney, Kogarah, New South Wales 2217, Australia. E-mail: a.diwan@spine-service.org

Access this article online	
Quick Response Code:	Website: www.ijoonline.com
	DOI: 10.4103/0019-5413.121569

application of BMP-7 produced 89% successful fusions in a small number of patients who had received at least one prior unsuccessful operation.[17] BMP-7 application has also reportedly aided successful bone fusion in open tibial shaft fractures and nonunions of the scaphoid, diaphyseal humerus and other long bones.[18,20]

Significantly higher posterolateral fusion rates have been achieved using BMP-7 composite application in the spines of ovarectomised rats.[21] Furthermore, BMP-7 stimulated proliferation, osteogenic differentiation and mineralization *in vitro* in human osteoporotic tissues[22] and in oestrogen-deficient rat fracture callus explant cultures.[23] With few therapeutic options and a high frequency of implant failure in osteoporotic fractures, we hypothesized that the application of a single dose of BMP-7 composite to fractured femur sites would promote enhanced fracture healing in an osteoporotic fracture. This was studied in a prospective randomized placebo-controlled rat femoral fracture model with blinded assessors.

Materials and Methods

Animal model

All experimental protocols and procedures were approved by the appropriate University Animal Care and Ethics Committee (ACEC). Sprague-Dawley rats (2-months-old, female) were surgically ovarectomised at the University Biological Resources Centre facility and obtained 3-months postovarectomy. Surgical procedures were conducted under halothane inhalation anesthesia. A mid-shaft osteotomy was performed with a Gigli saw and fixed with a 1.6-mm diameter stainless steel Kirchner wire-passed retrograde into the proximal fragment and then into the medullary canal of the distal fragment.

Rats were randomized to receive either a BMP-7 + collagen-I/CMC composite (BMP-7 Group, $n = 30$) at a dose of 69 mg/fracture (0.23 mg BMP-7 per 68.77 mg collagen-I) or collagen-I/CMC composite alone (0.2 ml, Control Group, $n = 30$) implanted at the fracture site. The BMP-7 is bound to bovine type I collagen and commercially available as OP-1. OP-1 and carboxy-methyl-cellulose (CMC) were provided by Stryker Biotech (Hopkinton, MA). The CMC, when mixed with saline, becomes putty-like and holds the sand-grain-like OP-I (BMP-7 + Collagen-I) more effectively. The dose of BMP-7 protein averaged 0.9 mg/kg, which was within the recommended human dosing range (0.5-1 mg/kg). The muscle and skin were then closed at the end of the procedure.

Rats from each group ($n = 10$) were euthanized at days 12, 20 and 31 postsurgery and then bilateral femora were harvested for experimental analyses. All assessments were performed in a blinded manner regarding the source of the tissue.

DEXA Scan

Bone mineral density (BMD) measurements were performed using a Lunar densitometer (Expert XL model with small animal research software options). Thawed specimens of both femora were evaluated by hand regional high-resolution mode (line spacing 0.254 and point resolution 0.89 mm). Point types were marked to delineate the bone and exclude soft tissue. The region of interest included the entire right femur without the K-wire, a 0.6 cm^2 rectangle centered on the fracture site and the entire left femur as an internal control.

Radiological examination

Specimens were radiographed in a frontal projection using a Faxitron X-ray apparatus (Model 8050-010; Faxitron X-ray Corporation, Wheeling, IL). For optimal observation of callus formation, bony continuity and remodeling, the specimens were exposed for 60 s at 20 kV. Images were examined qualitatively by three independent blinded observers who used two measurement tools devised for Faxitron assessment of fracture healing.[24] First, the radiographs were graded according to the following system and the mean ± SEM calculated: Grade 1-no callus visible; Grade 2-Small amounts of fluffy callus present at bone ends; Grade 3-external bridging callus visible with no new bone between bone ends; Grade 4-Bridging callus visible with callus between bone ends of specimens. Second, the following characteristics were observed on each radiograph and each was assigned one point, not to exceed the maximum points shown in parentheses: 1. Visible callus on one side of the fracture at one bone end (4); 2. Bridging callus on one side (2); 3. Callus between fracture fragments on one side (2); 4. Evidence of remodeling of callus on one side (2). The points were then added to produce a score between 0 and 10 for each radiograph.

