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의 학 박 사 학 위 논 문

골다공증 척추의 척추 유합 강화를 위한 전략들

Strategies for enhancing spine fusion in osteoporotic spine

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Sung Bae Park

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Abstract

Strategies for enhancing spine fusion in osteoporotic spine

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Osteoporosis is a degenerative disease with worldwide occurrence. Recently, surgical indications for elderly patients with osteoporosis have been increasing. De novo bone formation and remodeling takes place on the fusion bed in spine. Osteoporosis induces negative bone remodeling that can delay bone fusion. It is thus essential for spine surgeons who treat patients with osteoporosis to understand the differences between the osteoporotic and non-osteoporotic spinal fusion. However, only few strategies are available for osteoporotic patients who need spinal fusion. We described 3 fold strategies to increase vertebral fusion of osteoporotic spine including antiosteoporotic therapies, bone substitutes on the fusion bed and, augmented implants based on our published or unpublished research results and review articles. Of the common antiosteoporotic drugs, bisphosphonates (BPs) did not decrease the fusion rate. However, BPs inhibit the maturation of fusion mass. Selective estrogen receptor modulator (SERM) can accelerate

bone remodeling in an osteoporotic rat spine fusion model; and furthermore, bone fusion and formation can be enhanced by SERM treatment. Parathyroid hormone, an anabolic agent, may offer an advantage over agents such as BPs and SERM. The osteoinductive recombinant human bone morphogenetic protein (rhBMP) 2 enhances spinal fusion in ovariectomized rats during early bone formation. The rhBMP-2 might potentially improve the outcome of spinal fusion in the osteoporotic patient. Instrumentation and techniques with increased pullout strength may increase fusion rate through rigid fixation. Perioperative strategies in osteoporotic patients may affect the radiological and clinical outcomes. Therefore, surgeons should consider appropriate osteoporosis medication, instrumentation and technique before osteoporotic spine surgery.

Keywords: Osteoporosis, rat, antiosteoprotic therapy, fusion, remodeling
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Introduction

Spine fusion surgery including lumbar fusion is a common procedure for the treatment of degenerative spine disease, as degenerative change and instability in the spine segment could lead to pain (1). Instrumented posterolateral fusion and posterior interbody fusion have a long history of safety and effectiveness (2). Degenerative changes in the facet joint and intervertebral disc can induce spinal instability and increase at over 50 years of age. In addition, with the increased life expectancy and improved quality of life among the elderly population, the elderly desire to remain physically active. Therefore, indications of spine fusion in elderly patients have increased (3-5). The surgical outcome according to bone fusion and perioperative complication of spinal fusion surgery in elderly patients may be negatively affected by co-morbidities, such as diabetes mellitus, nutritional disorders, cardiopulmonary disease, renal disease, and bone metabolic disease including osteoporosis (6, 7). A successful bone fusion is achieved through balanced bone remodeling. The main role of screw fixation in spine fusion surgery is to provide immediate stability to the operated vertebrae until bridging bone is formed on the intervertebral fusion bed. Delay or failure in the formation of the bridging bone increases the risks of instrument failure and fusion failure. Osteoporosis induces negative bone remodeling and increases bone fragility, which can cause implant fixation failure and delayed bone graft healing (8, 9). Therefore, spinal surgeons must be informed of the pharmacological treatment plan for osteoporosis in the view of positive bone formation for bone fusion, and formulate appropriate surgical strategies for osteoporotic patients who need to undergo spinal fusion surgery. The objective of this article was to

recommend strategies for enhancing spine fusion in osteoporotic spine based on our published and unpublished research results and review articles.

Materials and Methods

The author studied the difference of spine fusion between osteoporosis and non-osteoporosis rats, the effects of anti-osteoporotic drugs including bisphosphonates (BPs), selective estrogen receptor modulator (SERM) and parathyroid hormone (PTH) on osteoporotic spine fusion, and the effect of osteoinductive material such as human recombinant bone morphogenetic protein (rhBMP) on osteoporotic spine using rats. Published animal studies were provided as Supplement 1 and 2. Non-published data were presented in Tables and Figures. The results obtained from the animal studies formed the experimental basis for the proposed strategies to enhance osteoporotic spine fusion. A PubMed literature search was conducted on publications between 1990 and 2014. The search strategy emphasized estrogen deficiency osteoporosis, osteoporosis medication in spine fusion, bone substitute for spine fusion, and augmented implant in spine fusion. All articles with animal and human studies were reviewed through the PubMed search. We considered our results and the data from the reviewed articles to reveal strategies for enhancing osteoporotic spine fusion. The methodologies and results of author's animal studies were described into Methods and Results, respectively.

Osteopenic Female Rat as a Model for Human Osteoporosis: Bone Mineral Density Changes and Stepwise Description of Surgical Technique

Twenty five 10-week-old female Sprague Dawley rats (Orientbio, Gyonggi, Korea) were used. Five rats were euthanized after two weeks, and BMD was measured in their femora. The other 20 rats were assigned to one of two groups: a sham group (n = 10), which underwent a sham operation and an OVX group (n = 10), which underwent bilateral OVX at 12 weeks of age (Fig 1). After six weeks, five rats from each group were euthanized, and BMD was measured in their femora. The same procedure were performed in the remaining rats form each group eight weeks later.

Spinal fusion process in living osteoporotic rats compared with non-osteoporotic rats: serial micro-computed tomography study.

Female Sprague Dawley rats (n = 12; 12 weeks of age; Orientbio, Gyonggi, Korea) were given either an ovariectomy (OVX) or sham operation and were randomized into two groups: non-OVX group (n = 6, sham operated + fusion) and OVX group (n = 6, OVX + fusion). Eight weeks after OVX, unilateral lumbar spinal fusion was performed using autologous iliac bone. Bone density (BD) was measured 2 days after fusion surgery. Microcomputed tomography was used to evaluate the process of bone fusion every week for 10 weeks after fusion surgery. The fusion rate, fusion process, and compactness of fusion mass were assessed between the two groups.

The time-dependent effect of ibandronate on bone graft remodeling in an ovariectomized rat spinal arthrodesis model.

Female Sprague Dawley rats (n=100; 12 weeks of age; Orientbio, Gyonggi, Korea) were ovariectomized (OVX, n=80) or non-OVX operated (n=20)and randomized into five

groups: non-OVX group, osteoporosis group, osteoporosis with early BP group, osteoporosis with simultaneous BP group, and osteoporosis with late BP group. Eight weeks after ovariectomy, lumbar spinal arthrodesis was performed using autologous tailbones. Animals were sacrificed 4 and 8 weeks after arthrodesis, and bone formation was assessed by measuring bone mineral density and mRNA expression, manual palpation, radiological evaluation, and histomorphometry.

Effect of a selective estrogen receptor modulator on bone formation in osteoporotic spine fusion using an ovariectomized rat model.

Female Sprague Dawley rats (n = 90; 12 weeks of age; Orientbio, Gyonggi, Korea) were OVX or sham operated, and randomized into three groups: Control (sham-operated + fusion procedure+ saline administration), OVX (OVX + fusion procedure + saline administration), and SERM (OVX + fusion procedure + administration of SERM). Eight weeks after OVX, a bilateral lumbar spinal fusion procedure was performed using autologous iliac bone. In each group, gene expression was evaluated at 2, 4, and 8 weeks after the fusion procedure, histological analysis was performed at 4 and 8 weeks after the procedure, and bone parameters were measured by microcomputed tomography at 2 days, 4 weeks, and 8 weeks after the procedure.

BMP-2 induced early bone formation in spine fusion using rat ovariectomy osteoporosis model

Female Sprague Dawley rats (n = 60; 12 weeks of age; Orientbio, Gyonggi, Korea) were ovariectomized or sham-operated, and randomized into three groups: Sham

(sham-operated + fusion), OVX (OVX + fusion) and BMP (OVX + fusion + BMP-2). Six weeks after ovariectomy, unilateral lumbar spine fusion was performed using autologous iliac bone with/without recombinant human bone morphogenetic protein (rhBMP)-2 delivered on a collagen matrix. For each group, gene expression and histology were evaluated at 3 and 6 weeks after fusion, and bone parameters were measured by micro-computed tomography at 3, 6, 9 and 12 weeks.

Results

Biology of osteoporotic spinal fusion

Although instrumentation and techniques for spinal fusion surgery have improved in recent years, a nonunion rate of 10–40% limits the success and may negatively affect clinical outcomes (3, 10, 11). De novo bone formation and remodeling occurs on the fusion bed in spine. Osteoporosis induces negative bone remodeling that can delay bone fusion. Therefore, the understanding of the differences in the bone fusion process between the osteoporotic and non-osteoporotic spinal fusion is essential for spine surgeons who treat patients with osteoporosis. Clinically, relevant lumbar fusion animal models provide important biological, histological, and radiological information about the fusion bed between the intertransverse processes (3, 12). Endochondral bone formation through a cartilage intermediate occurs centrally at the fusion bed between the upper and lower halves of the bridging bone, and intramembranous bone formation occurs near the decorticated transverse processes (2, 13). Also, fracture repair and spinal fusion have similar stages at the fracture site and fusion bed i.e., an inflammatory response, fibrocartilage formation, hard callus formation, and bone remodeling (14). In an animal

study, the fusion rate at 10 weeks after spinal fusion did not increase if the fusion had not occurred by 4 weeks after surgery (15). Therefore, to overcome fusion failure in patients with osteoporosis, it is important to understand whether there are differences in bone remodeling in the sites of endochondral and intramembranous ossifications and the early period of bone fusion between patients with and without osteoporosis. Few articles presented early period fracture healing under osteoporotic conditions in animal models. These studies showed that estrogen deficiency osteoporosis affects the fibrosis formation and the quantity and quality of callus during the early fracture healing process (16, 17). The difference between osteoporotic and non-osteoporotic spine fusion has not been reported. The author made the osteoporotic rat model using bilateral ovariectomy and showed that the significant decrease BMD appeared six weeks after bilateral ovariectomy. Also, the author investigated the difference of fusion process between the normal and osteoporotic spine in an ovariectomized rat model. In the non-OVX group, absorption of the grafted bone after surgery started 2 to 3 weeks after surgery, but the OVX group showed less bone absorption at the same time. Bone remodeling at the fusion bed in the late stage was less prominent in the OVX group, as compared with the non-OVX group (Fig. 1). In the non-OVX group, the fusion rate at 4, 8, and 10 weeks was 66.6% (4/6), 83.3% (5/6), and 83.3% (5/6), respectively. In contrast, the fusion rate in the OVX group was 50% (3/6) at 4, 8, and 10 weeks. Although the fusion rate was higher in the non-OVX group at all 3 time points, the difference was not significant. The presence of fusion masses connected to TPs and bridging bone between TPs at 4, 8, and 10 weeks also did not differ significantly between the 2 groups. However, compactness of grafted bones at 10 weeks was inferior in the OVX group, as compared with the non-OVX group

(Table 1 and Fig. 2). According to our study results, osteoporosis can affect bone remodeling at early and late stages. Strategies to induce balanced bone remodeling for mature fusion mass are needed to increase the fusion rate in the osteoporotic spine.

Strategies for enhancement of spine fusion in osteoporotic spine

Strategies to increase vertebral fusion in osteoporotic spine can be classified according to the target of action as antiosteoporotic therapies, bone substitutes on the fusion bed, and augmented implants.

The most common antiosteoporotic drugs in patients who underwent spine fusion are BPs, SERM, and PTH. The presence of bridged fusion mass, maturation degree within fusion mass and the osteointegration of implants with adjacent bone are evaluated for the effects of these drugs on osteoporotic spine fusion. BPs are the 1st line drugs that can increased bone mineral density (BMD) by reducing bone resorption through increasing apoptosis of the osteoclasts (16, 17). Therefore, many reports indicated that BPs increase BMD and improved implant integration in osteoporotic conditions (18, 19). However, osteoclast and osteoblast are members of basic multi-cellular unit (BMU) and osteoclast-osteoblast communications occurs during bone remodeling (20). Therefore, it remains controversial whether antagonistic effects of BPs in osteoclasts are beneficial for spinal fusion in osteoporotic patients. Most studies on the effect of BPs on osteoporotic spine fusion were conducted on rodent or rabbit models (21). There was one reported human study on the effect of BPs on osteoporotic spine fusion (22). In many animal studies, the fusion rate in the osteoporotic animal group using manual palpation and radiologic evaluation was not significantly different, as compared to the control group

(21, 23). In addition, the effect of BPs on osteointegration to screw and cage were positive (19, 24). However, the studies with histologically defined fusion indicated that BPs decreased fusion rate and delayed maturation of fusion mass (21, 25). One human study on the effect of BPs on osteoporotic spine fusion showed that BPs significantly increased fusion rate, although BPs suppressed bone formation after 6 months postoperatively (22). These findings indicate that BPs cannot inhibit the formation of bridging bone and osteointegration to implant via intramembranous ossification, but can delay the maturation and remodeling of grafted bone mass via endochondral ossification and balanced activity of osteoblasts and osteoclasts. We have previously investigated the effect of BPs administered at different times on the bone response to osteoporotic rat spine arthrodesis. Although there was no significant difference of fusion rate between groups, BPs increased bone volume and positively affected endochondral and intramembranous ossification. Therefore, early administration of BPs may not hinder the bone fusion of osteoporotic patients undergoing spinal arthrodesis (Fig. 3) (Sup. 1). SERMs are another first-line therapy for osteoporosis (26). SERM treatment prevents postmenopausal bone loss and decreases the incidence of vertebral fracture (27, 28). However, although several studies have reported a positive effect of SERM treatment on fracture healing, only 1 study looked at the effect of SERMs on spine fusion, concluding that SERM treatment was not effective on bone remodeling in an osteoporotic rat model (29, 30). Information on basic and clinical effects of SERMs on osteoporotic spine fusion is required, given the controversy concerning the effect of bisphosphonates on spine fusion. We studied the effect of SERMs on osteoporotic spine fusion using rats (unpublished data). Eight weeks after OVX, bilateral lumbar spine fusion was performed

using autologous iliac bone. Gene expression, histological analysis, and bone parameters were evaluated after fusion surgery in each group. In the SERM group, bone mineral density and trabecular quality of the vertebral body were significantly superior to those in the OVX group 16 weeks after OVX (table 2). At 8 weeks after fusion, the fusion rate and bone volume ratio in the fusion bed of the SERM group were higher than those of the OVX group (Table 3). Real-time reverse transcription polymerase chain reaction at 4 and 8 weeks after fusion showed increased expression of osteoblast-related markers (alkaline phosphatase, osteocalcin, Runx2, and transforming growth factor) in the SERM group, as compared with the OVX group (Fig. 4). The OVX group showed sparse bone mass between transverse processes. However, the SERM group had compact bridging fusion mass and matured trabecular within the fusion bed at 8 weeks after fusion (Fig 5 and 6). To the best of our knowledge, this was the first study to show that SERM treatment can accelerate bone remodeling in an osteoporotic rat spine fusion model, and that bone fusion and formation can be enhanced by SERM treatment. Studies using animal models have shown that PTH improves fusion rates at an early period (31, 32). The anabolic effect of PTH enhances the formation of a histologically mature fusion mass with a greater proportion of mineralization (31, 33). Although there is no clinical trial on the effect of PTH on osteoporotic spine fusion in humans, an osteoporotic spine fusion based study in humans showed that PTH can induce early spine fusion and improve remodeling of grafted bone.

The 3 critical biological processes involved in bone regeneration include osteogenic potential, osteoinductive factors, and osteoconductive scaffold (34). Osteogenic potential is the capability of cells that differentiate to osteoblasts, to form new bone.

