Effectiveness of combination hydroxyapatite and BMP-2 as alternatives to autologous bone graft in a male osteopenia rat model

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Doctoral Dissertation
Submitted to the Department of Medicine
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Medicine

Seung Jun Ryu

December 2016

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유승준 올림

<Table of contents>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Effectiveness of combination hydroxyapatite and BMP-2 as alternatives to autologous bone graft in a male osteopenia rat model

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Purpose: The aim of this study was to investigate the effect of not only orchiectomy on bone formation and resorption but also combination hydroxyapatite and BMP-2 as alternatives to autologous bone graft in a male osteopenia rat model.

Materials & Methods: A total of 41 Sprague-Dawley rats were randomized into 5 study groups. Group 1 have 9 rats and another group have 8 rats. Orchiectomy and posterior lumbar fusion were performed bilaterally on 10-week-old rats. Group 1 underwent posterior lumbar fusion with autologus bone graft, which was obtained

from the tail bone, Group 2 received a hydroxyapatite, beta Tricalcium Phosphate (HA/b-TCP) graft, and Group 3 received HA/b-TCP with BMP-2. Group 4 underwent orchiectomy without posterior spinal fusion, and Group 5 underwent sham surgery as the control group. Each group was euthanized 12 weeks post-operatively and lumbar vertebras were obtained. Serum bone markers including serum osteocalcin, alkaline phosphatase (ALP), and collagen type 1 cross-linked C-telopeptide (CTX) were analyzed 12 weeks post-operatively. Femoral and lumbar spine BMD were measured using micro computed tomography scans. Fusion assessment with manual palpation was used to compare the effects of combination hydroxyapatite and BMP 2 to those of autologous bone. We adopt the grading system from the literature and scored the fusion status, we were also able to divide between grade 0 and 1.

Results: After orchiectomy, serum osteocalcin, ALP, CTX decreased gradually; in contrast, N-terminal type 1 procollagen (P1NP) showed a slight increase but no significant change. Lumbar BMD in the orchiectomy group was 0.492 (±0.038) at week 12 post-surgery. Fusion scores showed moderate to good bone growth in the autologus bone graft group, moderate bone growth in the HA/b-TCP graft group, and excellent bone growth in the HA/b-TCP with BMP-2 graft group.

Conclusion: The effectiveness of autologous bone used for posterior spinal fusion is already well known. This study revealed that the combination of hydroxyapatite and BMP-2 is also effective for spinal bone fusion in male osteopenia.

Key words: osteoporosis, orchiectomy, bone mineral density, serum bone marker

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I. Introduction

Osteoporosis is becoming a global problem; as the Asian geriatric population rapidly increases, so does the total number of osteoporotic patients. Osteoporosis is a condition characterized by low bone mineral density, microarchitectural deterioration of bony tissue, and a consequent increase in the risk of fracture, predominantly in the hip, spine, and forearm.[1-3]

The incidence of osteoporosis is higher in females, who typically have a lower bone mass than males. Menopause leads to bone loss at a rate between 3 and 6% every year over a 5-year period. The prevalence of osteoporosis in spinal surgery patients older than 50 years was found to be 14.5% and 51.3% for males and females, respectively.[4] Thus, research regarding osteoporosis to date has primarily involved postmenopausal women.[5] The spinal fusion research so far was conducted primarily targeted animal models of osteoporosis in postmenopausal female.

However in the spinal field, male osteoporosis is also important. Among osteoporosis patients in whom fractures occur, fracture-associated morbidity and mortality is reported to be higher in males than in females.[6] To the best of our knowledge, this is the first study

looking into spinal fusion in orchiectomized rat model. Therefore, a study of male osteoporosis is important in the field of spinal surgery. Academic interest in male osteoporosis has increased in recent years.[7] There are clinical reports of an increased incidence of osteoporosis in elderly men[7] and prostate cancer patients following orchiectomy,[8] suggesting that androgen deprivation leads to osteoporosis.[9] Considering this, alternatives to autologous bone graft in spinal surgery are needed given the increasing incidence of male osteopenia.