Callus morphometry

Following the DEXA scanning, all femora were dissected clean from the surrounding soft tissue and the diameter of the callus was measured in two planes in the region where the callus is thickest using a Vernier calliper. Cross-sectional area of the fusion mass was calculated.

Biomechanical testing

Soft tissue was removed and each end of the femora was embedded into 12 × 8 mm aluminum cylinders using polymethyl methacrylate. Thawed specimens were subjected to a torsional load with a 20 Nm reaction torque cell and tested to failure at a rate of 2.0 degree/s on an AG-50KNE materials testing machine. The load displacement curves were acquired with a low load cell. The

force to failure, energy, stress and stiffness were measured from the resulting load-deformation curves.

Histological evaluation

Two rats from each group were randomized for histological analysis. At sacrifice, all soft tissue was removed from the right femur. The specimens were fixed in 10% buffered formalin for 48 h and decalcified with Rapid Decalcifier (48 h) (Lomb Scientific, Australia). The specimens were sectioned along the length of the femur and paraffin embedded. The blocks were cut into 4-mm thin sections and stained with hematoxylin-eosin (H and E), Alizarin Red-S stains and then evaluated under light microscopy.

Statistical analysis

Data analysis among multiple experimental groups was performed with analysis of variance (ANOVA) and unpaired Students *t* test using the statistical program SPSS (SPSS Inc., San Rafael, CA). All results are reported as a mean ± SEM (standard error of mean). Differences were considered significant at $P < 0.05$.

Results

Increased bone formation in bone morphogenetic protein-7 treated femurs visualized radiographically

Radiographs of rat femurs from BMP-7 treated and control groups were examined qualitatively using specific grading schemes to numerically describe the visual grade of callus formed. Visually, the radiographs in Figure 1a showed that more bone was present in the BMP-7 group as compared to the controls at both time points shown (days 12 and 31). This was particularly impressive at day 12 in the BMP-7 group, while little evidence of new bone material was visible in the control [Figure 1a].

When the grading scheme was applied, the results showed a significant enhancement in callus maturity in the BMP-7-treated group at all time points [Table 1]. Observations of bridging callus location and evidence of fracture remodeling are also visible in the radiographs, indicating more active fracture healing in the presence of BMP-7 than in the controls [Figure 1a].

Table 1: Radiological evaluation

Days	Treatment	Grade (1-4)	Score (0-10)
12	BMP-7 composite	3.54±0.40*	6.29±1.20*
	Control	1.08±0.24	0.13±0.35
20	BMP-7 composite	3.79±0.40*	7.21±2.04*
	Control	1.58±0.50	1.38±1.10
31	BMP-7 composite	3.88±0.35*	7.75±1.43*
	Control	1.92±0.50	2.83±1.38

Data demonstrates enhanced callus maturity in the BMP-7 treated group, The radiological grade determined by three independent observers was at least 2-fold higher in the BMP-7 treated group as compared to the controls (*$P \leq 0.05$), BMP-7 = Bone morphogenetic protein-7

BMP-7 increased BMD in an oestrogen-deficient environment

Dexa scanning of bone density showed that newly formed bone material present in the radiographs was more highly mineralized in the BMP-7 group. Administration