Osteoinductive factors are able to cause osteoblast differentiation from osteoprogenitor stem cells such as mesenchymal stem cells. In addition, osteoconductive scaffolds facilitate neovascularization and supports the ingrowth of bone. The ideal bone substitutes possess the 3 critical elements with optimal biological reaction and no harmful effect on human body. The common categories of bone substitutes that increase bone formation on fusion bed, include demineralized bone matrix, biologic graft extenders, osteoinductive factors, and mesenchymal stem cells. Many studies investigated the effect of bone substitutes, including ceramics, demineralized bone matrix, osteoinductive growth factor, autologous platelet concentrate, and mesenchymal stem cells on spine fusion (35-39). Hence, these bone substitutes can promote the fusion rate or bone formation. However, there was no earlier study on the effect of these materials on osteoporotic spine fusion. We accordingly studied the effect of recombinant rhBMP 2 on spine fusion in an ovariectomized (OVX) rat model. Although the Sham and OVX groups showed both sparse and compacted bone between transverse processes at 6 weeks, the BMP group had a significantly larger bone mass within the fusion bed at 3 weeks and later. All rats in the BMP group had bridging bone at 3 weeks; at 12 weeks, bridging bone in the Sham and OVX groups was about 50% and 25%, respectively, as compared to the BMP group. Histologic evaluation showed that the BMP group had high amounts of bridging bone and endochondral ossification with cartilage tissue at $\geq$ 3 weeks after surgery. In the BMP group, all gene expression in grafted tissues increased significantly at 3 weeks after surgery, as compared to other groups (Supp. 2). We concluded that rhBMP-2 enhances spinal fusion in OVX rats and acts during early bone formation (Fig. 7). Therapeutic BMP-2 may therefore improve the outcome of spinal fusion in the

osteoporotic patient.

Instrumentation using screws and rod provides the support the spinal loading and movement after spinal arthrodesis. Instrument failure at an early stage after spinal arthrodesis can induce fusion failure. Cancellous bone is more affected by osteoporosis than cortical bone, therefore lower BMD has been a major factor in poor screw fixation, screw loosening and fixation failure (40). Therefore, in osteoporotic spine fusion, it is important to find the way to increase pull-out strength of the screws. Several surgical techniques have been employed to enhance the pullout strength of the pedicle screw (41). The augmentation of screw fixation using polymethylmethacrylate and various calcium ceramics increased biomechanical support in osteoporosis spine surgery (42). The preparation for screw hole or minimization of tapping hole can affect the pullout strength in osteoporotic bone and, although the anatomical constraints vary with patients, bigger and longer screws may provide a good solution for fragile bones (43). In addition, the angulation of 2 screws and screw positioning in areas of higher BMD in the vertebrate may increase pullout strength (44). These techniques may enhance osteoporotic spine fusion through stabilization of fusion segments.

Discussion

Osteoporosis is a degenerative disease present that currently affects over 10 million people worldwide (45). In the Fourth Korea National Health and Nutrition Examination Survey 2008–2009, 39.1% of women aged 50 years or older in Korea were reported to have osteoporosis (46). In addition, the prevalence of osteoporosis in the lumbar spine in women between 50 and 59, 60 and 69, and over 70 years old are 15.4%, 44.5%, 60.9%,

respectively (46). Furthermore, the prevalence of osteoporosis in male and female patients over 50 years old who underwent spinal surgery were 14.5% and 51.3%, respectively (4). Although the skeletal system seems to be a static organ, the bone is a dynamic tissue microscopically. In other words, microcracks in bone occur continuously and repair of microcracks and bone remodeling develops in cancellous and cortical bone (47, 48). Basic mechanism of bone remodeling is performed by BMU, which comprises osteoclasts, osteoblasts, bone lining cells and osteocytes within the bone remodeling cavity (49). The dynamic nature of bone is achieved by bone remodeling harmonic relationship of BMU. Positive remodeling occurs in the growing skeleton, and negative remodeling causes reduced BMD and osteoporosis. Among several causes of osteoporosis, menopause is the most common etiology. The estrogen deficiency causes shorter osteoblast and osteocytes lifespan, and prolongation of osteoclast lifespan (50, 51). The longer lifespan of osteoclasts is responsible for deeper absorption of microcracks and the increased osteocyte apoptosis may impair the osteocytes-canalicular mechanoreceptor for skeletal signals in the detection of microdamage and repair (50). Therefore, in postmenopausal osteoporosis, the rate of osteoblast-mediated bone formation is lower than the rate of osteoclast-related bone resorption and negative remodeling of bone in postmenopausal osteoporosis can cause skeletal fragility and delayed bone fusion in the graft site (3, 52). To overcome the negative bone remodeling in osteoporotic spine, appropriate anti-osteoporotic medication and osteogenic local administration in fusion bed should be considered in osteoporotic patients who undergo spine fusion surgery. For systematic treatment of osteoporotic patients with spine fusion, the understanding and appropriate prescription of anti-osteoporotic drugs is important.

Data from other articles and our own research studies indicate that anabolic medication such as PTH may offer an advantage over antiresorptive agents such as BPs and SERM. In addition, although BPs do not significantly decrease the fusion rate, the degree of maturation and remodeling of grafted bone mass in response to SERM may be superior to BPs. However, before the selection of anti-osteoporotic medication, personalized therapy should be considered according to the cost and the complication of drugs. The human is a bipedal animal. However, axial loading was not considered in all animal studies using osteoporotic spine fusion. Hence, the animal model cannot fully reproduce the clinical filed. In the present article, there were few reports on local administration into fusion bed, as compared to the effect of anti-osteoporotic drug on spine fusion. Because early bone formation is important to decrease fusion failure in the osteoporotic spine, the local administration into the fusion bed will be considered in the future study.

Conclusion

Osteoporosis, which results in fragile bone and negative bone remodeling, is a risk factor of fusion failure in spine surgery. Therefore, prior to performing osteoporotic spinal fusion, surgeons should consider multidisciplinary strategies, including the use of the effective antiresorptive agents and anabolic agent as PTH, proper instrumentation and bone substitutes. Perioperative strategies in osteoporotic patients may affect the radiological and clinical outcomes.

References

1. Helenius I, Lamberg T, Osterman K, Schlenzka D, Yrjonen T,Tervahartiala P, et al: Posterolateral, anterior, or circumferential fusion in situ for high-grade spondylolisthesis in young patients: a long-term evaluation using the Scoliosis Research Society questionnaire. Spine (Phila Pa 1976). 2006; 31:190-196.

2. Totoribe K, Tajima N, Chosa E: A biomechanical study ofposterolateral lumbar fusion using a three-dimensional nonlinear finite element method. J Orthop Sci. 1999; 4:115-126.

3. Park SB, Chung CK. Strategies of spinal fusion on osteoporotic spine. *J Korean Neurosurg Soc* 2011;49: 317-22.

4. Chin DK, Park JY, Yoon YS, Kuh SU, Jin BH, Kim KS, et al. Prevalence of osteoporosis in patients requiring spine surgery: incidence and significance of osteoporosis in spine disease. *Osteoporos Int* 2007;18: 1219-24.

5. Fraizer DD, Lipson SJ, Fossel AH, Katz JN : Associations between spinal deformity and outcomes after decompression for spinal stenosis. Spine (Phila Pa 1976). 1997; 22: 2025-2029.

6. Carreon LY, Puno RM, Dimar JR 2nd, Glassman SD, Johnson JR : Perioperative complications of posterior lumbar decompression and arthrodesis in older adults. J Bone Joint Surg Am. 2003; 85 : 2089-2092.

7. Lee JH, Chun HJ, Yi HJ, Bak KH, Ko Y, Lee YK : Perioperative risk factors related to lumbar spine fusion surgery in korean geriatric patients. J Korean Neurosurg Soc. 2012 ;51(6):350-8

8. 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 2008;33:242–9.

9. Kanakaris NK, Petsatodis G, Tagil M, Giannoudis PV. Is there a role for bone morphogenetic proteins in osteoporotic fractures? Injury 2009;40(3Suppl):S21–6.

10. Boden SD. Biology of lumbar spine fusion and use of bone graft substitutes: present, future, and next generation. Tissue engineering 2000;6:383-399.

11. Bridwell KH, Sedgewick TA, O'Brien MF, Lenke LG, Baldus C The role of fusion and instrumentation in the treatment of degenerative spondylolisthesis with spinal stenosis. Journal of spinal disorders 1993;6:461-472.

12. Bae HW, Zhao L, Kanim LE, Wong P, Marshall D, Delamarter RB. Bone marrow enhances the performance of rhBMP-2 in spinal fusion: a rodent model. The Journal of bone and joint surgery American volume 2013;95:338-347. doi: 10.2106/JBJS.K.01118.

13. Zipfel GJ, Guiot BH, Fessler RG. Bone grafting. Neurosurgical focus 2003;14:e8.

14. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: The cellular picture. Seminars in cell & developmental biology. 2008;19:459-466

15. Boden SD, Schimandle JH, Hutton WC. An experimental lumbar intertransverse process spinal fusion model. Radiographic, histologic, and biomechanical healing characteristics. Spine 1995;20:412-420.

16. Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. J Clin Invest 1996;97:2692–6.

17. Frith JC, Rogers MJ. Antagonistic effects of different classes of bisphosphonates in

osteoclasts and macrophages in vitro. J Bone Miner Res 2003;18:204–12.

18. Kurth AH, Eberhardt C, Muller S, Steinacker M, Schwarz M, Bauss F. The bisphosphonate ibandronate improves implant integration in osteopenic ovariectomized rats. Bone 2005;37:204–10.

19. Xue Q, Li H, Zou X, Dalstra M, Lind M, Christensen FB, et al. Alendronate treatment improves bone-pedicle screw interface fixation in posterior lateral spine fusion: an experimental study in a porcine model. Int Orthop 2010;34:447–51.

20. Matsuo K, Irie N. Osteoclast-osteoblast communication. Arch Biochem Biophys. 2008 May 15;473(2):201-9.

21. Hirsch BP, Unnanuntana A, Cunningham ME, Lane JM. The effect of therapies for osteoporosis on spine fusion: a systematic review. Spine J. 2013 Feb;13(2):190-9.

22. Nagahama K, Kanayama M, Togawa D, Hashimoto T, Minami A. Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. J Neurosurg Spine 2011;14:500–7.

23. Gezici AR, Ergun R, Gurel K, Yilmaz F, Okay O, Bozdoğan O. The effect of risedronate on posterior lateral spinal fusion in a rat model. J Korean Neurosurg Soc 2009;46:45–51.

24. Zou X, Xue Q, Li H, Bünger M, Lind M, Bünge C. Effect of alendronate on bone ingrowth into porous tantalum and carbon fiber interbody devices: an experimental study on spinal fusion in pigs. Acta Orthop Scand 2003;74:596–603.

25. Lehman RA Jr, Kuklo TR, Freedman BA, Cowart JR, Mense MG, Riew KD. The effect of alendronate sodium on spinal fusion: a rabbit model. Spine J 2004;4:36–43.

26. Schuiling KD, Robinia K, Nye R. Osteoporosis update. Journal of midwifery &

women's health. 2011;56(6):615-27.

27. Riggs BL, Hartmann LC. Selective estrogen-receptor modulators -- mechanisms of action and application to clinical practice. The New England journal of medicine. 2003;348(7):618-29.

28. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. Jama. 1999;282(7):637-45.

29. Takahata M, Ito M, Abe Y, Abumi K, Minami A. The effect of anti-resorptive therapies on bone graft healing in an ovariectomized rat spinal arthrodesis model. Bone. 2008;43(6):1057-66.

30. Spiro AS, Khadem S, Jeschke A, Marshall RP, Pogoda P, Ignatius A, et al. The SERMS raloxifene improves diaphyseal fracture healing in mice. Journal of bone and mineral metabolism. 2013;31(6):629-36.

31. O'Loughlin PF, Cunningham ME, Bukata SV, Tomin E, Poynton AR, Doty SB, et al. Parathyroid hormone (1-34) augments spinal fusion, fusion mass volume, and fusion mass quality in a rabbit spinal fusion model. Spine 2009;34: 121–30.

32. Lehman RA Jr, Dmitriev AE, Cardoso MJ, Helgeson MD, Christensen CL, Raymond JW, et al. Effect of teriparatide [rhPTH(1,34)] and calcitonin on intertransverse process fusion in a rabbit model. Spine 2010;35:146–52.

33. Abe Y, Takahata M, Ito M, Irie K, Abumi K, Minami A. Enhancement of graft bone healing by intermittent administration of human parathyroid hormone (1-34) in a rat spinal arthrodesis model. Bone 2007;41:775–85.

34. Miyazaki M1, Tsumura H, Wang JC, Alanay A. An update on bone substitutes for spinal fusion. Eur Spine J. 2009;18(6):783-99

35. An HS, Simpson JM, Glover JM, Stephany J. Comparison between allograft plus demineralized bone matrix versus autograft in anterior cervical fusion. A prospective multicenter study. Spine. 1995;20:2211–2216.

36. Boden SD, Zdeblick TA, Sandhu HS, Heim SE. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine. 2000;25:376–381.

37. Kanayama M, Hashimoto T, Shigenobu K, Yamane S, Bauer TW, Togawa D. A prospective randomized study of posterolateral lumbar fusion using osteogenic protein-1 (OP-1) versus local autograft with ceramic bone substitute: emphasis of surgical exploration and histologic assessment. Spine. 2006;31:1067–1074.

38. Ransford AO, Morley T, Edgar MA, Webb P, Passuti N, Chopin D, et al. Synthetic porous ceramic compared with autograft in scoliosis surgery. A prospective, randomized study of 341 patients. J Bone Joint Surg Br. 1998; 80:13–18.

39. Wang T, Dang G, Guo Z, Yang M. Evaluation of autologous bone marrow mesenchymal stem cell-calcium phosphate ceramic composite for lumbar fusion in rhesus monkey interbody fusion model. Tissue Eng. 2005;11:1159–1167.

40. Coe JD, Warden KE, Herzig MA, McAfee PC : Influence of bone mineral density on the fixation of thoracolumbar implants. A comparative study of transpedicular screws, laminar hooks, and spinous process wires. Spine (Phila Pa 1976) 1990;15: 902-907.

41. Lehman RA Jr, Kang DG, Wagner SC. Management of Osteoporosis in Spine Surgery. J Am Acad Orthop Surg. 2015 Apr;23(4):253-263.

42. Elder BD, Lo SF, Holmes C, Goodwin CR, Kosztowski TA, Lina IA, et al. The Biomechanics of Pedicle Screw Augmentation with Cement. Spine J. 2015 Jun 1;15(6):1432-45.

43. Cook SD, Barbera J, Rubi M, Salkeld SL, Whitecloud TS 3rd : Lumbosacral fixation using expandable pedicle screws : an alternative in reoperation and osteoporosis. Spine J 2001;1: 109-114.

44. Reinhold M, Schwieger K, Goldhahn J, Linke B, Knop C, Blauth M : Influence of screw positioning in a new anterior spine fixator on implant loosening in osteoporotic vertebrae. Spine (Phila Pa 1976) 2006;31: 406-413.

45. Hart RA, Prendergast MA : Spine surgery for lumbar degenerative disease in elderly and osteoporotic patients. Instr Course Lect. 2007;56 : 257-272.

46. Kim KH, Lee K, Ko YJ, Kim SJ, Oh SI, Durrance DY, et al. Prevalence, awareness, and treatment of osteoporosis among Korean women: The Fourth Korea National Health and Nutrition Examination Survey. Bone. 2012 May;50(5):1039-47

47. Khosla S, Westendorf JJ, Oursler MJ : Building bone to reverse osteoporosis and repair fracture. J Clin Invest. 2008;118 : 421-428.

48. Manolagas SC : Birth and death of bone cells : basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev. 2000;21 : 115-137.

49. Sims NA, Gooi JH : Bone remodeling : multiple cellular interactions required for coupling of bone formation and resorption. Semin Cell Dev Biol. 2008;19: 444-451.

50. Manolagas SC, Weinstein RS, Bellido T, Bodenner DL : Opposite effects of estrogen on the life span of osteoblasts / osteocytes vs. ostoclasts in vivo and in vitro : an

explanation of the imbalance between formation and resorption in estrogen deficiency. J Bone Miner Res. 1999;14 : S169.

51. Parfitt AM, Mundy GR, Roodman GD, Hughes DE, Boyce BF : A new model for the regulation of bone resorption, with particular reference to the effects of bisphosphonates. J Bone Miner Res. 1996;11 : 150-159.

52. Li X1, Ominsky MS, Warmington KS, Morony S, Gong J, Cao J, et al. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis. J Bone Miner Res. 2009;24(4):578-88

Supplement 1. Park SB, Park SH, Kang YK, Chung CK. The time-dependent effect of ibandronate on bone graft remodeling in an ovariectomized rat spinal arthrodesis model. Spine J. 2014 Aug 1;14(8):1748-57.