Hydroxyapatite (HA) is a ceramic with chemical composition similar to the mineral component of bone that has received attention for its biocompatibility and osteoconductivity. Researches using BMP-2 on Beta TCP/HA carriers is already actively underway both on human and animal models.

Bone morphogenic protein-2 (BMP-2) has been demonstrated in spinal fusion surgery by induction of osteogenesis[10,11,12] and it can be used for the treatment of open tibial fractures[13] or femoral defects in rats, tibial and ulnar defects in rabbits, femoral defects in sheep, mandibular defects in dogs, spinal fusion in dogs, and porous ingrowth in rats.[14] In vitro studies revealed several mechanisms of action in which BMP-2 stimulates osteogenesis.[15,16,17] These effects have been observed in not only normal bone mineral density animals but also osteoporotic animal models.[18] While it is obvious that BMP-2 increases bone fusion in the animal tibia, ulnar, femur, and vertebra, the relative importance of improved microarchitecture and improved intrinsic bone tissue quality on the vertebra fusion strength in a male osteopenia rat model are still to be examined.

The aim of this study was to investigate the effect of combination hydroxyapatite and BMP-2 as alternatives to autologous bone graft in a male osteopenia rat model.

This study's hypotheses are as follows;

1) Orchiectomy has a negative effect on bone formation and a positive effect on bone resorption in young male rats.

2) The combination of hydroxyapatite and BMP-2 have a positive effect on spinal fusion compared with autologous bone graft in a male osteopenia rat model.

II. Materials and Methods

1. Experimental animals

10-week-old male Sprague-Dawley rats (n=41) were purchased and acclimated separately in pathogen-free ventilated cages in a controlled environment (temperature 22±4℃, humidity 65±5%, day-night cycle 6:00-18:00). The rats were permitted free intake of tap water and standard rodent chow (SAFE, Augy, France) containing 8g/kg calcium, 4.2g/kg phosphorus, and 1,000IU/kg Vitamin D3. A total of 41 Sprague-Dawley rats were randomized into five study groups. Bilateral orchiectomy and posterior lumbar fusion were performed on 10-week-old rats. Group 1 underwent posterior lumbar fusion with autologus bone graft, which was obtained from its tail bone. After surgery one rat was expired. Group 2 received hydroxyapatite and beta Tricalcium Phosphate (HA/b-TCP) graft, and Group 3 received HA/b-TCP with BMP-2. Each group was euthanized 12 weeks post-operatively and lumbar vertebras were obtained. Group 4 underwent orchiectomy without posterior spinal fusion, and Group 5 underwent sham surgery as the control group.

Group 1: OCX + PSF (autologous bone graft from tail) (n = 9, one rat was expired)
Group 2: OCX + PSF (HA/b-TCP) (n = 8)
Group 3: OCX + PSF (HA/b-TCP + BMP-2) (n = 8)
Group 4: OCX + no PSF (n = 8)
Group 5: Sham surgery (no OCX, PSF) (n = 8)

Figure 1. Experimental groups and surgery performed
(OCX; orchiectomy, PSF; posterior spinal fusion)

2. Surgical procedures

All surgical procedures were in concordance with the regulations and authorization of the animal trial department in the Clinical Trial Center of Gangnam Severance Hospital.
Bilateral orchiectomy was performed in 10-week-old rats and posterior lumbar fusion was performed 1 week after orchiectomy. After orchiectomy, there is no complications. However, after posterior lumbar fusion of 25 rats, one rat was expired. Mortality rate was 4% on posterior lumbar fusion.