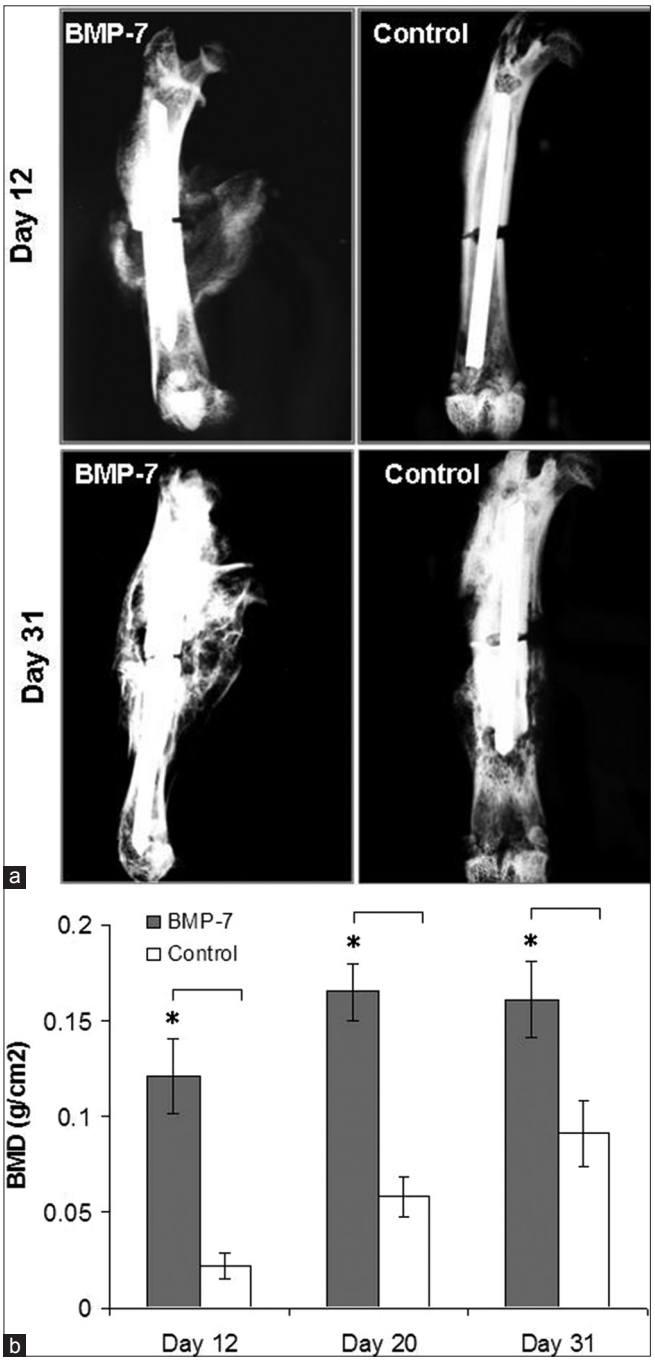

Figure 1: (a) Radiographic images of fractured rat femurs. Representative radiological images of fractured femurs from BMP-7 treated (left panels) or control (right panels) groups from days 12 (upper panels) and 31 (lower panels). Enhanced callus formation was consistently observed in the BMP-7 treatment group at both time points. (b) Fracture callus bone mineral density. The bone mineral density (BMD) of calluses as measured using a Lunar Expert XL densitometer centered on the fracture site. The entire left femur was used as an internal control ($n = 6$, mean ± SEM, *$P < 0.01$)

of the BMP-7 composite to fractured femurs resulted in significant increases in BMD measurements as compared to controls ($P < 0.01$, Figure 1b), particularly at the earliest time point (day 12), where a 5.5-fold increase was measured.

Both groups recorded increases in BMD over time, with controls increasing from 0.02 g/cm^2 to 0.09 g/cm^2 at 31 days [Figure 1b] and the BMP-7 composite increasing from 0.12 g/cm^2 to 0.16 g/cm^2 by day 20. The data was normalized using the BMD of the non-operated femurs, where no statistical difference between those groups was measured.

BMP-7 treatment leads to biomechanically stronger healing

The application of BMP-7 composite to fracture sites in osteoporotic rats resulted in significantly larger callus cross-sectional areas at all time points tested [Figure 2a]. The mean cross-sectional area of the control group for all time points was approximately 57 mm^2, while the BMP-7 group calluses all measured more than twice this area at 140 mm^2 [Figure 2a]. At later time points, there was a trend for decreasing area of callus consistent with effective bone remodeling, whereas the non-fractured femurs in both groups recorded unchanged cross-sectional areas.