Supplement 2. Park SB, Park SH, Kim NH, Chung CK. BMP-2 induced early bone formation in spine fusion using rat ovariectomy osteoporosis model. Spine J. 2013 Oct;13(10):1273-80.

국문 초록

골다공증 척추의 척추 유합 강화를 위한 전략들

서울대학교 대학원

의학과 뇌신경과학 과정

박성배

골다공증은 전세계적인 퇴행성 질환이다. 최근 골다공증이 동반된 노령인구의 수술 적응증이 늘어나고 있다. 새로운 골 형성과 개조가 척추의 유합 장소에서 발생되며, 골다공증은 골 개조에 음성적인 측면을 보여서 척추유합을 지연시킨다. 그래서 골다공증과 비 골다공증 척추 유합에서 골 유합 과정의 차이에 대한 이해는 골다공증 환자를 치료하는 척추외과의사에서 필수적이다. 그러나 골다공증 환자의 척추 유합에 대한 치료 전략에 관한 논문은 소수이다. 저자는 항 골다공증 치료 약제, 골대체제 및 기구 강화의 측면에서 골다공증 척추의 골유합을 강화할 수 있는 방법들에 대해서 저자의 실험 결과들과 기존 논문들의 고찰을 통하여 기술하였다. 흔한 골다공증 약제들 중에서 비스포스포네이트는 골 유합을 방해하지는 않으나, 유합골의 성숙을 방해한다. 선택적 에스트로겐 수용체 조절자는 골다공증 척추 융합 모델을 이용한 실험에서

골 개조를 강화시켰으며, 골 유합 및 골 형성이 이 약제를 통하여 강화될 수 있다. 동화제제인 부갑상선 호르몬은 골 유합 및 골 형성 부분에서 비스포스포네이트와 선택적 에스트로겐 수용체 조절자를 넘어서는 장점을 제공해줄 것이다. 골 유도 물질인 재 조합형 인간 골 형성 단백질 2는 난소제거 쥐의 척추 유합을 강화시키고, 조기 골 형성을 유도한다. 따라서, 골 유도 물질인 재 조합형 인간 골 형성 단백질 2는 골다공증 환자에서 척추 유합의 결과를 향상시킬 수 있다. 뽑힘 강도를 강화시킨 기구들이나 기술들은 견고한 고정을 통하여 유합율을 향상 시킬 수 있다. 골다공증 환자의 수술 전, 후 전략들은 방사선학적으로나 임상적인 결과에 영향을 키 칠 수 있다. 따라서, 골다공증 척추 수술 전에 외과의사는 적절한 골다공증 약제, 골대체제, 기구 및 기술에 대해서 반드시 고려해야 한다.

주요어: 골다공증, 쥐, 항 골다공증 치료, 유합, 개조

학번: 2009-30563

Tables

Table 1. The comparison of fusion rate and compactness

		4 weeks	8 weeks	10 weeks
`Fusion rate	Non-osteoporotic group	66.6% (4/6)	83.3% (5/6)	83.3% (5/6)
	Osteoporotic group	50% (3/6)	50% (3/6)	50% (3/6)
	P value	0.567	0.505	0.505
Compactness	Non-osteoporotic group	16.6% (1/6)	83.3% (5/6)	100% (6/6)
	Osteoporotic group	33.3% (2/6)	50% (3/6)	50% (3/6)
	P value	0.500	0.505	0.046

Table 2. The value of bone mineral density (BMD) density in three groups

After OVX	Control group	OVX group	SERMS group	P value (OVX vs. SERMS)
8 weeks (g/cm^2)	**1.287 ± 0.1812**	**1.241 ± 0.0224***	**1.231 ± 0.1578***	**0.432**
16 weeks (g/cm^2)	**1.298 ± 0.0304**	**1.199 ± 0.0285***	**1.249 ± 0.0332***	**0.003**

***, p** < 0.05 vs Control group

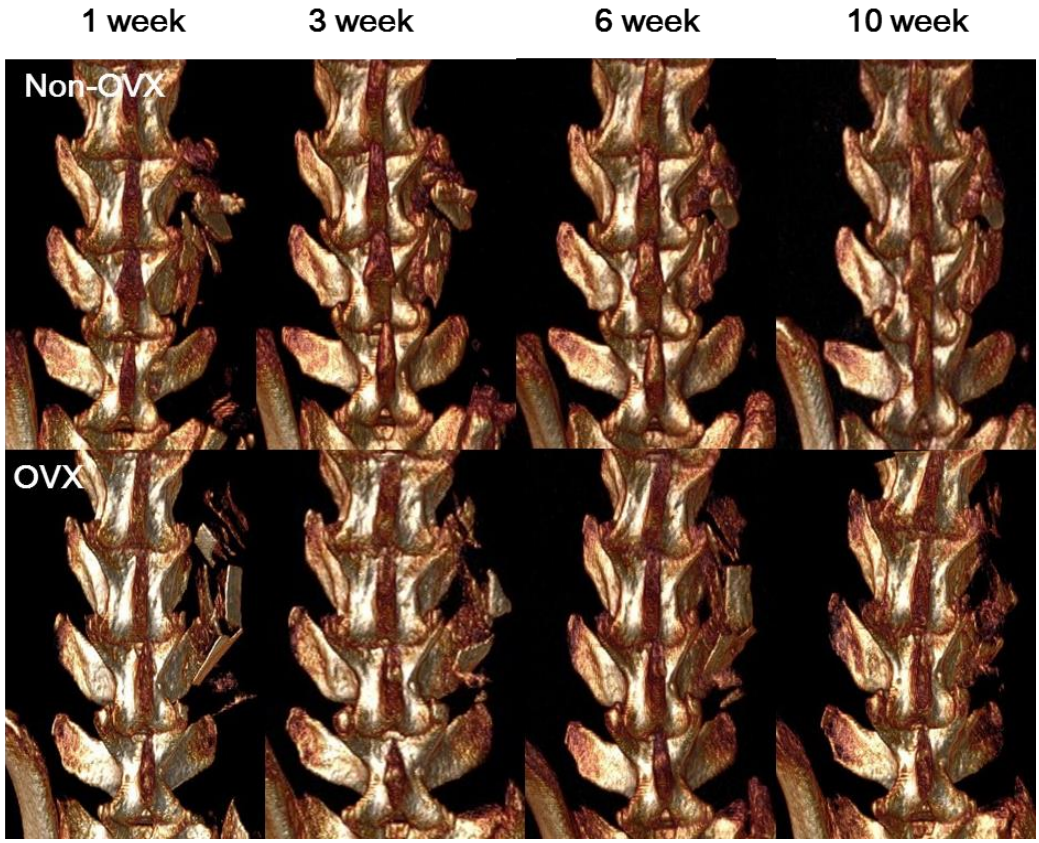
Table 3. The fusion rate and bone volume ratio in three groups

After OVX (16wks)		Manual palpation	Right side	Left side	Bone volume / Total volume %	
					8 wks	16 wks
Control group		13/16 (81.2%)	12/16 (75%)	13/16 (81.2%)	2.00 ± 0.451	2.16 ±0.706
OVX group		6/16 (37.5%)	6/16 (37.5%)	4/16 (25%)	1.57 ± 0.319	1.52 ± 0.395
SERMS group		10/16 (62.5%)	9/16 (56.2%)	10/16 (62.5%)	1.55 ± 0.493	2.23 ± 0.703
P value	Control vs. OVX vs. SERMS	0.040	0.102	0.005	0.003	0.002
	OVX vs. SERMS	0.157	0.288	0.037	1.000	0.002

Figure 1. Procedures of ovariectomy in rat.

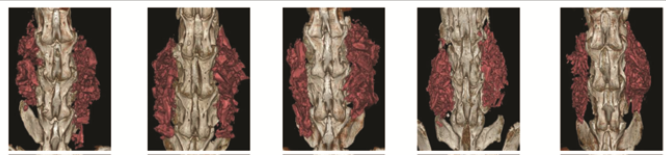
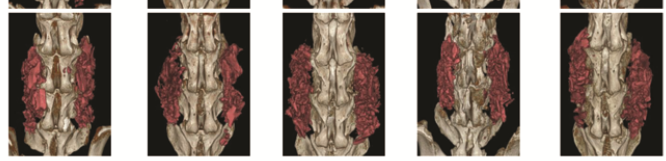
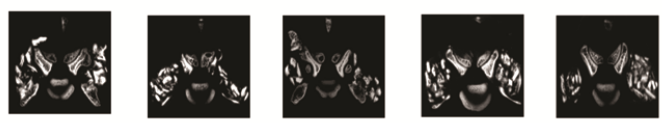
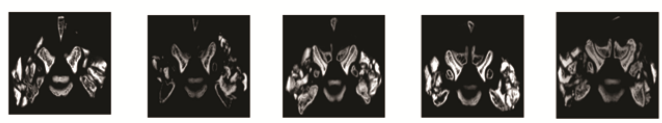
Anesthetized rat is laid prone on operating table. Thick black arrow: shaving site (A). Skin incision point is located just medial portion of the most bulged area (★) or a thumb used to find the incision point (dotted arrow) (B). External oblique muscle is exposed after skin incision. Thick black arrow: External oblique muscle (C). After the muscle dissection, peritoneal space and adipose tissue surrounding ovary are exposed. Dotted arrow : adipose tissue surrounding ovary (D). The surrounding fat must be gently pulled to avoid detachment of small pieces of ovary (E). This shows ovary (thick black arrow) and uterine horn (dotted arrow) surrounded by fat (F). Ligation must undergo at distal uterine horn in order to get rid of total ovary at a time (G). Ovary surrounded by fat is removed totally (thick black arrow) (H).

Figure 2. Microcomputed tomography scanning of fusion masses



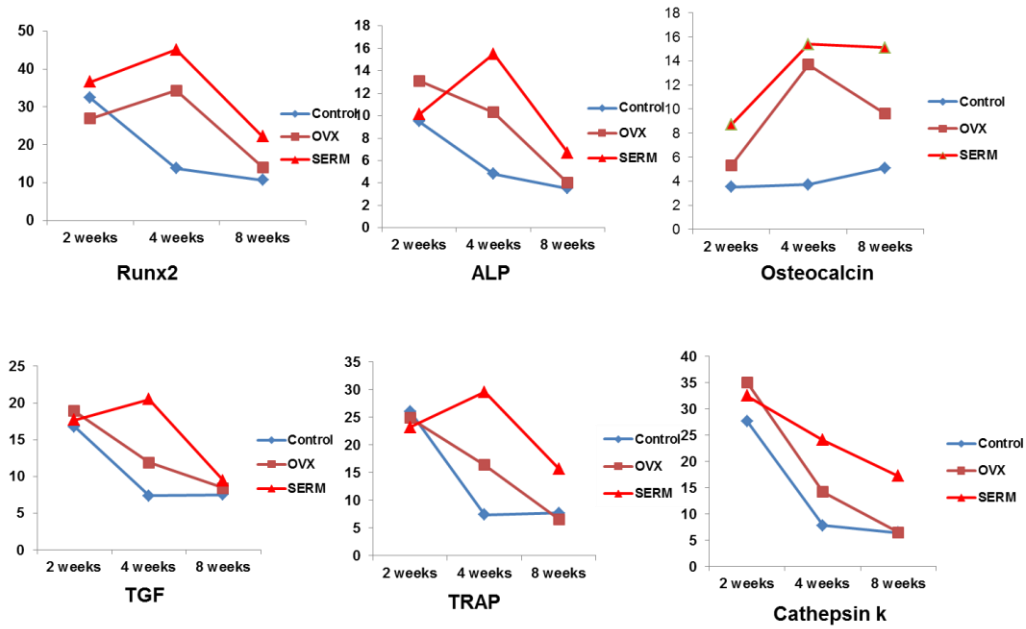
The figure shows three dimensional (3D) reconstructed images from both groups after surgery. The pictures in the upper row show serial 3D-reconstructed images from rat in the non-OVX group. The grafted bone materials were inserted on the fusion bed between the L4 and L5 transverse processes. The shape of the bone fragments 10 weeks after surgery was more compact compared with that in the early period after surgery. The pictures in the lower row are 3D-reconstructed images from rat in the OVX group. The shape of the grafted bone at 10 weeks was less compact in the OVX group than in the non-OVX group. (OVX = ovariectomy).

Figure 3. Microcomputed tomography scanning of fusion masses

parameters	Postoperative time	Sham group	Non-BP group	Simultaneous BP group	Early BP group	Late BP group
Bone volume (mm^3)	4 weeks (n=5)	214 ± 30.8	229± 88.9	256± 89.2	220± 54.2	269± 66.3
	8 weeks (n=5)	199 ± 27.3	186± 68.3	218± 71.8	205± 114.2	197± 80.0
	P value	0.442	0.473	0.524	0.743	0.008
3-dimental views	4 weeks					
	8 weeks					
Axial views	4 weeks					
	8 weeks					

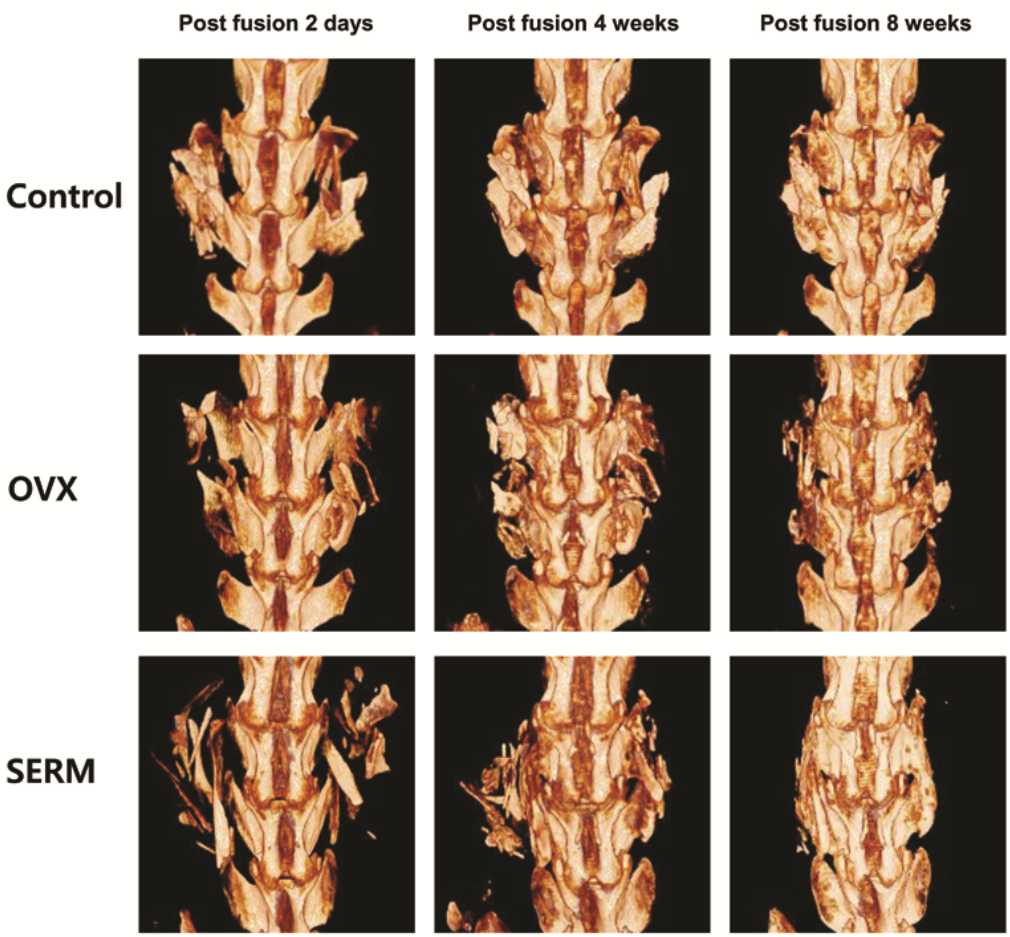
The upper row shows the measured bone volume in the fusion bed 4 and 8 weeks after arthrodesis. The middle and lower rows show 3D-reconstructed and axial images in all groups after surgery. Red color indicates the grafted and newly formed bone. Four weeks after surgery, only the sham group had new bone formation near the transverse processes. Eight weeks after surgery, the late BP group had new bone formation and incorporation into the transverse processes similar to that in the sham group. Eight weeks after surgery, the sham and early BP groups had a more compact fusion mass compared with the non-BP and late BP groups. (BP = bisphoshponate)