가. Method of orchiectomy prior to the posterior lumbar fusion

We performed orchiectomy on the rats 1 week prior to bone fusion surgery, using surgical equipment in the Biomedical research center of our hospital. We used the Scrotal approach, described as follows:
First, we administered intraperitoneal anesthesia using ketamine (80-100 mg/kg) and then, additional inhaled anesthesia (Halothane (1.5-3%) in 100% oxygen) on anesthetized rats via a coaxial nose cone for anesthesia maintenance was administered. Bilateral orchiectomy was performed via a scrotal approach. The anesthetized rat was placed supine on the operating table and its position was fixed using sticking tape. The scrotal hair was bilaterally shaved. (Figure 2A.)
Betadine prep was performed as an aseptic maneuver. (Figure 2B.) If the cremaster muscle was stimulated during the betadine prep resulting in ascension of the testes, a downward stroke was performed to lower the testes back into place. A small, 1.0-cm median incision was made through the skin at the tip of the scrotum. (Figure 2C.) The cremaster muscles were opened with a small, 7-mm incision. At the entrance to the scrotal cavity, the testicular fat pad was located and pulled through the incision using

blunt forceps. The caudal epididymis was pulled out along with the testis, followed by the caput epididymis, the vas deferens, and the testicular blood vessels. (Figure 2D.) After identifying the testis, epididymis, vas deferens, and testicular blood vessels, a single ligature was placed on the spermatic cord around the vas deferens and the blood vessels (Figure 2E.). The testis and epididymis were removed. This procedure was repeated on the other testis and epididymis. The cremaster muscle and scrotal skin were sutured layer by layer. (Figure 2F.) The same preparation was performed on animals in the sham operation group, allowing the authors to visually identify the testis, epididymis, vas deferens, and testicular blood vessels. After visual identification, the cremaster muscle and skin were sutured without ligation or resection.

나. Posterior lumbar fusion technique

We performed the operation on 6 rats per day for quality control with lack of staff, 1 rat from each group to maintain homogeneity of the surgical environment. We first administered intraperitoneal anesthesia using ketamine (80-100mg/kg). To obtain the maintenance of anesthesia effect on every rat, so we used additional inhaled anesthesia (Halothane (1.5-3%) in 100% oxygen) on anesthetized rats. We performed bilateral posterior lumbar fusion on the rats, removed the fur on the broad area, and made preparations using betadine. (Figure 2G.) After midline skin incision, we detached the muscles around the vertebrae using a blade and exposed the lamina, facet joint, and the transverse process. We exposed the bilateral transverse processes of the two vertebral bodies, decorticated the periosteum using the blade, and placed the predetermined bone substitute and bone fusion material. (Figure 2H.) Group 1 underwent posterior lumbar fusion with autologus bone graft, which was obtained from its tail bone, Group 2 received a hydroxyapatite, beta Tricalcium Phosphate (HA/b-TCP) graft, and Group 3 received HA/b-TCP with BMP-2. Finally, the wound was sutured layer by layer. (Figure 2I.)

다. Post-operational management

After the surgery we administered antibiotics (gentamycin sulfate, 4mg/kg/day), put the rats in 15 minutes of convalescence in a warm basket, put them in cages, and moved them to the animal lab facility. The rats received antibiotics and analgesics, and their movements, appetite, wound, and neurological status were examined twice daily.