Measurement of biomechanical parameters stiffness [Figure 2b] and maximum torque [Figure 2c] showed that the fracture callus strength increased significantly in the presence of BMP-7 as compared to the controls. In particular, at day 31 postsurgery, BMP-7 composite-treated calluses had a 5-fold higher stiffness ($P < 0.01$) and 3-fold greater torque ($P < 0.01$) than controls. While the enhancement of parameters like callus area and mineral density in the BMP-7-treated group was most marked in the early stages, the biomechanical strength and torque continued to be improved by the single BMP-7 application in the later stages of fracture healing [Figures 2b, c]. This is an important consideration in an osteoporotic environment, where fractures are subject to frequent surgical implant failure.[4,25]

Histological analysis of BMP-7-treated bony unions

The histological appearance of the callus suggests that BMP-7 promotes accelerated tissue formation with potentially greater extracellular matrix deposition and cellularity [Figure 3a]. At day 21 of healing, calcified tissue around the fractured sites was visible in the BMP-7 groups, but there appeared to be only minor new bone formation in the control group. At the later time point (day 31) in the BMP-7 group, there was evidence of a bridging fusion mass between and around the fractured sites that was absent in the control group.

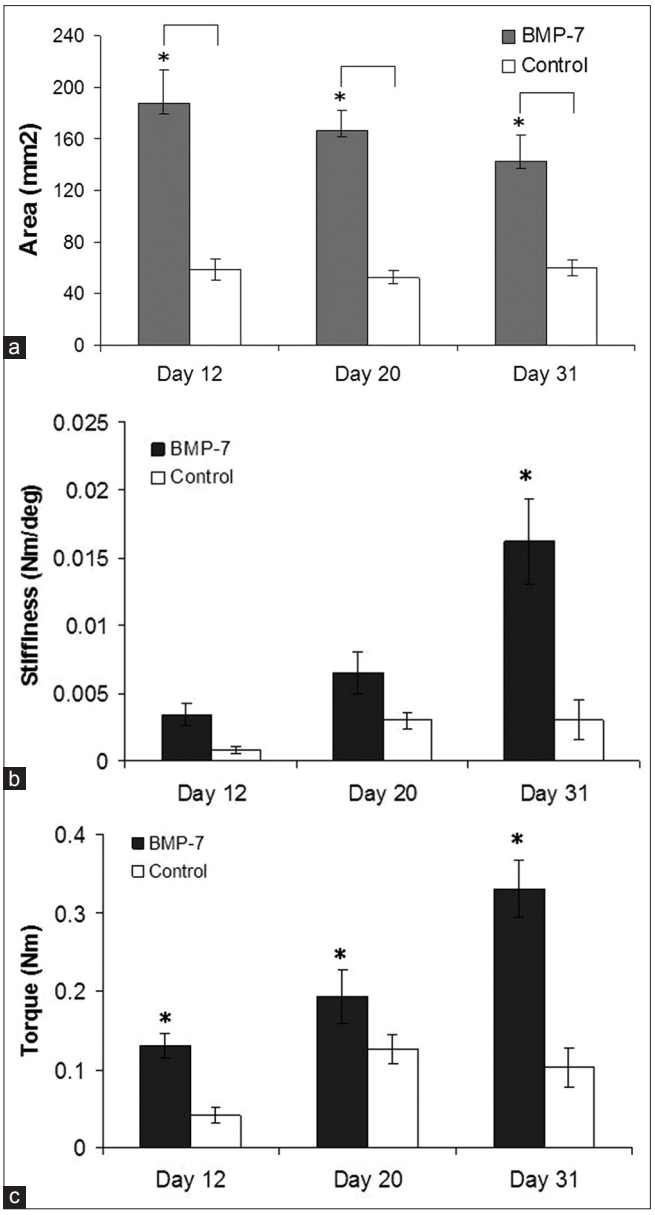

Figure 2: Bar diagrams showing (a) Fracture callus cross-sectional area. Callus cross-sectional area (mm^2) calculated from measurements of dissected calluses in two planes using a Vernier calliper demonstrates a significantly larger callus in the BMP-7 group ($n = 6$, mean ± SEM, $^*P < 0.01$). (b) Biomechanical strength parameters in fractured rat femurs. Forces applied to the fractured femurs at days 12, 20 and 31, following fracture illustrate increasing stiffness (Nm/deg) over time. Data was collected from deformation curves when specimens were subjected to forces in a materials testing machine ($n = 6$, mean ± SEM, $^*P < 0.01$). (c) Biomechanical strength of fractured femurs. Forces applied to the fractured femurs at days 12, 20 and 31 postfracture show increasing "Maximum Torque" (Nm), determined from load-deformation curves when specimens were subjected to forces in a materials testing machine ($n = 6$, mean ± SEM, $^*P < 0.01$)