Figure 4. The value of mRNA expression in fusion mass



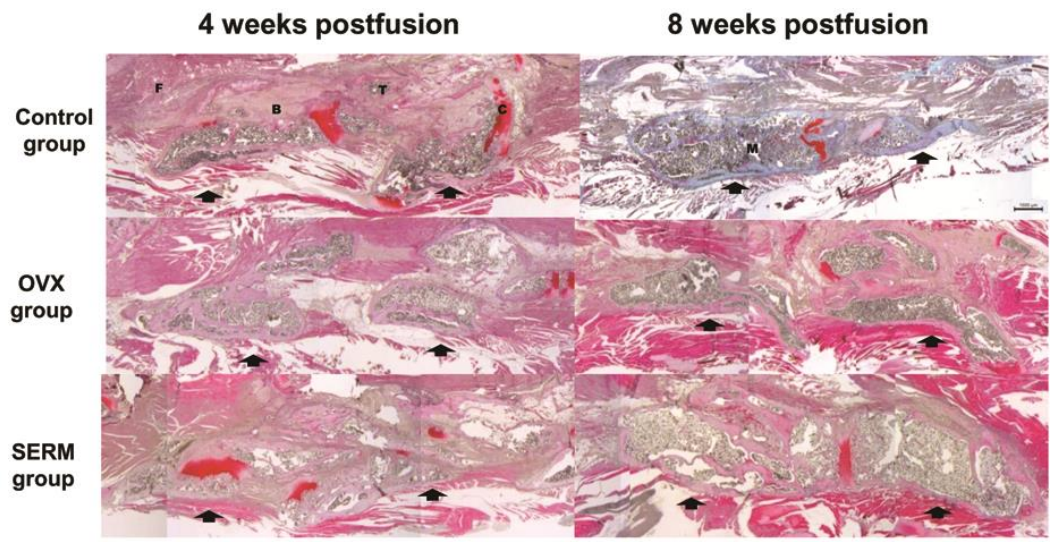
The mRNA expression levels relative to GAPDH showed that expression levels of all genes were higher in the SERM group at 4 weeks after fusion surgery than in the OVX group. The expression of osteocalcin in the SERM group increased up to 8 weeks after fusion surgery. The OVX group showed a similar pattern of changes in mRNA to that of the Control group

Figure 5. Microcomputed tomography scanning of fusion masses



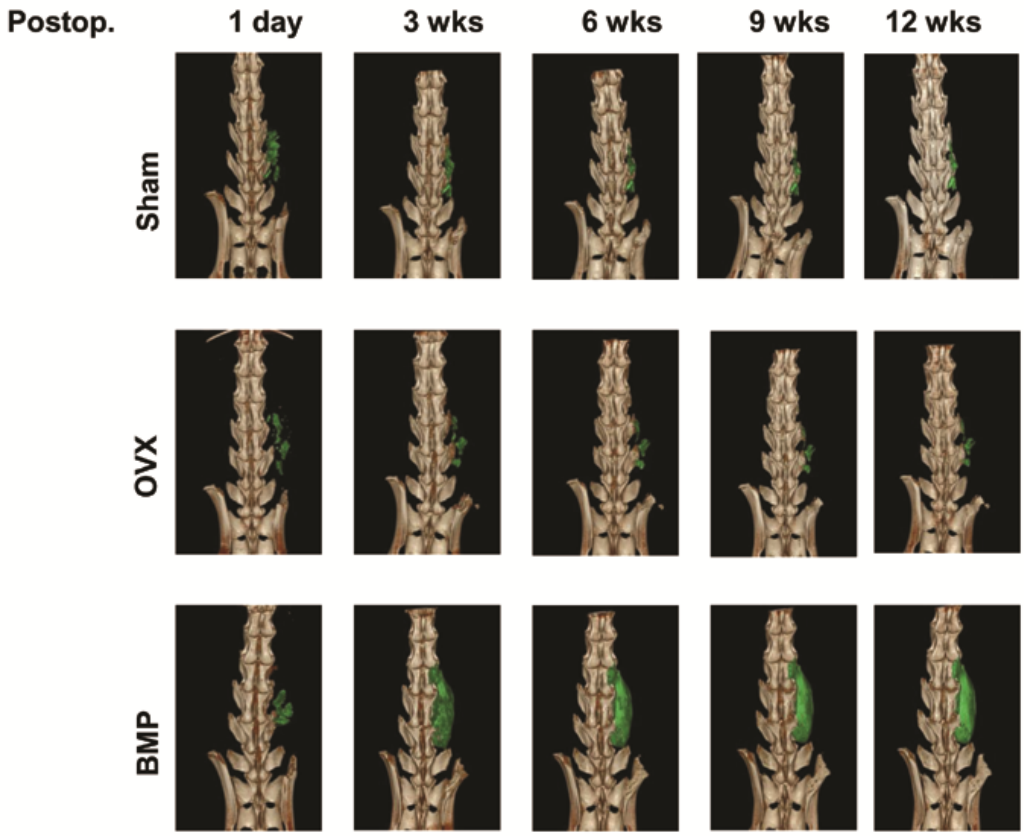
The figure shows 3-D reconstructed images from all groups after surgery. The consolidation of the bone fragment in the fusion bed in the OVX group was weaker than that in the SERM group. The bone contour in the SERM group was more compact than that in the OVX group at 8 weeks after fusion surgery. (OVX = ovariectomy, SERM = selective estrogen receptor modulator).

Figure 6. Histological examination of fusion mass



At 4 weeks, active endochondral ossification with cartilage tissue (bright red-stained tissue, “C”) was observed in the grafted site in the Control and SERM groups. However, the OVX group showed weak endochondral ossification with cartilage tissue. At 8 weeks after fusion surgery, the Control and SERM groups showed a large matured trabecular area (“M”) with healed cortical bone of the transverse processes. However, in the OVX group, there was a large amount of fibrous tissue among the bone fragments at 8 weeks postfusion. (B = bone, C = cartilage, F = fibrous tissue, T = trabecular area, M = matured trabecular area, arrow = transverse process).

Figure 7. Microcomputed tomography scanning of fusion masses



The figure show 3D-reconstructed images in all groups after surgery. There is the grafted and new bone between intertransverse processes. (OVX = ovariectomy)

Supplement 1.

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Basic Science

The time-dependent effect of ibandronate on bone graft remodeling in an ovariectomized rat spinal arthrodesis model

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Abstract

BACKGROUND CONTEXT: In osteoporotic patients undergoing spinal arthrodesis, the use of bisphosphonates (BPs) remains controversial with regard to bone fusion. There is no consensus about the appropriate time to give BPs to patients with osteoporosis undergoing spinal arthrodesis.
PURPOSE: We aimed to study the effect of BPs, given at different times, on the bone response to osteoporotic spinal arthrodesis.
STUDY DESIGN/SETTING: Radiological, histologic, and molecular assessments of bone formation after the different administration time of ibandronate in an ovariectomized (OVX) rat spinal fusion model.
METHODS: Female Sprague-Dawley rats (n=100) were OVX (n=80) or non-OVX operated (n=20) and randomized into five groups: non-OVX, osteoporosis, and osteoporosis with early, simultaneous, and late BP groups. Eight weeks after ovariectomy, lumbar spinal arthrodesis was performed using autologous tailbones. Animals were killed 4 and 8 weeks after arthrodesis, and bone formation was assessed by measuring bone mineral density (BMD), messenger RNA expression, manual palpation, radiological evaluation, and histomorphometry.
RESULTS: Compared with late administration, early administration of ibandronate increased femur BMD in OVX rats and did not hinder bone fusion. Radiological analysis showed that groups given early ibandronate had increased bone volume in the grafted site 8 weeks after surgery. Histomorphometric analysis showed that ibandronate positively affected endochondral and intramembranous ossification. In the OVX groups, ibandronate increased bone turnover to a level similar to that in the non-OVX group. These findings suggested that early administration of ibandronate did not inhibit osteogenesis, including endochondral and intramembranous ossification and fusion rate.
CONCLUSIONS: Our results suggest that the early administration of BPs may not hinder the bone fusion of osteoporotic patients undergoing spinal arthrodesis. © 2014 Elsevier Inc. All rights reserved.

Keywords: Osteoporosis; Rat; Spine; Bisphosphonate; Bone; Ibandronates; Fusion; Osteoblast; Osteoclast; Remodeling

Introduction

Advances in surgical techniques and the greater expectation to remain physically active in older people have increased the demand for spinal fusion in elderly patients [1,2]. The increasing number of elderly patients implies that the number of patients with osteoporosis has increased [3,4]. Osteoporosis is characterized by a decrease of bone mass with consequential fragility, which increases the susceptibility to fracture, most commonly in the spine, wrist, and hip [3,5]. Because osteoporosis increases bone fragility,

FDA device/drug status: Approved (ibandronate).

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None of the authors have any conflicts of interest to report related to the study.

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which can cause implant fixation failure and delay bone graft healing, osteoporosis is detrimental to spinal fusion [6,7]. Medical treatment using bisphosphonates (BPs) or human parathyroid hormone has been tried to improve the bone quality and fusion rate [8].

A successful fusion at the bone-grafting site is achieved through the balance between bone formation and resorption. In osteoporotic patients, BPs are first-line drugs that can increase bone mineral density (BMD) by reducing bone resorption function of osteoclast [9]. Previous studies suggested that BPs increase BMD and improve implant integration in osteoporotic conditions [10]. However, it remains controversial whether antagonistic effects of BPs in osteoclasts are beneficial for spinal fusion in osteoporotic patients. To our knowledge, only a few articles have addressed the effect of BPs on spinal fusion in osteoporotic conditions [4,11].

We studied the effect of ibandronate on spinal fusion in the ovariectomized (OVX) rat, an experimental model that mimics postmenopausal osteoporosis. The drug was given at times including before, during, and after the fusion operation.

Materials and methods

Animals and reagents

Female Sprague-Dawley rats (n=100, 10 weeks of age; Orientbio, Gyeonggi, Korea) were housed, one per ventilated cage, in a specific pathogen-free room with a 12-hour light/dark cycle. The average weight of all rats was around 350 g at 10 weeks of age. The rats were allowed free access to tap water and commercially available standard rodent food (Cargill, Gyeonggi, Korea) containing 1.35 g calcium, 0.44 g phosphorus, and no vitamin D per 100 g dry weight. Although a low-calcium (<0.3%) diet can affect the BMD negatively, the amount of calcium (1.35%) in our rodent food did not influence BMD [12]. Ibandronate (Bonviva; Roche, Basel, Switzerland) was used as the antiresorptive drug.

Experimental design

Eighty 12-week-old rats were OVX, and 20 rats were given a non-OVX operation. After 8 weeks, all 20-week-old rats underwent spinal arthrodesis using autologous tailbones. Rats were randomized into five groups: non-OVX (non-OVX operated+arthrodesis alone), non-BP (OVX+arthrodesis alone), simultaneous BP (OVX+arthrodesis with simultaneous administration of ibandronate), early BP (OVX+early administration of ibandronate 2 weeks before arthrodesis+arthrodesis), and late BP (OVX+arthrodesis+the late administration of ibandronate 4 weeks after arthrodesis). The rats in the simultaneous, early, and late BP groups were injected subcutaneously with ibandronate (25 μg/1 kg per 25 days) [10,13,14]. The rats in the non-OVX and non-BP groups received subcutaneous injections of saline (25 μg/1 kg per 25 days). The average weight of all rats was less than 1 kg. Therefore, we converted the adequate concentration of saline and BP according to the standard administration (eg, 25 μg/1 kg for each rat every 25 days) and administered the converted concentration of saline and BP to all rats at every time point.

Ten rats in each group were euthanized at 4 and 8 weeks after the arthrodesis. Their BMD was analyzed, and fusion was assessed using manual palpation 4 and 8 weeks after the operation. Molecular analysis was performed in 5 of the 10 rats. Radiological and histologic analyses were performed from the other five rats in each group (Fig. 1). This experimental protocol was approved by the Institutional Animal Care and Use Committee of the Clinical Research Institute, Seoul National University Hospital.

Surgical procedures

Anesthesia was induced with 5% isoflurane and maintained with 2.5% isoflurane. The surgical procedure performed was an L4–L5 posterolateral intertransverse process fusion [15]. After the bilateral dorsal incision, the L4 and L5 transverse processes were exposed using a muscle-splitting approach between the multifidus and erector spinae muscles [16]. The cortical bone of the transverse processes of L4 and L5 was decorticated. The autograft bone (~0.5 g) was harvested from the tail. The tailbones were implanted in the decorticated fusion beds located between the decorticated transverse process of L4 and L5.

Bone mineral density

Both femora from each rat were harvested after euthanization. Bone mineral density was measured in the femora with a dual-energy X-ray absorptiometer (Lunar PIXImus, Madison, WI, USA) using Lunar PIXImus 2 2.0 software. Absolute BMD values are expressed in grams per square millimeter (g/mm^2).

Fusion assessment

Fusion was assessed by manual palpation and soft-tissue X-ray at the L4–L5 segment [15,17,18]. The lateral bending motion at the L4–L5 level of the harvested lumbar spine was checked and compared with the motion at the superior and inferior adjacent levels. Each spine was classified as a fusion (no bending motion) or nonfusion (bending motion). The specimens were evaluated by two spine surgeons who classified them as fusion when no motion was observed and as nonfusion when motion was detected regardless of the presence of bridging bone. X-ray radiographs were taken under equal conditions (NFR-Polaris-G90; NanoFocusRay, Iksan, Korea). The bony continuity within the intertransverse process at L4–L5 was evaluated by two spine surgeons. In manual palpation and X-ray testing, the case was considered as fusion only when there was total agreement between two spine surgeons about fusion. The fusion rate is expressed as a ratio of the number of fusion cases to the total number in the subgroup (n=10).

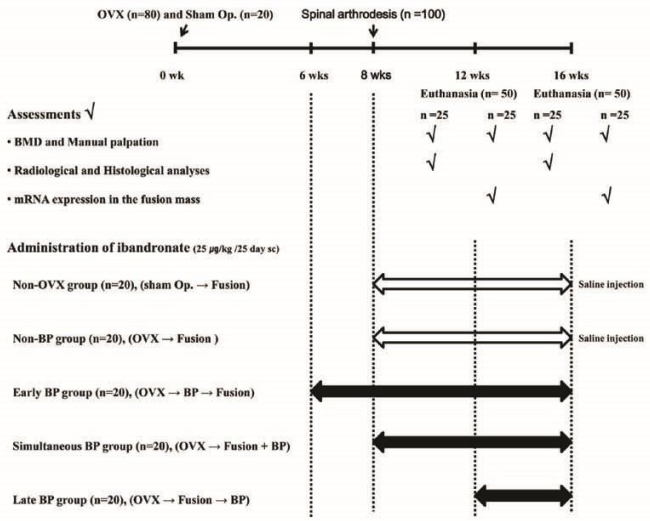


Fig. 1. Experimental groups, time schedule, and assessment tools. Eighty rats in the non-bisphosphonates (non-BPs) and simultaneous, early, and late BP groups received a bilateral ovariectomy, and 20 rats underwent a non-ovariectomized (non-OVX) operation group. After 8 weeks, all rats underwent spinal arthrodesis using autologous tailbones. To assess bone mineral density (BMD), manual palpation, radiological and histomorphometric analyses, and molecular assessment were applied in 10 rats in each group killed 4 and 8 weeks after arthrodesis. Bone mineral density and manual palpation were assessed in all rats. Radiological, histologic, and molecular analyses were performed in five rats in each group 4 and 8 weeks after surgery. Op, operation.

Three-dimensional volume and microstructure analyses

To evaluate the microstructure and measure the bone volume between intertransverse areas, three-dimensional (3D) microcomputed tomography (micro-CT) was performed using a CT scanner (NFR-Polaris-G90; NanoFocusRay). The scans started from the lower end plate of the L2 vertebral body and proceeded caudally to the femoral head, and images were acquired at 70 kVp, 80 μA, and 100 ms per frame and included 720° views. The reconstruction image size was 1,024×1,024 pixels, the voxel size was 56.179×56.179×123.594 μm, and 512 slices were acquired. To measure the volume at the grafted site and to assess bone formation, the axial image was converted to the Digital Imaging and Communications in Medicine format to create a 3D image using 3D-rendering software (Lucion; Infinite, Seoul, Korea). The total bone volume of the intertransverse area was calculated 4 and 8 weeks after spinal arthrodesis. The volume is expressed in cubic millimeters (mm^3).