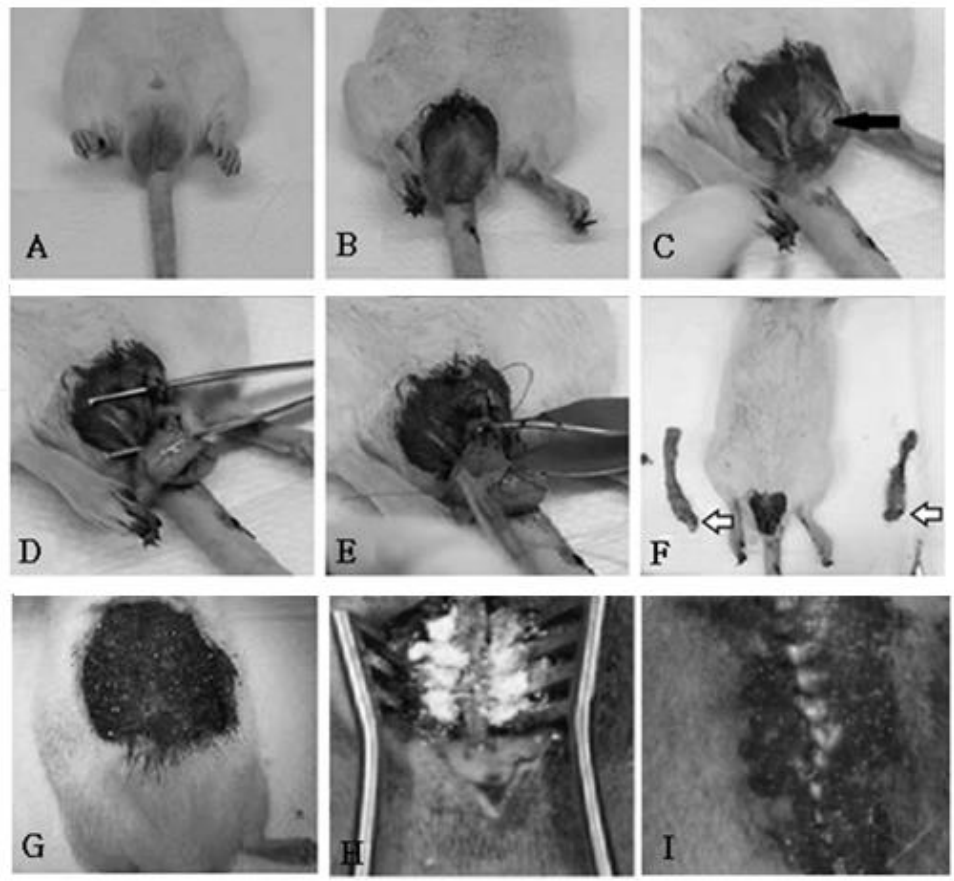

Figure 2. Procedures for orchiectomy and posterior spinal fusion in rat

A: scrotum was exposed and betadine preparation was performed. C: midline incision on scrotum, D: Identifying the testis, epididymis, vas deferens, and testicular blood vessels, E: a single ligature was placed on the spermatic cord around the vas deferens and the blood vessels, F: The bilateral testis and epididymis were removed, G: Removed the fur on the dorsal broad area, and made preparations using betadine, H: Posterior lumbar fusion was performed, I: Skin was sutured

3. Serum bone turnover marker analysis

Rats were maintained in a fasting state after midnight and placed in a single cage for ether anesthesia. Blood (about 5 cc) was collected via cardiac puncture (CP) in the morning under sufficient artificial light. Blood samples were clotted for 10 minutes at room temperature and then centrifuged for 15 min at 1000 G and 4°C. The serum was obtained, and stored at -20°C. Serum levels of P1NP, osteocalcin, and CTX were measured using ELISA kits (MyBioSource Inc., kit: MBS2506450, San Diego, CA, USA; Biomedical Technologies Inc., kit: BT-490, Stoughton, MA, USA; IDS Inc., kit: AC-06F1, Boldon, North-East England, UK). The ELISA kits all used a 96-well plate format. Serum samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. A biotinylated detection antibody specific to P1NP and an Avidin Horseradish Peroxidase (HRP) conjugate were then added to each microplate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only the wells that contained P1NP, biotinylated detection antibody, and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color turned yellow. Serum osteocalcin and CTX measurement were performed in a similar manner using a different ELISA kit.
Serum total alkaline phosphatase (ALP) and bone-specific ALP activities were determined by protein electrophoresis. The blood serum was placed into liquid in a capillary tube and exposed to an electric current in order to separate the serum protein components into five major fractions by size and electrical charge. Protein electrophoresis was performed at Seoul Medical Science Institute, and protein electrophoresis data were confirmed by a laboratory medicine specialist.

4. Micro-computed tomographic analysis

Rats were euthanized and the femur and lumbar spine were obtained from each rat. BMD was measured and fusion assessment was also performed in the femur and lumbar spine using micro computed tomography (SkyScan1173, Bruker-CT, Kontich, Belgium) and the NRecon (Ver. 1.6.9.4, Bruker, Kontich, Belgium) software. To measure Bone Volume (BV) (mm3) at the graft side, the axial image was converted to 3D by the NRecon software. Pixel images are 2240 x 2240 with a resolution of 27.70 ㎛. Areas of analysis were the L5 vertebral body from the lower end plate to 3 mm cranial to evaluate the calcified fusion mass at the inter spinous and transverse process area where original bone did not exist and the femora from the distal femora to 3 mm proximal.