Similarly, reduced Alizarin Red-S staining suggested a reduction in calcium mineralization in the early stages of bone healing in the control group as compared to the BMP-7 group [Figure 3b]. Greater positive staining with Alizarin Red-S was noticed at day 31 in the BMP-7 group, while

negative staining persisted in the control group [Figure 3b]. This staining represents calcium mineralization remaining after a 48-h decalcification period.

Discussion

This study shows the accelerated healing potential of BMP-7 application in large bone fractures in an osteoporotic rat model. BMP-7 stimulated new bone growth, greater mineral density and perhaps most importantly, enhanced the biomechanical strength of healed fractures as compared to the controls.

BMP-7 is known to stimulate osteogenesis[22,26-28] and to promote fracture healing *in vivo* in a number of different models,[17,19,29,30] including osteoporotic rats.[21,31,32] Furthermore, BMP-7 stimulation of bone cell proliferation, osteogenic differentiation and expression of early (alkaline phosphatase) and late (calcium mineralisation) markers of bone formation have been reported *in vitro* in an osteoporotic environment.[22,23] The BMD measurements and histological studies shown here provide a relative indication of new bone formation at the fracture site, showing accelerated new tissue formation and mineralization in the BMP-7 group. There may be potential for BMP-7 application to offer an alternative approach to osteoporotic fractures where bone fragility and instrumentation failure rates are high.

Improved and lasting biomechanical strength for osteoporotic fractures represents a considerable advantage and evaluation of the long term benefits of BMP-7 application to fracture sites may be worthy of further study. BMP-7 application also has enhanced bone biomechanical strength in fractures in chronic infection[15] and estrogen depletion,[32] and such an effect may be dependent on the dosage delivered to the fracture site. Doses of 20-30 μg BMP-7 in some studies failed to show enhancement in spinal fusion[2] and in fractures in a diabetic rat model,[16] yet administration of 90-200 μg produced considerable improvement in bone fusion.[15,21]

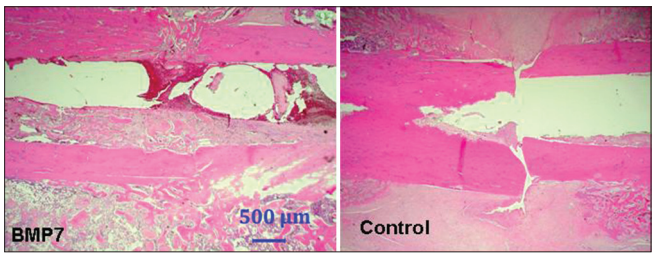

Figure 3a: Histological images of fracture callus tissue. H and E stained micrographs (2.5× magnification) showing significant new bone formation in the BMP-7 group (left panel) not controls (right panel), day-12 post-fracture (upper panels). Complete healing (lower panels) shown in day-31 fractures in the BMP-7 group, control group fractures remained un-united