Histologic evaluation

The specimens, including the L4–L5 intertransverse process fusion area and adjacent vertebral bodies, were fixed with 4% paraformaldehyde. The specimens were decalcified in 5% nitric acid for 3 days, washed in distilled water, and embedded in paraffin. The specimens were sectioned sagittally at 5 μm at the midline of the intertransverse process mass bilaterally. The sections were stained with Safranin O with hematoxylin and Fast Green and observed under light microscopy. To evaluate early- and late-stage bone formation at the grafted site, the fibrous tissue area, cartilage area, trabeculation area, and bone area/tissue area ratio were assessed. The parameters are expressed as a ratio percentage. We defined trabeculation area as the cavities with bony septum and marrow tissue except the transverse process, and we have marked the trabeculation area in Fig. 4. Histomorphometric analysis was performed using MacBiophotonics ImageJ software [19].

Reverse transcription and real-time reverse transcription-polymerase chain reaction

To assess bone turnover activity related to osteoblasts, osteoclasts, and osteocytes in the grafted site, the messenger RNA (mRNA) expression level within the extracted specimen was measured using quantitative real-time reverse transcription-polymerase chain reaction (PCR) [11]. The extracted specimen for molecular analysis was harvested at postoperative 4 and 8 weeks. Total RNA was extracted from the specimen using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For complementary DNA synthesis, 1 μg of RNA was reverse transcribed using

Table
The value of mRNA expression in fusion mass

Parameters	Postoperative time (wk)	Non-OVX group	Non-BP group	Simultaneous BP group	Early BP group	Late BP group
ALP	4 (n=5)	0.28±0.075	0.32±0.083	0.35±0.232	0.04±0.031	0.08±0.051
	8 (n=5)	0.99±0.689	0.14±0.090	0.60±0.216	0.20±0.105	0.39±0.217
	p Value	.123	.118	.174	.043	.061
Collagen	4 (n=5)	5.67±6.958	2.45±0.525	4.22±2.361	0.67±0.032	0.34±0.111
	8 (n=5)	0.50±0.313	3.47±2.431	1.32±0.312	0.27±0.091	0.22±0.096
	p Value	.327	.547	.165	.000	.146
OC	4 (n=5)	0.10±0.093	0.09±0.078	0.21±0.175	0.28±0.338	0.01±0.022
	8 (n=5)	0.11±0.098	0.36±0.319	0.35±0.243	0.41±0.432	1.37±0.674
	p Value	.862	.277	.355	.640	.005
Runx2	4 (n=5)	1.02±0.600	1.80±1.314	1.65±0.256	0.79±0.663	0.88±0.243
	8 (n=5)	0.33±0.167	2.00±1.546	1.26±0.552	0.92±0.376	0.76±0.422
	p Value	.812	.891	.309	.730	.679
Sclerostin	4 (n=5)	0.35±0.149	0.32±0.083	0.54±0.483	0.04±0.031	0.08±0.051
	8 (n=5)	0.99±0.689	0.14±0.090	0.60±0.216	0.20±0.105	0.39±0.217
	p Value	.105	.118	.822	.043	.061
TRAP	4 (n=5)	1.16±0.693	1.84±1.207	2.85±1.479	0.33±0.138	0.21±0.060
	8 (n=5)	0.30±0.224	1.79±1.173	1.92±0.968	0.30±0.178	0.39±0.164
	p Value	.162	.959	.317	.760	.117

ALP, alkaline phosphatase; BP, bisphosphonate; OC, osteocalcin; mRNA, messenger RNA; OVX, ovariectomized; TRAP, tartrate-resistant acid phosphatase.

random hexamer primers (Invitrogen) and ImProm II reverse transcriptase (Invitrogen).

For the Sequence Detection System 7,000 system reactions, a master mix of the following components was prepared: 6.25 μL water, 1.25 μL probe (2.5 M), and 12.5 μL TaqMan PCR 2× master mixture (Applied Biosystems, Lincoln, CA, USA). Fifty nanograms of reverse-transcribed total RNA in 5 μL was added as the PCR template. Relative quantitative real-time PCR was performed in 96-well optical plates using the previously mentioned reagents, and the results were analyzed on an ABI Prism 7,000 Sequence Detection System (Perkin-Elmer Applied Biosystems, Lincoln, CA, USA). After initial activation of uracil-*N*-glycosylase at 50°C for 2 minutes, AmpliTaq Gold (AmpliTaq Gold Applied Biosystems, Weiterstadt, Germany) was activated at 95°C for 10 minutes. The subsequent PCR condition comprised 45 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 1 minute per cycle. During the PCR amplification, the amplified products were measured continuously by measuring the fluorescence emission. The PCR primer and probe sets of *alkaline phosphatase* (ALP), *Type I collagen*, *osteocalcin* (OC), *Runx2*, *sclerostin*, *tartrate-resistant acid phosphatase* (TRAP), *and glyceraldehyde-3-phosphate dehydrogenase* (GAPDH) as housekeeper genes used in the real-time PCR investigation are listed in the Table. The expression level of the target gene was normalized to the internal control rat GAPDH and is represented as relative expression. To confirm a constant housekeeping gene expression level in the total RNA extractions, GAPDH real-time PCR was performed. Real-time PCR was quantified using the SDS 7,000 (Applied Biosystems) with the GAPD (GAPDH) Endogenous Control (FAM/MGB Probe, Primer Limited).

Statistical analyses

The results are presented as mean±standard deviation. Differences between groups in fusion status assessed by manual palpation were analyzed using the chi-square test. To identify significant differences between groups in BMD, CT-based bone area, histomorphometry, and bone metabolic markers, one-way analysis of variance and post hoc analysis were used. A p value less than .05 was considered significant. A power analysis was performed before the study. The statistician had suggested that more than 188, 252, 272, and 380 rats were needed according to 0%, 25%, 30%, and 50% of dropout rate (power=0.8, α=0.05, β=0.2), respectively, in the study.

Results

Bone mineral density

Four weeks after the fusion operation, the mean femoral BMD was greater in the non-OVX and early BP groups than in the non-, simultaneous, and late BP groups. Eight weeks after the operation, BMD was similar in the simultaneous and early BP groups. However, the mean BMD was lower in the late BP group compared with the simultaneous BP group (Fig. 2).

Fusion assessment using manual palpation

Four weeks after surgery, the fusion rate did not differ significantly between groups (p=.095). The fusion rates of the non-OVX and non-, simultaneous, early, and late BP groups were 70% and 30%, 30%, 60%, and 20%, respectively. The non-OVX and non-, simultaneous, early,

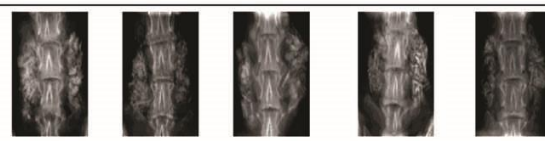
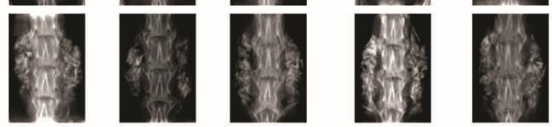
Parameters	Postoperative time	Non-OVX group	Non-BP group	Simultaneous BP group	Early BP group	Late BP group
BMD (g/mm²)	4 wks (n=10)	0.29 ± 0.051	0. 25 ± 0.044	0.25 ± 0.042	0.28 ± 0.042	0.23 ± 0.029
	8 wks (n=10)	0.29 ± 0.051	0.25 ± 0.035	0.27 ± 0.038	0.27 ± 0.026	0.26 ± 0.039
Fusion rate	4 wks (n=10)	70% (7/10)	30% (3/10)	30% (3/10)	60% (6/10)	20% (2/10)
	8 wks (n=10)	80% (8/10)	60% (6/10)	70% (7/10)	80% (8/10)	70% (7/10)
Soft X-ray	4 wks					
	8 wks					

Fig. 2. Bone mineral density (BMD) and fusion assessment results. The early bisphosphonate (BP) and non-ovariectomized (OVX) groups had greater BMD 4 and 8 weeks after arthrodesis. At 8 weeks, the simultaneous and late BP groups had increased BMD. Although the fusion rate did not differ significantly between groups at 8 weeks, the non-OVX and early BP groups had higher fusion rates 4 weeks after arthrodesis. Soft-tissue X-ray images of non-OVX and simultaneous, early, and late BP groups at 8 weeks showed denser fusion masses compared with those 4 weeks after arthrodesis. By contrast, the non-BP group had sparser fusion masses in X-ray images at 8 weeks compared with 4 weeks after the fusion operation.

and late BP groups had similar fusion rates at postoperative 8 weeks (80% and 60%, 70%, 80%, and 70%, respectively). X-ray images showed that the bone mass on the fusion bed was greater in the non-OVX and simultaneous, early, and late BP groups at 8 weeks compared with 4 weeks after arthrodesis. The non-BP group had a sparser fusion mass in X-ray images 8 weeks after, compared with 4 weeks after the fusion operation (Fig. 2).

Three-dimensional micro-CT images

At 4 weeks, bone volume on the fusion bed did not differ significantly between groups (p=.253) and the bone volume was lower 8 weeks after surgery than it was 4 weeks after surgery and did not differ between groups (p=.724). However, the late BP group had significantly lower bone volume at 8 weeks than at 4 weeks (197±80.0 vs. 269±66.3 mm^3, respectively, p=.008) (Fig. 3, top). At 8 weeks, 3D micro-CT showed that the non-OVX group had dense and small fusion masses with well-remodeled bone structure compared with the other groups (Fig. 3, middle). The small fusion mass at 8 weeks suggests remodeling. Four weeks after surgery, axial micro-CT views showed that only the non-OVX group exhibited new bone formation near the transverse processes. Eight weeks after surgery, the early BP group had new bone formation and incorporation into the transverse processes, as observed in the non-OVX group. The non-OVX and simultaneous and early BP groups exhibited more closely packed fusion masses compared with the non- and late BP groups (Fig. 3, bottom).

Histologic evaluation

Four weeks after surgery, all groups exhibited the cartilage matrix between transverse processes, denoting the early stage of endochondral ossification and the healing process as shown by membranous bone formation on the surface of the decorticated transverse processes. However, the non-BP groups had scarce cartilage matrix retention and membranous bone formation. The simultaneous and early BP groups had a pattern of endochondral and intramembranous bone formation similar to that in the non-OVX group. The bone formation processes in the graft bed were weaker in the late BP group than in the early BP group. Eight weeks after spinal arthrodesis, the non-OVX and early BP groups had healed and fused transverse processes and abundant

parameters	Postoperative time	Non-OVX group	Non-BP group	Simultaneous BP group	Early BP group	Late BP group
Bone volume (mm^3)	4 weeks (n=5)	214 ± 30.8	229± 88.9	256± 89.2	220± 54.2	269± 66.3
	8 weeks (n=5)	199 ± 27.3	186± 68.3	218± 71.8	205± 114.2	197± 80.0
	p value	.442	.473	.524	.743	.008
3-dimental views	4 weeks					
	8 weeks					
Axial views	4 weeks					
	8 weeks					

Fig. 3. Microcomputed tomography scanning of fusion masses. The top row shows the measured bone volume in the fusion bed 4 and 8 weeks after arthrodesis. The middle and bottom rows show three-dimensional–reconstructed and axial images in all groups after surgery. Red color indicates the grafted and newly formed bone. Four weeks after surgery, only the non-ovariectomized (non-OVX) group had new bone formation near the transverse processes. Eight weeks after surgery, the late bisphosphonate (BP) group had new bone formation and incorporation into the transverse processes similar to that in the non-OVX group. Eight weeks after surgery, the non-OVX and early BP groups had a more compact fusion mass compared with the non-BP and late BP groups.

trabeculation masses between adjacent transverse processes. There were fewer trabeculation masses and unhealed transverse processes in the simultaneous BP group. The late BP group had weak bone formation in a pattern similar to that of the simultaneous BP group. Bone formation in the fusion bed and healing of decorticated transverse processes were delayed in the non-BP group compared with the simultaneous and early BP groups (Fig. 4).

Histomorphometric analysis showed that the fibrous tissue area, cartilage area, and trabeculation area ratio decreased in the non-BP group 8 weeks after spinal arthrodesis. The non-OVX group showed the opposite effect. The fibrous tissue area, cartilage area, and bone area ratio increased 8 weeks after surgery in the early BP group, and the trabeculation ratio was maintained at a high level. The cartilage and trabeculation ratio increased 8 weeks after surgery in the late BP group. In the simultaneous BP group, the fibrous tissue area and trabeculation ratio increased and the cartilage and bone ratio decreased (Fig. 5). There was no significant difference in comparing subpopulation groups.

mRNA expression in the fusion mass

The mRNA levels of osteoblast-related genes (ALP, Type I collagen, and Runx2) and osteoblast inhibitor–related gene (sclerostin) changed in opposite directions in the non-BP and non-OVX groups. In the non-OVX and simultaneous, early, and late BP groups, mRNA expression of collagen and Runx2 decreased from 4 to 8 weeks after fusion surgery. Messenger RNA expression of ALP and sclerostin increased from 4 to 8 weeks after spinal arthrodesis. The mRNA expression of the mature osteoblast-related gene OC increased in all groups except the non-OVX group. In the non-BP group, the relative mRNA expression level of the osteoclast-related gene TRAP was maintained at a high level. However, the other groups had low or decreased mRNA expression of TRAP (Table).

Discussion

The results of the present study indicate that the administration of ibandronate did not inhibit bone formation and bone healing compared with no administration. Also the results suggest that endochondral and intramembranous ossification was not inferior in rats treated early with ibandronate compared with those treated later.

Bisphosphonates would increase microcrack density in the trabecular bone microstructure of the normal skeleton [20–22]. Many people are diagnosed with osteoporosis or osteopenia throughout the world, and BPs have been proven safe and effective in the treatment of osteoporosis [23–25]. Bisphosphonates slow bone remodeling [26–28].

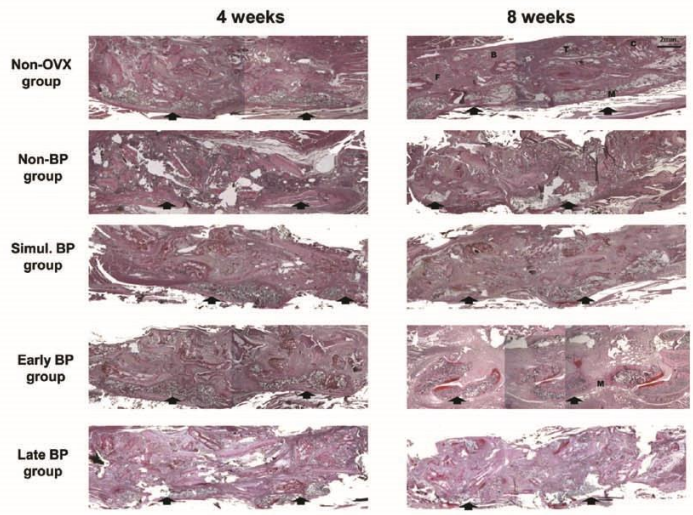


Fig. 4. Histologic features of fusion mass. At 4 weeks, all groups had transverse processes that were decorticated and healed (arrow). Differences in cartilage formation (bright red-stained tissue) were observed between the transverse processes. Cartilage formation (C) was more extensive in the non-ovariectomized (non-OVX) and early bisphosphonates (BP) groups than in the other groups. Trabeculation mass (T) was abundant in the non-OVX and simultaneous and early BP groups 8 weeks after arthrodesis. Membranous bone formation (M) was prominent in the non-OVX and early BP groups. Simul., simultaneous; F, fibrous tissue, B, bone.

Bisphosphonates have been shown to improve fracture healing and to increase the pullout strength of implants in animal studies [28,29].