5. Manual palpation analysis

Fusion assessment with manual palpation was used to compare the effects of combination hydroxyapatite and BMP-2 to those of autologous bone. The fused lumbar spine was manually palpated, and a lateral side-bending motion at the L4-5 level was analyzed with the motion at the adjacent levels above (L3-4) and below (L5-6).[19] Two independent observers, including the first author, a spine-neurosurgeon, and another researcher who were blinded to the grouping of animals tested the instability of the lateral side-bending motion at the operative spinal segment. We chose to adopt the grading system published by K. Sing et al. in the 2007 spine journal[20] and scored the fusion status; we were also able to divide between grade 0 and 1 as follows. (Table 1)

Table 1. Posterior spinal fusion scoring index

Grade	Description	Modified
0	No bone growth	No growth/No restricted motion
1	Minimal bone growth	1-40% bone growth/restricted motion
2	Moderate bone growth	41-70% bone growth/restricted motion
3	Good bone growth	71-90% bone growth/restricted motion
4	Excellent bone growth	91-100% bone growth/restricted motion

Grade	Description
0	Unrestricted motion in either plane
1	No segmental motion between adjacent vertebrae in lateral bending and flexion and extention planes

6. Statistical analysis

The results are presented as the mean ± standard deviation in the tables and as the mean ± standard error in the graphs. Statistical analysis was performed using SPSS software (version 20.0, IBM, Chicago, IL, USA). Differences in the fusion status between groups were analyzed using the one-way ANOVA test. The Mann-Whitney test was used to compare values between the Group 4 and 5. P-values less than 0.05 were considered statistically significant.

III. Results

1. Serum bone turnover marker findings

To assess the chemical factors associated with changes in BMD, we measured serum levels of the markers osteocalcin, total and bone specific ALP, and P1NP as bone formation markers, and CTX as a bone resorption marker. Comparison between Group 4 and Group 5 at 12 weeks post-operatively showed a difference in serum bone markers P1NP, osteocalcin, and CTX ($p=0.05$); however, serum bone-specific and total ALP did not show a significant difference ($p=0.513$ and $p=0.827$). Group 4 showed elevated levels of bone markers. (Table 2, Figure 3.)

When we observing the bone marker tracking, serial changes in serum bone markers resulted that serum levels of CTX and bone-specific and total ALP showed an abrupt decrease in Group 4 at POD 1 and 4 week, serum P1NP increased gradually, and serum osteocalcin showed a gradual increase and decrease. Serum Osteocalcin, Bone specific, total ALP and CTX were diminished, and Serum CTX and Bone Specific, total ALP were statistically significant ($p=0.015$, $p=0.014$, $p=0.019$) (Table 3)

Table 2. Levels of serum bone markers

	Group 4	Group 5	*p*-value
PINP(pg/10^{-4}L)	19.5±1.3	16±0.9	0.05
CTX(ng/mL)	22.2±1.7	15.9±1.6	0.05
Osteocalcin(ng/mL)	19.7±2.8	13.2±0.4	0.05
Bone ALP(U/dL)	7.7±1.1	6.5±0.1	0.513
Total ALP(U/dL)	8.4±1.6	7.4±3.3	0.827

P1NP, N-terminal type 1 procollagen; CTX, C-telopeptide; ALP, alkaline phosphatase.