BMP-7 has well documented effects on bone-forming progenitors including stimulation of differentiation,[22,28] chemotactic migration,[11] and osteogenic gene expression.[28] Indeed, BMP-7 was superior to other BMPs and growth factors in stimulating osteogenesis and enzyme function in bone tissue derived from elderly, osteoporotic fractures[22] and induced mineralization in serum-free callus explant cultures.[23] What remains unknown is whether BMP-7 may have the ability to reverse some of the adverse effects of oestrogen deficiency, such as osteoblast apoptosis, in the later stages of healing, to bring about effective bone repair. Certainly, BMP-7 has shown an anti-apoptotic effect in cartilaginous cells *in vitro*.[33]

The present fracture model tests the effect of BMP-7 on fracture healing in osteoporotic rats. The use of ovariectomized animals is well-established, providing a powerful approach to understand bone healing in osteoporosis.[34] Early studies demonstrated that ovariectomized rats showed a significant decrease in both serum 17-β-estradiol and BMD.[35] Bone loss in this model shares many similarities with bone loss in early postmenopausal women, including an increase in bone turnover with bone resorption in excess of formation.[36] However, the total loss of BMD is less in this model than in humans. The impairment of fracture healing is considered to be the result of combined effects of prolonged endochondral calcification, highly activated osteoclast cells and deceleration of bone mineralization density.[37] Clearly, there are some biological differences in bone composition between the ovariectomised rats and osteoporotic humans,[38] and the results from these studies should be cautiously applied to human osteoporotic fractures. However, osteoporosis is a growing health problem, characterized by a high risk of bone fracture and a retarded healing process and there are only few

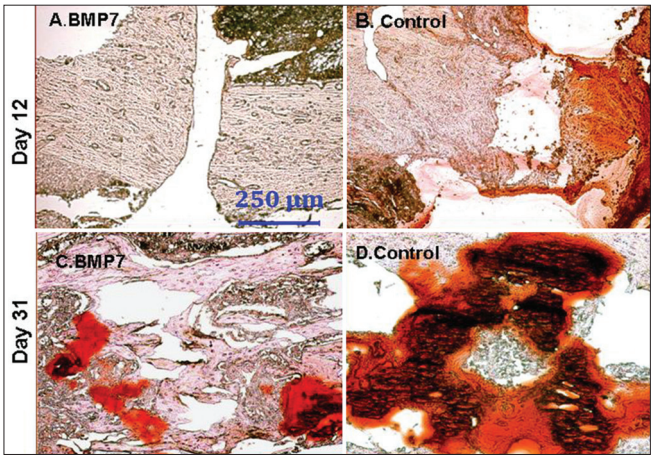

Figure 3b: Histological images of fracture callus tissue stained with Alizarin red-S. Images show greater calcium mineralization (red areas) in calluses of the BMP-7 group at days 12 (A) and 31 (C) as compared to controls (B, D). Tissues were de-calcified for 48 h prior to staining and were captured at 100× magnification

biological therapeutic options available. While fracture prevention strategies are a public health necessity, with an aging population, the burden of osteoporotic fractures will continue to rise. Given the frequent failure of orthopedic implants in osteoporotic bone, it seems promising that BMP-7 delivered locally to fracture sites with carrier material may be an effective adjunct to the treatment of fractures in osteoporosis.

Acknowledgment

The authors wish to thank Dr. Allan Turnbull of the Department of Orthopaedic Surgery, St. George's Hhospital, for his expert interpretation of the radiography images in this study; Dr. Lisa Williams for her extensive efforts in assembling and editing this manuscript; and St. George's Hospital Nuclear Medicine Department, Kogarah, Sydney for expert performance of DEXA scanning.