However, it is controversial whether BPs are beneficial in patients undergoing osteoporotic spinal fusion. Several studies have suggested that normal or supranormal doses of BPs inhibit or delay spinal fusion by reducing bone incorporation between the grafted and host bone and delay the maturation and remodeling of the fracture callus [11,23,24,29–31]. By contrast, some studies have demonstrated favorable effects of BPs on spinal fusion in osteoporosis [4,32].

In this study, we tried to reflect the clinical circumstance of early, simultaneous, or late administration of BPs.

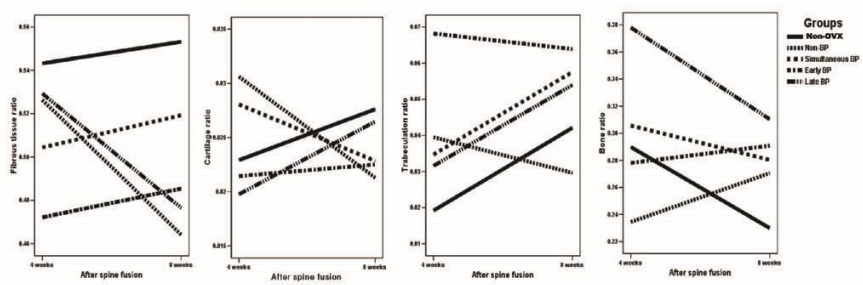


Fig. 5. Histomorphometric analysis of fusion mass. The non-ovariectomized (non-OVX) group showed increased fibrous, cartilage, and trabeculation ratio and decreased bone ratio; the non-bisphosphonate (BP) group showed the opposite pattern. The late BP groups showed similar patterns for cartilage, trabeculation, and bone ratio as the non-OVX group. The changes in fibrous, cartilage, and trabeculation in the early BP group were close to those of the non-OVX group.

Therefore, the ibandronates were given at different times according to the groups of rats used in this study. Saline or BP was not administered to the simultaneous BP group before 8 weeks after ovariectomy or in the late BP group before 4 weeks after spinal arthrodesis. We tried to create the same stress circumstances that might follow the injection of BP in the non-OVX and non-BP groups by using the saline injection. Bone mineral density was similar in the early BP and non-OVX groups 4 weeks after arthrodesis. At 8 weeks, the simultaneous and early BP groups had BMD similar to that of the non-OVX group.

In preclinical studies with OVX rats, intermittent and continuous dosing of ibandronates produced similar effects. The optimal dosage of ibandronates for OVX rats is 0.2 to 1.0 μg/1 kg per day, which approximately reflects the optimum dose in osteoporosis patients [10,13]. Therefore, we selected an intermittent administration of 25 μg/1 kg every 25 days, which is equivalent to the upper limit of 1 μg/1 kg per day.

Although a few studies have reported that BPs can inhibit cell proliferation and induce apoptosis of osteoclasts, several other studies have also suggested that BPs can increase osteoblast differentiation and lead to a positive bone balance [33–36]. In this study, molecular analysis revealed that the changes in ALP, Type I collagen, Runx2, and sclerostin gene expressions in the non-BP group were opposite to those in BP groups. In the BP groups, the values for collagen and Runx2 decreased over time and the value for OC did not increase significantly. These changes are secondary coupling effects because of the decreases in resorption induced by BPs and are unlikely to be primary effects of the BPs.

Good bone quality is a function of the balance between remodeling and the coupled reactions of osteoblasts and osteoclasts [37]. In osteoporosis, the coupling bone formation does not persist and fracture healing is delayed [38,39]. Bisphosphonates might increase the fusion mass and fusion rate [30,40]. In the non-OVX group, which was included as a model of nonosteoporotic spinal fusion, bone mass decreased 8 weeks after surgery. In the early BP group, early injection of ibandronate increased bone mass as measured in micro-CT imaging and histomorphometric analysis. Although the fusion rate does not differ between nonosteoporotic and osteoporotic spinal fusions after ibandronate injection, the fusion quality, such as incorporation of bone, may differ. Fibrous tissue, soft callus including cartilage, and hard callus with new bone matrix appear sequentially in fracture healing [37]. Therefore, the effect of BPs on fusion mass can be evaluated by histologically estimating the volume ratios of fibrous, cartilage, and trabecular areas. The comparison of tissue ratios can be used to estimate the effect of BPs on bone matrix at different fusion stages. In other studies, the fusion mass was analyzed by estimating the trabecular thickness, number, and separations [11,41]. In this study, there was not enough bridging bone mass between the transverse process in almost all rats, and we could not evaluate the trabecular thickness, number, and separation to compare the fusion masses radiologically. The incomplete fusion mass 8 weeks after arthrodesis could be attributed to the age of the rats and to the use of tailbones as grafted bone [42–44].

There are some limitations to this study. The power analysis value was meaningful only for the comparison of the trabeculation ratio at 4 weeks ($p=.012$, $\alpha=0.05$, power=0.915). For the comparison of fusion rate at 4 and 8 weeks, the power values were 0.587 and 0.130, respectively. The power values for the micro-CT analysis at 4 and 8 weeks were 0.153 and 0.071, respectively. To assess the primary outcome in this study, analysis of an adequate number of rats is necessary. Because of the limited number of animals tested, we could not definitively present our results in terms of the differences in ultimate fusion rate (as the primary outcome), bone volume, and histomorphometric analysis; thus, we have presented only the trends. However, the ultimate fusion rate of the early BP group was not inferior to that of the late BP group. In addition, bone remodeling and histomorphometric analysis were similar to that of the non-OVX group. In histology, maturation of trabecular bone in the early BP group seems to be superior to that of the late BP group. These results could be valuable in the clinical setting. In the clinical field, fusion is defined as the continuity of bone called bridging bone between intertransverse processes, not maturation of trabecular bone. In the present study, the early administration of BPs did not hinder the formation of bridging bone between intertransverse processes. Therefore, based on the results of the present study, early administration cannot hinder the bone fusion in humans. Therefore, we propose that the observed trend may provide meaningful information about the administration of BPs to osteoporotic patients who need to undergo spine fusion surgery. Further studies with a large number of rats are needed to provide more affirmative information about the timing of BP administration in relation to osteoporotic bone fusion. Second, although bone remodeling in rat and mice is similar to the Haversian remodeling in larger animals, the limitations of the rat spinal fusion model should be considered before translating the laboratory findings to clinical conditions [42]. Further studies with greater number of animals and in larger animals such as the rabbit are needed to identify the clinical implications of our findings. Third, there was no complete bridging bone between transverse processes. In the clinical setting, bridging bone formation is a critical point for determining whether fusion is complete. The use of tailbones, which have less bone marrow compared with iliac bones, might have interfered with formation of the complete bridging bone. Fourth, the histomorphometric analysis may have limited our ability to draw definitive conclusions because only one surgeon analyzed the volume ratio using sagittal sections of tissue and the limited number of animals at each evaluation step. Fifth, although all rats were housed under the same environmental condition, we should have

measured the blood levels of calcium and vitamin D because these can affect bone remodeling. In future studies, blood levels of calcium and vitamin D should be measured. To reproduce the clinical situation of osteoporotic patient with spine fusion, a future study using older rats should be considered. The study by Balkarli et al. [45] was "Does application time of alendronate sodium effect the spinal fusion in ovariectomy rats?" Although the limited number of animals could hinder our ability to emphasize our results in terms of the differences in ultimate fusion rate, the present study included more rats (n=100 vs. n=50 in the study by Balkarli et al.), four kinds (vs. three kinds in the study by Balkarli et al.) of analytical methods performed at two time points (4 and 8 weeks postarthrodesis vs. only 6 weeks after fusion in the study by Balkarli et al.), more injection times for bisphosphonate (early, simultaneous, and late vs. early and simultaneous in the study by Balkarli et al.), and a sham group. Hence, the present study better reflects the real clinical circumstances and the practical implications than does the study by Balkarli et al. [45]. Therefore, we believe that our study is the most comprehensive investigation of different administration times of BPs as used in the clinical practice and may be helpful to spine surgeons considering the use of BPs to treat patients with osteoporosis undergoing spinal fusion. Further investigations using large animal models are needed to study the incorporation between grafted bones and the recipient bed and factors that can improve fusion by influencing osteoblasts.

In conclusion, the effect of early administration of ibandronates on bone formation may not be inferior to those of simultaneous or delayed administration of ibandronates. In addition, because the present study was only able to suggest trends in the results, a future study with a greater number of rats and fewer groups is needed.

Acknowledgment

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References

[1] Deyo RA, Gray DT, Kreuter W, et al. United States trends in lumbar fusion surgery for degenerative conditions. Spine 2005;30:1441–5; discussion 1446–7.
[2] Katz JN. Lumbar spinal fusion. Surgical rates, costs, and complications. Spine 1995;20:78S–83S.
[3] Chin DK, Park JY, Yoon YS, et al. Prevalence of osteoporosis in patients requiring spine surgery: incidence and significance of osteoporosis in spine disease. Osteoporos Int 2007;18:1219–24.
[4] Nagahama K, Kanayama M, Togawa D, et al. Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. J Neurosurg Spine 2011;14:500–7.
[5] Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377:1276–87.
[6] Tarantino U, Cerocchi I, Scialdoni A, et al. Bone healing and osteoporosis. Aging Clin Exp Res 2011;23:62–4.
[7] Cortet B. Bone repair in osteoporotic bone: postmenopausal and cortisone-induced osteoporosis. Osteoporos Int 2011;22:2007–10.
[8] Burval DJ, McLain RF, Milks R, Inceoglu S. Primary pedicle screw augmentation in osteoporotic lumbar vertebrae: biomechanical analysis of pedicle fixation strength. Spine 2007;32:1077–83.
[9] Frith JC, Rogers MJ. Antagonistic effects of different classes of bisphosphonates in osteoclasts and macrophages in vitro. J Bone Miner Res 2003;18:204–12.
[10] Kurth AH, Eberhardt C, Muller S, et al. The bisphosphonate ibandronate improves implant integration in osteopenic ovariectomized rats. Bone 2005;37:204–10.
[11] Takahata M, Ito M, Abe Y, et al. The effect of anti-resorptive therapies on bone graft healing in an ovariectomized rat spinal arthrodesis model. Bone 2008;43:1057–66.
[12] Omi N, Ezawa I. Animal models for bone and joint disease. Low calcium diet-induced rat model of osteoporosis. [in Japanese]. Clin Calcium 2011;21:173–80.
[13] Bauss F, Lalla S, Endele R, Hothorn LA. Effects of treatment with ibandronate on bone mass, architecture, biomechanical properties, and bone concentration of ibandronate in ovariectomized aged rats. J Rheumatol 2002;29:2200–8.
[14] Bauss F, Wagner M, Hothorn LH. Total administered dose of ibandronate determines its effects on bone mass and architecture in ovariectomized aged rats. J Rheumatol 2002;29:990–8.
[15] Salamon ML, Althausen PL, Gupta MC, Laubach J. The effects of BMP-7 in a rat posterolateral intertransverse process fusion model. J Spinal Disord Tech 2003;16:90–5.
[16] Wiltse LL, Spencer CW. New uses and refinements of the paraspinal approach to the lumbar spine. Spine 1988;13:696–706.
[17] Hidaka C, Goshi K, Rawlins B, et al. Enhancement of spine fusion using combined gene therapy and tissue engineering BMP-7-expressing bone marrow cells and allograft bone. Spine 2003;28:2049–57.
[18] Erulkar JS, Grauer JN, Patel TC, Panjabi MM. Flexibility analysis of posterolateral fusions in a New Zealand white rabbit model. Spine 2001;26:1125–30.
[19] Collins TJ. ImageJ for microscopy. Biotechniques 2007;43:25–30.
[20] O'Neal JM, Diab T, Allen MR, et al. One year of alendronate treatment lowers microstructural stresses associated with trabecular microdamage initiation. Bone 2010;47:241–7.
[21] Allen MR, Iwata K, Phipps R, Burr DB. Alterations in canine vertebral bone turnover, microdamage accumulation, and biomechanical properties following 1-year treatment with clinical treatment doses of risedronate or alendronate. Bone 2006;39:872–9.
[22] Allen MR, Burr DB. Three years of alendronate treatment results in similar levels of vertebral microdamage as after one year of treatment. J Bone Miner Res 2007;22:1759–65.
[23] Lehman RA Jr, Kuklo TR, Freedman BA, et al. The effect of alendronate sodium on spinal fusion: a rabbit model. Spine J 2004;4:36–43.
[24] Liberman UA, Weiss SR, Broll J, et al. Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis. The Alendronate Phase III Osteoporosis Treatment Study Group. N Engl J Med 1995;333:1437–43.
[25] Black DM, Cummings SR, Karpf DB, et al. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet 1996;348:1535–41.
[26] Hughes DE, Wright KR, Uy HL, et al. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. J Bone Miner Res 1995;10:1478–87.
[27] Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 2000;21:115–37.
[28] Parfitt AM, Mundy GR, Roodman GD, et al. A new model for the regulation of bone resorption, with particular reference to the effects of bisphosphonates. J Bone Miner Res 1996;11:150–9.

[29] Peter CP, Cook WO, Nunamaker DM, et al. Effect of alendronate on fracture healing and bone remodeling in dogs. J Orthop Res 1996;14:74–9.
[30] Huang RC, Khan SN, Sandhu HS, et al. Alendronate inhibits spine fusion in a rat model. Spine 2005;30:2516–22.
[31] Sama AA, Khan SN, Myers ER, et al. High-dose alendronate uncouples osteoclast and osteoblast function: a study in a rat spine pseudarthrosis model. Clin Orthop Relat Res 2004;425:135–42.
[32] Nakao S, Minamide A, Kawakami M, et al. The influence of alendronate on spine fusion in an osteoporotic animal model. Spine 2011;36:1446–52.
[33] Mackie PS, Fisher JL, Zhou H, Choong PF. Bisphosphonates regulate cell growth and gene expression in the UMR 106-01 clonal rat osteosarcoma cell line. Br J Cancer 2001;84:951–8.
[34] Fromigue O, Body JJ. Bisphosphonates influence the proliferation and the maturation of normal human osteoblasts. J Endocrinol Invest 2002;25:539–46.
[35] Im GI, Qureshi SA, Kenney J, et al. Osteoblast proliferation and maturation by bisphosphonates. Biomaterials 2004;25:4105–15.
[36] Ponader S, Brandt H, Vairaktaris E, et al. In vitro response of hFOB cells to pamidronate modified sodium silicate coated cellulose scaffolds. Colloids Surf B Biointerfaces 2008;64:275–83.
[37] Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: the cellular picture. Semin Cell Dev Biol 2008;19:459–66.
[38] Namkung-Matthai H, Appleyard R, Jansen J, et al. Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone 2001;28:80–6.
[39] Park SB, Chung CK. Strategies of spinal fusion on osteoporotic spine. J Korean Neurosurg Soc 2011;49:317–22.
[40] Bransford R, Goergens E, Briody J, et al. Effect of zoledronic acid in an L6-L7 rabbit spine fusion model. Eur Spine J 2007;16:557–62.
[41] Abe Y, Takahata M, Ito M, et al. Enhancement of graft bone healing by intermittent administration of human parathyroid hormone (1-34) in a rat spinal arthrodesis model. Bone 2007;41:775–85.
[42] Histing T, Garcia P, Holstein JH, et al. Small animal bone healing models: standards, tips, and pitfalls results of a consensus meeting. Bone 2011;49:591–9.
[43] Meyer RA Jr, Tsahakis PJ, Martin DF, et al. Age and ovariectomy impair both the normalization of mechanical properties and the accretion of mineral by the fracture callus in rats. J Orthop Res 2001;19:428–35.
[44] Mehta M, Strube P, Peters A, et al. Influences of age and mechanical stability on volume, microstructure, and mineralization of the fracture callus during bone healing: is osteoclast activity the key to age-related impaired healing? Bone 2010;47:219–28.
[45] Balkarlı H, Dönmez OB, Ozbey O, et al. Does application time of alendronate sodium effect the spinal fusion in ovariectomized rats? [in German]. Eklem Hastalik Cerrahisi 2011;22:94–9.

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Basic Science

BMP-2 induced early bone formation in spine fusion using rat ovariectomy osteoporosis model

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Abstract

BACKGROUND CONTEXT: Bone morphogenetic proteins (BMPs) enhance bone formation. Numerous animal studies have established that BMPs can augment spinal fusion. However, there is a lack of data on the effect of BMP-2 on spinal fusion in the osteoporotic spine.