References

1. Walsh WR, Sherman P, Howlett CR, Sonnabend DH, Ehrlich MG. Fracture healing in a rat osteopenia model. Clin Orthop Relat Res 1997:218-27.
2. Moazzaz P, Gupta MC, Gilotra MM, Gilotra MN, Maitra S, Theerajunyaporn T, *et al*. Estrogen-dependent actions of bone morphogenetic protein-7 on spine fusion in rats. Spine (Phila Pa 1976) 2005;30:1706-11.
3. Namkung-Matthai H, Appleyard R, Jansen J, Hao Lin J, Maastricht S, Swain M, *et al*. Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone 2001;28:80-6.
4. McLain RF, McKinley TO, Yerby SA, Smith TS, Sarigul-Klijn N. The effect of bone quality on pedicle screw loading in axial instability. A synthetic model. Spine (Phila Pa 1976) 1997;22:1454-60.
5. Halvorson TL, Kelley LA, Thomas KA, Whitecloud TS 3rd, Cook SD. Effects of bone mineral density on pedicle screw fixation. Spine (Phila Pa 1976) 1994;19:2415-20.
6. Leboy PS. Regulating bone growth and development with bone morphogenic proteins. Ann N Y Acad Sci 2006;1068:14-8.
7. Urist MR. Bone formation by autoinduction. Science 1965;150:893-9.
8. Xiao YT, Xiang LX, Shao JZ. Bone morphogenetic protein. Biochem Biophys Res Commun 2007;362:550-3.
9. Yamaguchi A, Katagiri T, Ikeda T, Wozney JM, Rosen V, Wang EA, *et al*. Recombinant human bone morphogenic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation *in vitro*. J Cell Biol 1991;113:681-7.
10. Wozney JM. The bone morphogenic protein family and osteogenesis. Mol Reprod Dev 1992;32:160-7.
11. Lee DH, Park BJ, Lee MS, Lee JW, Kim JK, Yang HC, *et al*. Chemotactic migration of human mesenchymal stem cells and MC3T3-E1 osteoblast-like cells induced by COS-7 cell line expressing rhBMP-7. Tissue Eng 2006;12:1577-86.
12. von Bubnoff A, Cho KW. Intracellular BMP signaling regulation in vertebrates: Pathway or network? Dev Biol 2001;239:1-14.
13. Bilic R, Simic P, Jelic M, Stern-Padovan R, Dodig D, van Meerdervoort HP, *et al*. Osteogenic protein-1 (BMP-7) accelerates healing of scaphoid nonunion with proximal pole sclerosis. Int Orthop 2006;30:128-34.
14. Giannoudis PV, Tzioupis C. Clinical applications of BMP-7: The UK perspective. Injury 2005;36 Suppl 3:S47-50.
15. Chen X, Schmidt AH, Tsukayama DT, Bourgeault CA, Lew WD. Recombinant human osteogenic protein-1 induces bone formation in a chronically infected, internally stabilized segmental defect in the rat femur. J Bone Joint Surg Am 2006;88:1510-23.
16. Kidder LS, Chen X, Schmidt AH, Lew WD. Osteogenic protein-1 overcomes inhibition of fracture healing in the diabetic rat: A pilot study. Clin Orthop Relat Res 2009;467:3249-56.
17. Giannoudis PV, Psarakis S, Kanakaris NK, Pape HC. Biological enhancement of bone healing with Bone Morphogenetic Protein-7 at the clinical setting of pelvic girdle nonunions. Injury 2007;38 Suppl 4:S43-8.
18. White AP, Vaccaro AR, Hall JA, Whang PG, Friel BC, McKee MD. Clinical applications of BMP-7/OP-1 in fractures, nonunions and spinal fusion. Int Orthop 2007;31:735-41.
19. Ristiniemi J, Flinkkila T, Hyvonen P, Lakovaara M, Pakarinen H, Jalovaara P. RhBMP-7 accelerates the healing in distal tibial fractures treated by external fixation. J Bone Joint Surg Br 2007;89:265-72.
20. Friedlaender GE, Perry CR, Cole JD, Cook SD, Cierny G, Muschler GF, *et al*. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. J Bone Joint Surg Am 2001;83-A Suppl 1:S151-8.
21. Lu J, Bhargav D, Wei AQ, Diwan A. Posterolateral intertransverse spinal fusion possible in osteoporotic rats with BMP-7 in a higher dose delivered on a composite carrier. Spine (Phila Pa 1976) 2008;33:242-9.
22. Pountos I, Georgouli T, Henshaw K, Bird H, Jones E, Giannoudis PV. The effect of bone morphogenetic protein-2, bone morphogenetic protein-7, parathyroid hormone and platelet-derived growth factor on the proliferation and osteogenic differentiation of mesenchymal stem cells derived from osteoporotic bone. J Orthop Trauma 2010;24:552-6.
23. Wei A, Leong A, Williams L, Chung S, Shen B, Bhargav D, *et al*. BMP-7 in combination with estrogen enhances bone formation in a fracture callus explant culture. Tohoku J Exp Med 2010;221:61-8.
24. Rose T, Peng H, Shen HC, Usas A, Kuroda R, Lill H, *et al*. The role of cell type in bone healing mediated by *ex vivo* gene therapy. Langenbecks Arch Surg 2003;388:347-55.
25. Snider RK, Krumwiede NK, Snider LJ, Jurist JM, Lew RA, Katz JN. Factors affecting lumbar spinal fusion. J Spinal Disord 1999;12:107-14.
26. Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop Relat Res 1998:26-37.
27. Geesink RG, Hoefnagels NH, Bulstra SK. Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. J Bone Joint Surg Br 1999;81:710-8.
28. Shen B, Wei A, Whittaker S, Williams LA, Tao H, Ma DD, *et al*. The role of BMP-7 in chondrogenic and osteogenic differentiation of human bone marrow multipotent mesenchymal stromal cells *in vitro*. J Cell Biochem 2010;109:406-16.
29. Nauth A, Ristiniemi J, McKee MD, Schemitsch EH. Bone morphogenetic proteins in open fractures: Past, present and future. Injury 2009;40 Suppl 3:S27-31.
30. Kanakaris NK, Lasanianos N, Calori GM, Verdonk R, Blokhuis TJ, Cherubino P, *et al*. Application of bone morphogenetic proteins to femoral nonunions: A 4-year multicentre experience. Injury 2009;40 Suppl 3:S54-61.