PURPOSE: To investigate whether recombinant human BMP-2 (rhBMP-2) enhances spine fusion in an ovariectomized rat model.

STUDY DESIGN: In vivo animal study.

METHODS: Female Sprague-Dawley rats (n=60) were ovariectomized or sham operated and randomized into three groups: Sham (sham operated+fusion), ovariectomy (OVX) (OVX+fusion), and BMP (OVX+fusion+BMP-2). Six weeks after ovariectomy, unilateral lumbar spine fusion was performed using autologous iliac bone with/without rhBMP-2 delivered on a collagen matrix. For each group, gene expression and histology were evaluated at 3 and 6 weeks after fusion, and bone parameters were measured by microcomputed tomography at 3, 6, 9, and 12 weeks.

RESULTS: Real-time reverse-transcription polymerase chain reaction at 3 weeks showed markedly increased expression of osteoblast-related markers (namely alkaline phosphatase, osteocalcin, Runx2, Smad1, and Smad5) in the BMP group compared with the other groups (p=.0005, .0005, .003, .009 and .012, respectively). Although the Sham and OVX groups showed both sparse and compacted bones between transverse processes at 6 weeks, the BMP group had a significantly larger bone mass within the fusion bed at 3 weeks and later. All rats in the BMP group had bridging bone at 3 weeks; at 12 weeks, bridging bones in the Sham and OVX groups were about 50% and 25%, respectively, of that in the BMP group.

CONCLUSIONS: Recombinant human BMP-2 enhances spinal fusion in OVX rats and acts during early bone formation. Therapeutic BMP-2 may therefore improve the outcome of spinal fusion in the osteoporotic patient. © 2013 Elsevier Inc. All rights reserved.

Keywords: Bone morphogenetic protein; Osteoporosis; Spine; Rat

Introduction

The number of individuals with age-related osteoporosis continues to increase. Osteoporosis is characterized by a decrease in bone mass and an increase in susceptibility to fracture, which occur most commonly in the spine, wrist, and hip [1–3]. The prevalence of osteoporosis in spinal surgery patients more than 50 years of age was found to be 14.5% and 51.3% for males and females, respectively [1]. Because bone strength is compromised in osteoporosis, achieving successful spinal bone fusion in the elderly is an increasing challenge [4,5]. Although instrumentation

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with its techniques have improved and the fusion rate in elderly patients is more than 90%, 5% to 35% of patients still exhibit nonunion, resulting in unsatisfactory clinical outcomes of spine fusion surgery [6–9]. Patients with osteoporosis may experience more frequent problems involving instrumentation failure or delayed or collapsed fusion because of poor bone quality [1,10,11]. In spinal arthrodesis, the main role of screw fixation is to provide immediate stability to the instrumented vertebrae until bridging bone is formed on the fusion bed. Delay or failure in the formation of the bridging bone increases the risks of instrument failure and pseudoarthrosis. Therefore, fusion enhancement techniques such as facilitation of early bone formation could be beneficial for patients with osteoporosis who need to undergo spinal arthrodesis.

Bone morphogenetic proteins (BMPs) bind to specific cell-surface receptors and modify the behavior of many different types of cells [12]. For example, the activation of BMP receptors induces the differentiation of osteoblasts, resulting in increased potential for bone formation [13]. Of the approximately 40 members of the transforming growth factor beta superfamily, BMP-2, -4, -6, and -7 have been studied the most often for their effects on bone remodeling [5,14]. Clinical studies with recombinant human BMP-2 (rhBMP-2) and rhBMP-7 have shown that rhBMPs can accelerate bone fusion. However, there are negative side effects, including pseudoarthrosis, heterotopic ossification, and neural tissue compression because of bone overgrowth [15–20]. Although rhBMPs have undesirable side effects in nonosteoporotic conditions, rhBMPs may produce favorable effects in patients with estrogen-deficient osteoporosis needing spinal fusion. To date, there has been no clinical study evaluating the usefulness of BMPs specifically in the osteoporotic spine, although the effect of BMPs on the osteoporotic animal spine has been investigated to a very limited extent [4,21].

In ovariectomized rats, BMP-7 can overcome the detrimental effects of estrogen deficiency on posterolateral spinal fusion and generate a relatively robust fusion [4]. In addition, although 30 μg of BMP-7 exhibited only partial fusion, a higher dose (90 μg) showed significantly higher fusion rates than in control animals [4,21]. These data suggest that spinal fusion using rhBMP-7 was able to overcome the inhibitory effects of estrogen deficiency on spinal fusion. Furthermore, it is well established from clinical and basic studies that rhBMP-2 can enhance bone formation and achieve excellent fusion rates in normal individuals [16,17,22]. However, despite the potentially important clinical implications for osteoporotic patients undergoing spinal fusion, there have been no reports on the effects of rhBMP-2 on the rate of fusion in osteoporotic animals. Therefore, to provide this information, the present study was designed to determine the effect of rhBMP-2 on spinal fusion rates, as determined by microcomputed tomography (micro-CT) in osteoporotic rats.

Methods

Animals

Ten-week-old female Sprague-Dawley rats (n=60) were obtained from Orient Bio (Gyonggi, Korea) and housed singly in pathogen-free ventilated cages with a 12-hour light/dark cycle. The rats were allowed free access to tap water and standard rodent chow (Cargill, Gyonggi, Korea) that contained 1.35% (wt/wt) calcium and 0.44% (wt/wt) phosphate and no vitamin D. Although a low-calcium diet (<0.3% wt/wt) can lower bone mineral density (BMD), the chow used here did not affect BMD [23].

Experimental design and surgery

After 2 weeks of acclimatization, 40 rats were ovariectomized to induce osteoporosis and 20 were sham operated. All rats in the ovariectomy (OVX) and BMP groups underwent bilateral ovariectomy, and those in the Sham group underwent the same procedure, except that the ovaries were identified but not removed.

After 6 weeks, all rats underwent unilateral posterolateral intertransverse fusion with autologous iliac bone (AIB). Anesthesia was induced with 5% isoflurane and maintained with 2.5% isoflurane; during maintenance, oxygen was supplied through a coaxial nose cone. A posterior midline incision was made over the spinous process on L4–L5. After the right-side dorsal incision, the L4 and L5 transverse processes were exposed by muscle splitting between the multifidus and erector spinae muscles [24,25]. The cortical bone of the transverse processes of L4 and L5 was decorticated with an electronic bur under microscopy, and about 0.25 g of AIB was harvested from the ipsilateral iliac bone. Collagen gels (3.7 mg porcine skin type I; Bioland, Chungnam, Korea) were soaked in either 90 μg saline or rhBMP-2 (Daewoong Bio, Gyeonggi, Korea) and placed between the decorticated transverse processes of L4 and L5, and AIB was laid on the collagen.

Three groups of 20 rats were studied: Sham (sham operated+fusion using saline-soaked collagen and AIB), OVX (OVX+fusion using saline-soaked collagen and AIB), and BMP (OVX+fusion using rhBMP-2–soaked collagen and AIB) (Fig. 1). Twelve rats in each group were allocated for the assessment of bone turnover activity by real-time reverse-transcription polymerase chain reaction (RT-PCR), with six used at 3 and 6 weeks and two were allocated for histology, one each at 3 and 6 weeks. The remaining six rats in each group had micro-CT images taken at 3, 6, 9, and 12 weeks. Bone fusion rates and BMD were obtained from the serial micro-CT images. This experimental protocol was approved by the Institutional Animal Care and Use Committee of the Clinical Research Institute, National University Hospital, Seoul, Korea.

Bone density, bone volume, and fusion assessment

To assess fusion (the formation of bridging bone between transverse processes) and to measure the bone

density (BD) and bone volume (BV) of intertransverse areas, three-dimensional (3D) micro-CT was performed (NFR-Polaris-G90; NanoFocusRay, Jeonbuk, Korea) at postoperative Day 1 and at weeks 3, 6, 9, and 12. Scans were from the lower end plate of the S2 vertebral body and proceeded cranially with images, including 720° views, acquired at 70 kVp, 80 μA, and 100 ms per frame. The reconstructed image size was 1,024×1,024 pixels, the voxel size was 56.179×56.179×123.594 μm, and 512 slices were acquired. To measure BV (mm^3) at the graft site, the axial image was converted to 3D by Digital Imaging and Communications in Medicine software (Lucion; Infinite, Seoul, Korea). For BD, we measured the area between the upper end plate of L4 and the lower end plate of L5 with a threshold of more than 700, and BD was expressed as the ratio of this area/the total area.

Analysis of gene expression by real-time RT-PCR

Total intertransverse areas were harvested by microscopic dissection, and RNA was extracted in TRIzol reagent (Invitrogen, Carlsbad, CA, USA). For complementary DNA synthesis, 1 μg of RNA was reverse transcribed using random hexamer primers (Invitrogen) and Impron II reverse transcriptase (Invitrogen).

For analysis on the Applied Biosystems sequence detection system 7,000, a master mix was prepared containing 6.25 μL water, 1.25 μL probe (2.5 M), and 12.5 μL TaqMan PCR 2× master mixture. Fifty nanograms of reverse-transcribed total RNA in 5 μL was added as the PCR template in 96-well optical plates, and the results were analyzed on the Prism 7,000 Sequence Detection System (Perkin-Elmer Applied Biosystems, Lincoln, CA, USA). The following PCR conditions were used. After initial activation of uracil-*N*-glycosylase at 50°C for 2 minutes, AmpliTaq Gold was activated at 95°C for 10 minutes. This was followed by 45 cycles of denaturation at 95°C for 15 seconds and annealing and extension at 60°C for 1 minute per cycle. Polymerase chain reaction primer and probe sets were obtained from Invitrogen for *alkaline phosphatase* (*ALP*), *osteocalcin*, *Runx2*, *Smad1*, *Smad5*, and *tartrate-resistant acid phosphatase* (*TRAP*). The expression levels of target genes were normalized to the internal control rat *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) (FAM/MGB Probe, primer limited), and fold changes were relative to the data obtained at Day 1.

Histological evaluation

Samples including the L4–L5 intertransverse process fusion area and adjacent vertebral bodies were fixed with 4% paraformaldehyde. The specimens were decalcified in 5% nitric acid for 3 days, washed in distilled water, and embedded in paraffin. Unilateral sagittal sections (5 μm) were obtained from the midline of the intertransverse process area. The sections were stained with Safranin O with hematoxylin and fast green and observed under light microscopy.

Statistical analysis

The results are presented as mean±standard deviation. Differences between groups in fusion status were analyzed

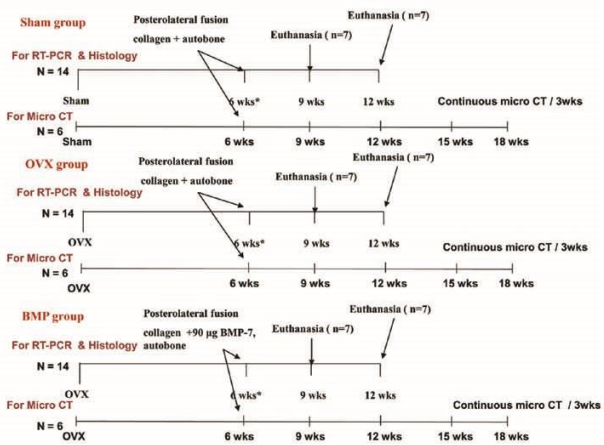

Fig. 1. Experimental groups and time schedule. Forty rats in the ovariectomy (OVX) and bone morphogenetic protein (BMP) groups received a bilateral ovariectomy, and 20 rats underwent a sham operation (Sham group). Six weeks after OVX, all rats underwent unilateral spine fusion using autologous iliac bone. To assess molecular and histological analyses, seven rats in each group were euthanized 3 and 6 weeks after fusion. The sequential computed tomography (CT) evaluations in the remaining rats were obtained every 3 weeks until 12 weeks after surgery. RT-PCR, real-time reverse-transcription polymerase chain reaction.

Table 1
The value of bone density in three groups

After fusion surgery	Sham group	OVX group	BMP group	p Value (Sham vs. OVX vs. BMP)
1 d	0.90±0.041	0.77±0.029	0.75±0.057	.004
3 wk	0.96±0.049	0.81±0.022	0.93±0.047	.002
6 wk	0.93±0.088	0.78±0.031	0.92±0.033	.004
9 wk	0.92±0.081	0.74±0.028	0.92±0.047	.004

OVX, ovariectomy; BMP, bone morphogenetic protein.

using the chi-square test. To identify significant differences between groups in BD, BV, and gene expression, one-way analysis of variance and post hoc analysis were used. A p value <.05 was considered significant.

Results

Bone density

As expected, there was a significant difference in BD between the Sham group and the other two groups at 1 day after fusion surgery (0.90±0.041, 0.77±0.029, and 0.75±0.057, respectively; p=.004, Sham vs. OVX vs. BMP). Thus, the OVX and BMP groups, both of which were ovariectomized, were osteoporotic relative to the Sham group. In addition, the BD values for the BMP group only recovered to Sham levels; this was seen at 3, 6, and 9 weeks after fusion surgery. In summary, the BMP group exhibited rapid new bone formation at the graft site, whereas for the OVX group, all BD values obtained after fusion were below those of the Sham and BMP groups (Table 1).

Bone volume

At spinal fusion for all groups, AIB (about 0.25 g) was grafted onto the intertransverse processes, and predictably at 1 day after surgery, the BV on the fusion bed did not differ significantly between groups (p=.599). However, at 3 weeks and thereafter, the BMP group had significantly increased BV at the graft site relative to the OVX group (p=.0005) (Table 2). Indeed, 3D micro-CT images showed that the BMP group had incorporated new bone into the transverse processes at 3 weeks. This could imply that both intramembranous and endochondral ossifications occurred in this early period. After 3 weeks, the Sham and OVX groups exhibited a progressive decline in BV; however, the BV of the Sham group remained higher than that of the OVX group at every time point. In addition, at 12 weeks after fusion, all groups exhibited more closely packed fusion masses compared with those at the early periods (Fig. 2).

Fusion assessment

In the Sham group, bridging bone was seen across the intertransverse area in 1 of 6 rats at 9 weeks and in 3 of 6 rats at 12 weeks. The OVX group had bridging in 1 of 6 rats at 9 weeks and in 2 of 6 rats at 12 weeks. However, all six rats in the BMP group exhibited bridging at 3 weeks and thereafter (Table 3).

Analysis of gene expression in the intertransverse area

In the Sham and OVX groups, the expression of osteoblast-related genes (*ALP, Runx2*), the osteoclast-related gene (*TRAP*), and BMP signaling mediators (*Smad1 and Smad5*) showed a decreasing trend between 3 and 6 weeks after fusion surgery (Fig. 3), whereas the expression of osteocalcin was maintained or increased over this period. The osteoblast/osteoclast-related genes were higher in the Sham than the OVX group at 3 weeks but lower in the Sham at 6 weeks. This might mean that bone remodeling in the Sham group progressed earlier than that in the OVX group. However, although there was an apparent difference in the expression levels of these genes between the Sham and OVX groups at 3 and 6 weeks after fusion surgery, this was not significant (p>.05).

Histological evaluation

At 3 and 6 weeks after surgery, a range of tissues including fibrous, cartilaginous, trabecular, and bone were seen in the space between the superior and the inferior transverse

Table 2
The fusion rate in three groups

After fusion surgery	Sham group	OVX group	BMP group	p Value (Sham vs. OVX vs. BMP)
1 d	0/6 (0%)	0/6 (0%)	0/6 (0%)	
3 wk	0/6 (0%)	0/6 (0%)	6/6 (100%)	.000
6 wk	0/6 (0%)	0/6 (0%)	6/6 (100%)	.000
9 wk	1/6 (16.6%)	1/6 (16.6%)	6/6 (100%)	.004

OVX, ovariectomy; BMP, bone morphogenetic protein.