31. Khosla S, Westendorf JJ, Oursler MJ. Building bone to reverse osteoporosis and repair fractures. J Clin Invest 2008;118:421-8.
32. Blokhuis TJ, Buma P, Verdonschot N, Gotthardt M, Hendriks T. BMP-7 stimulates early diaphyseal fracture healing in estrogen deficient rats. J Orthop Res 2012;30:720-5.
33. Wei A, Brisby H, Chung SA, Diwan AD. Bone morphogenetic protein-7 protects human intervertebral disc cells *in vitro* from apoptosis. Spine J 2008;8:466-74.
34. Lelovas PP, Xanthos TT, Thoma SE, Lyritis GP, Dontas IA. The laboratory rat as an animal model for osteoporosis research. Comp Med 2008;58:424-30.
35. Ruenitz PC, Shen Y, Li M, Liang H, Whitehead RD Jr, Pun S, *et al*. Specific bone-protective effects of metabolites/derivatives of tamoxifen and clomiphene in ovariectomized rats. Bone 1998;23:537-42.
36. Bagi CM, Mecham M, Weiss J, Miller SC. Comparative morphometric changes in rat cortical bone following ovariectomy and/or immobilization. Bone 1993;14:877-83.
37. Egermann M, Goldhahn J, Schneider E. Animal models for fracture treatment in osteoporosis. Osteoporos Int 2005;16 Suppl 2:S129-38.
38. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density and quality: Potential implications for *in vivo* bone research. Endocrinology 1998;139:663-70.

How to cite this article: Diwan AD, Leong A, Appleyard R, Bhargav D, Fang ZM, Wei A. Bone morphogenetic protein-7 accelerates fracture healing in osteoporotic rats. Indian J Orthop 2013;47:540-6.

Source of Support: The authors acknowledge that a research fellowship supported by Stryker Australia was provided to AL. OP-1 test material was provided gratis by Stryker Biotech (MA)—now Olympus Biotech. Spine Service and the University of New South Wales provided funds for the animal studies and the South Eastern Illawara Health Service, NSW, provided the remainder of the funding for this work, **Conflict of Interest:** None.