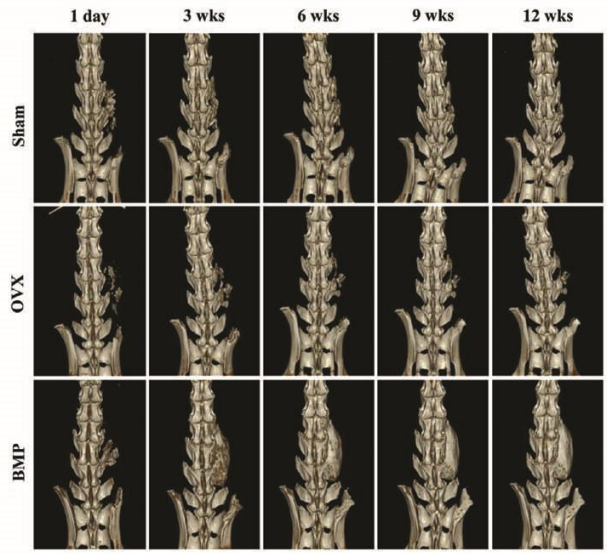

Fig. 2. Microcomputed tomography scanning of fusion masses and three-dimensional reconstructed images in all groups after surgery. There is a grafted and new bone between intertransverse processes. OVX, ovariectomy; BMP, bone morphogenetic protein.

processes. At 3 weeks, the Sham and OVX groups also had scattered bone fragments intermingled with fibrous tissue between transverse processes. In the Sham group at 6 weeks, the bone fragments were decreased in size and compacted into a density similar to the bridging bone. However, in the OVX group at this time, there was a large amount of fibrous tissue among the bone fragments. In the BMP group at 3 weeks, the mature bone, together with trabecular and cartilaginous tissues, merged with the cortical bone of the transverse processes. This could mean that with BMP-2 in this system, endochondral and intramembranous ossifications can occur simultaneously. Last, at 6 weeks in the BMP group, the bone mass encompassing the transverse processes contained mature trabecular areas and thick cortical bone, and there was no evidence of cartilaginous remnants (Fig. 4).

Discussion

The present study showed that the use of rhBMP-2 achieved early fusion, with an increased fusion rate, in the osteoporosis rat spine fusion model.

Bone morphogenetic proteins are known to induce mesenchymal stem cells into osteogenic cells and bone formation in a stepwise fashion [12,26]. Indeed, systemic administration of rhBMP-2 increased bone formation in Type I and Type II osteoporosis animal model [27]. Although there was a report that rhBMP-7 can overcome the negative effects of estrogen deficiency on osteoporosis rat spine fusion [4], there have been no studies showing augmentation of spine fusion by rhBMP-2 in OVX rats. The present study has revealed that rhBMP-2 can have a positive effect on spine fusion in estrogen-deficient rats.

Table 3
The bone volume in three groups

After fusion surgery	Sham group	OVX group	BMP group	p Value (Sham vs. OVX vs. BMP)
1 d	59.9±8.70	53.7±5.71	53.3±18.82	.599
3 wk	41.8±15.16	34.37±14.45	364.8±180.11	.000
6 wk	32.5±12.10	24.0±7.65	300.8±152.26	.000
9 wk	25.5±11.84	18.0±5.50	314.2±147.50	.000

OVX, ovariectomy; BMP, bone morphogenetic protein.

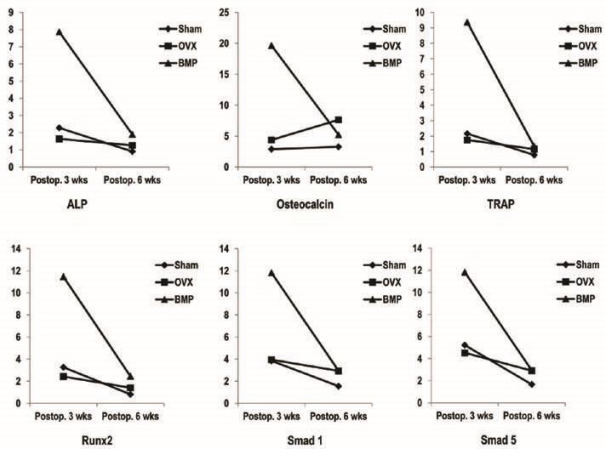

Fig. 3. The value of messenger RNA (mRNA) expression in fusion mass. The mRNA expression levels relative to *glyceraldehyde 3-phosphate dehydrogenase* showed that the all mRNA expression levels of bone morphogenetic protein (BMP) groups at 3 weeks after surgery increased significantly compared with those of Sham and ovariectomy (OVX) groups. However, all levels of the BMP groups decreased as much as those of Sham and OVX groups at 6 weeks after surgery. Sham group had a similar pattern in the change of mRNA as that of the OVX group.

One report showed that both BMP-2 and -7 can induce the osteogenic differentiation of mesenchymal stem cells from patients with osteoporosis, and it was reported in another study that 90 μg of rhBMP-7, but not 30 μg, could overcome the negative effects of estrogen deficiency on spinal fusion in osteoporotic rats [4,28]. Therefore, as we are not aware of any publications on the effective dose of rhBMP-2 in the OVX rat spine fusion model, we selected the 90-μg dose.

Consistent with our earlier work [10], the present study showed that BD was significantly decreased after 6 weeks in the bilateral OVX model, making it feasible to examine spinal fusion in osteoporotic rats. For this purpose, we performed a power analysis that suggested, assuming a 10% drop-out rate, that 45 rats were needed (power=0.8) for the present study. Therefore, we started this study with 60 rats, and because there were no dropouts, the results are statistically robust. Because excessive bilateral removal of iliac bone can have long-term effects on rat locomotion, only the minimum amount required (around 0.25 g) was removed for AIB grafting.

It has been reported that evaluation of fusion status can be achieved by manual palpation or biomechanical bending tests and that these parameters might give more reproducible results than imaging [29,30]. However, despite relatively poor reproducibility, micro-CT is noninvasive and can therefore provide time-course data on single animals [31]. It will therefore be important for future studies on fusion status to compare data obtained by micro-CT, manual palpation, and biomechanical bending tests.

The micro-CT study described here showed that in the BMP group alone, a large bridging bone mass appeared between intertransverse processes as early as 3 weeks after surgery and that the BMP group exhibited 100% fusion at the same time. In addition, in this group, the expression of osteoblast/osteoclast-related genes was significantly greater at 3 weeks compared with the Sham and OVX groups. Histological examination showed that more bone may have been formed through intramembranous than through endochondral ossification. These results reveal that, in the presence of rhBMP-2, bone healing and formation progressed quickly by activation of intracellular signal mediators such as Smad1 and Smad5. Although the mere presence of cortical continuity without mature trabeculation does not imply that the bone formation is of good quality, as in osteopetrosis, rhBMPs are effective in achieving early spine arthrodesis in patients with estrogen-deficient osteoporosis. However, to our concern, there appeared to be excessive bone mass at the graft site in the BMP-2–treated osteoporotic group. This suggests that, even in the presence of osteoporosis, the control of excessive and detrimental bone formation will be the key to the clinical value of BMPs [18–20]. Further studies are needed to identify the best methods to curtail the undesirable side effects of rhBMPs.

Although the mean BV of the fusion bed was greater in the Sham group than in the ovariectomy group at 3, 6, 9, and 12 weeks, the difference in BV of the fusion bed between the Sham and ovariectomy groups was not significant at 3, 6, and 9 weeks after fusion surgery. This result raises

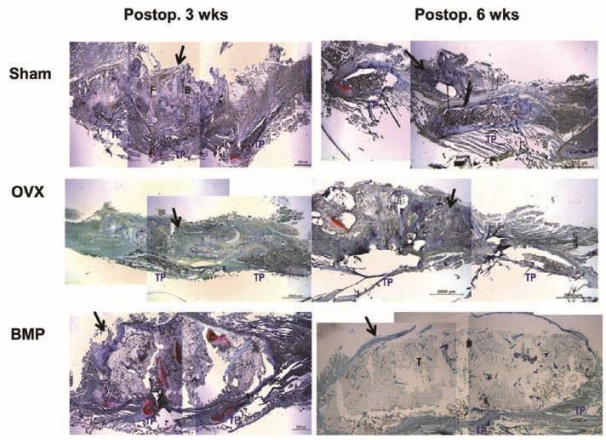

Fig. 4. Histological examination of fusion mass. At 3 weeks, there was no endochondral ossification with cartilage tissue (bright red-stained tissue) on the grafted site (arrow) of Sham and ovariectomy (OVX) groups. However, the bone morphogenetic protein (BMP) group had an enormous endochondral ossification with cartilage tissue from 3 weeks after surgery. At 6 weeks after fusion surgery, in the Sham group, bone fragments were decreased in size and compacted in density similar to the bridging bone. However, in the OVX group, there was a large amount of the fibrous tissue among bone fragments at 6 weeks postfusion. However, there was a huge bone mass with the matured trabecular area in the BMP group. B, bone; C, cartilage; F, fibrous tissue; T, trabecular area; TP, transverse process.

a similar question to the one you ask about whether the osteopenic condition in the ovariectomy group is meaningful comparing the bone quality with that in the Sham group. However, we think that the following three results address the observation of no difference in BV between the two groups. First, the difference in BD between the Sham and ovariectomy groups from Day 1 to 9 weeks after the fusion surgery was statistically significant. This result means that the osteopenic or osteoporotic condition in the ovariectomy model was recreated successfully. Second, histological evaluation at 6 weeks after the fusion surgery showed that, in the Sham group, bone fragments were smaller and of more compact density and therefore similar to bridging bone. In the ovariectomy group, a large amount of fibrous tissue was observed among bone fragments, indicating a delayed fusion process in the ovariectomy group. Third, analysis of gene expression in the fusion bed showed that bone remodeling had progressed earlier in the Sham group than in the ovariectomy group. These three results suggest strongly that the bone biology differed between the Sham and ovariectomy groups in this study. However, we acknowledge that further studies with a larger number of rats are needed to confirm whether there is a real difference in BV in the fusion bed between the two groups.

One limitation of this study is the absence of a detailed histomorphometric analysis. Such an analysis could reveal novel details of the endochondral and intramembranous ossification processes. Furthermore, bilateral spine fusion could be performed in the future to more accurately reflect the clinical setting. In addition, although all rats were housed under the same environmental conditions, it would be useful to measure blood levels of calcium and vitamin D to determine whether these play a part in the bone remodeling observed. Future studies could also more completely address intracellular mechanisms in OVX rats and the specific changes that occur in both osteoblasts and osteoclasts.

Nevertheless, the present study has revealed, for the first time as far as we are aware, that 90 μg of rhBMP-2 delivered locally on a collagen sponge has a positive effect in the OVX rat spinal fusion model described here. Specifically, with rhBMP-2, bone fusion was accelerated and both intramembranous and endochondral ossification were simultaneously affected. It is clear that further studies are required to establish an effective and safe dose of BMPs for improving outcomes of spinal fusion surgery.

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References

[1] Chin DK, Park JY, Yoon YS, et al. Prevalence of osteoporosis in patients requiring spine surgery: incidence and significance of

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osteoporosis in spine disease. Osteoporos Int 2007;18: 1219–24.
[2] Nagahama K, Kanayama M, Togawa D, et al. Does alendronate disturb the healing process of posterior lumbar interbody fusion? A prospective randomized trial. J Neurosurg Spine 2011;14: 500–7.
[3] Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. Lancet 2011;377:1276–87.
[4] 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 2008;33:242–9.
[5] Kanakaris NK, Petsatodis G, Tagil M, Giannoudis PV. Is there a role for bone morphogenetic proteins in osteoporotic fractures? Injury 2009;40(3 Suppl):S21–6.
[6] Boden SD. Overview of the biology of lumbar spine fusion and principles for selecting a bone graft substitute. Spine 2002;15: S26–31.
[7] Bridwell KH, Sedgewick TA, O'Brien MF, et al. The role of fusion and instrumentation in the treatment of degenerative spondylolisthesis with spinal stenosis. J Spinal Disord 1993;6:461–72.
[8] Okuda S, Miyauchi A, Oda T, et al. Surgical complications of posterior lumbar interbody fusion with total laminectomy in 251 patients. J Neurosurg Spine 2006;4:304–9.
[9] Glassman SD, Polly DW, Bono CM, et al. Outcome of lumbar arthrodesis in patients sixty-five years of age or older. J Bone Joint Surg Am 2009;91:783–90.
[10] Park SB, Lee YJ, Chung CK. Bone mineral density changes after ovariectomy in rats as an osteopenic model: stepwise description of double dorso-lateral approach. J Korean Neurosurg Soc 2010;48: 309–12.
[11] Coe JD, Warden KE, Herzig MA, McAfee PC. Influence of bone mineral density on the fixation of thoracolumbar implants. A comparative study of transpedicular screws, laminar hooks, and spinous process wires. Spine 1990;15:902–7.
[12] Granjeiro JM, Oliveira RC, Bustos-Valenzuela JC, et al. Bone morphogenetic proteins: from structure to clinical use. Braz J Med Biol Res 2005;38:1463–73.
[13] Deal C. Future therapeutic targets in osteoporosis. Curr Opin Rheumatol 2009;21:380–5.
[14] Wu X, Shi W, Cao X. Multiplicity of BMP signaling in skeletal development. Ann N Y Acad Sci 2007;1116:29–49.
[15] Rogozinski A, Rogozinski C, Cloud G. Accelerating autograft maturation in instrumented posterolateral lumbar spinal fusions without donor site morbidity. Orthopedics 2009;32:809.
[16] Rihn JA, Makda J, Hong J, et al. The use of RhBMP-2 in single-level transforaminal lumbar interbody fusion: a clinical and radiographic analysis. Eur Spine J 2009;18:1629–36.
[17] Burkus JK, Gornet MF, Schuler TC, et al. Six-year outcomes of anterior lumbar interbody arthrodesis with use of interbody fusion cages and recombinant human bone morphogenetic protein-2. J Bone Joint Surg Am 2009;91:1181–9.
[18] Shen HX, Buchowski JM, Yeom JS, et al. Pseudarthrosis in multilevel anterior cervical fusion with rhBMP-2 and allograft: analysis of one hundred twenty-seven cases with minimum two-year follow-up. Spine 2010;35:747–53.
[19] Shah RK, Moncayo VM, Smitson RD, et al. Recombinant human bone morphogenetic protein 2-induced heterotopic ossification of the retroperitoneum, psoas muscle, pelvis and abdominal wall following lumbar spinal fusion. Skeletal Radiol 2010;39:501–4.
[20] Smoljanovic T, Bojanic I, Vlahovic Z. Safety of posterior interbody fusions of the lumbar spine using rhBMP-2. J Spinal Disord Tech 2010;23:78.
[21] Moazzaz P, Gupta MC, Gilotra MM, et al. Estrogen-dependent actions of bone morphogenetic protein-7 on spine fusion in rats. Spine 2005;30:1706–11.
[22] Itoh H, Ebara S, Kamimura M, et al. Experimental spinal fusion with use of recombinant human bone morphogenetic protein 2. Spine 1999;24:1402–5.
[23] Omi N, Ezawa I. Animal models for bone and joint disease. Low calcium diet-induced rat model of osteoporosis. [in Japanese]. Clin Calcium 2011;21:173–80.
[24] Boden SD, Schimandle JH, Hutton WC. An experimental lumbar intertransverse process spinal fusion model. Radiographic, histologic, and biomechanical healing characteristics. Spine 1995;20:412–20.
[25] Wiltse LL, Spencer CW. New uses and refinements of the paraspinal approach to the lumbar spine. Spine 1988;13:696–706.
[26] Salamon ML, Althausen PL, Gupta MC, Laubach J. The effects of BMP-7 in a rat posterolateral intertransverse process fusion model. J Spinal Disord Tech 2003;16:90–5.
[27] Turgeman G, Zilberman Y, Zhou S, et al. Systemically administered rhBMP-2 promotes MSC activity and reverses bone and cartilage loss in osteopenic mice. J Cell Biochem 2002;86:461–74.
[28] Pountos I, Georgouli T, Henshaw K, et al. 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.
[29] Erulkar JS, Grauer JN, Patel TC, Panjabi MM. Flexibility analysis of posterolateral fusions in a New Zealand white rabbit model. Spine 2001;26:1125–30.
[30] Hidaka C, Goshi K, Rawlins B, et al. Enhancement of spine fusion using combined gene therapy and tissue engineering BMP-7-expressing bone marrow cells and allograft bone. Spine 2003;28: 2049–57.
[31] Boutroy S, Bouxsein ML, Munoz F, Delmas PD. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. J Clin Endocrinol Metab 2005;90:6508–15.