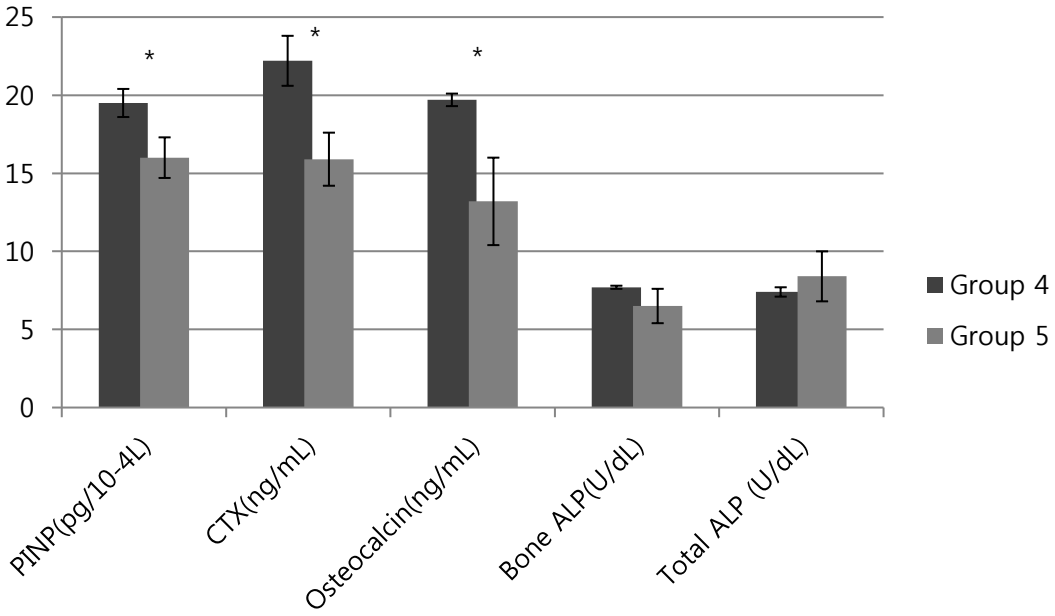

Figure 3. Comparison of serum bone markers at 12 weeks orchiectomy.

Group 4 showed elevated levels of bone markers, P1NP, osteocalcin, and CTX, which showed a significant difference.(*, *p*-value = 0.05)

Table 3. The Serial changes of Serum bone markers

	Pre-op	OCX 1 w	OCX 4 w	OCX 8 w	OCX 12 w	*p*-value
P1NP (pg/mL)	1213.7±177.6	1445.3±98.9	1605.8±145.0	1735.6±98.3	1948.6±130.8	0.098
CTX (ng/mL)	39.2±2.4	38.9±0.8	26.0±1.7	16.3±2.2	22.2±1.7	0.015*
Osteocalcin (ng/mL)	21.6±1.2	23.2±1.0	23.8±1.1	21.4±2.8	19.7±2.8	0.857
Bone ALP (Units/L)	198.0±9.7	161.8±11.2	59.8±2.8	55.7±2.9	76.6±11.7	0.014*
Total ALP (Units/L)	206±9.0	167±10.9	63±3.5	59.7±2.6	84±16.1	0.019*

OCX, orchiectomy; P1NP, N-terminal type 1 procollagen; CTX, C-telopeptide; ALP, alkaline phosphatase. *- statistically significant (*p*-value < 0.05)

2. Micro-computed tomographic findings

가. Bone mineral density

There was a statistically significant difference in BMD between the Group 4 and 5. Femoral BMD in the Group 4 and 5 were 0.28±0.01 g/cm^3 and 0.39±0.06 g/cm^3, respectively. Lumbar BMD in the Group 4 and 5 were 0.53±0.04 g/cm^3 and 0.64±0.05 g/cm^3, respectively. (Table 4) Micro CT axial images show low density of trabecular bone at the femur and vertebra body. (Figure 4.)

Table 4. BMD value of femur and lumbar

Group (POD)		BMD (g/cm^3)	
		Mean±SD	P
Femur	Group 4 (12 weeks)	0.28±0.01	0.008*
	Group 5 (12 weeks)	0.39±0.06	
Lumbar	Group 4 (12 weeks)	0.53±0.04	0.008*
	Group 5 (12 weeks)	0.64±0.05	

POD – post operation date, *- statistically significant (p-value < 0.05)

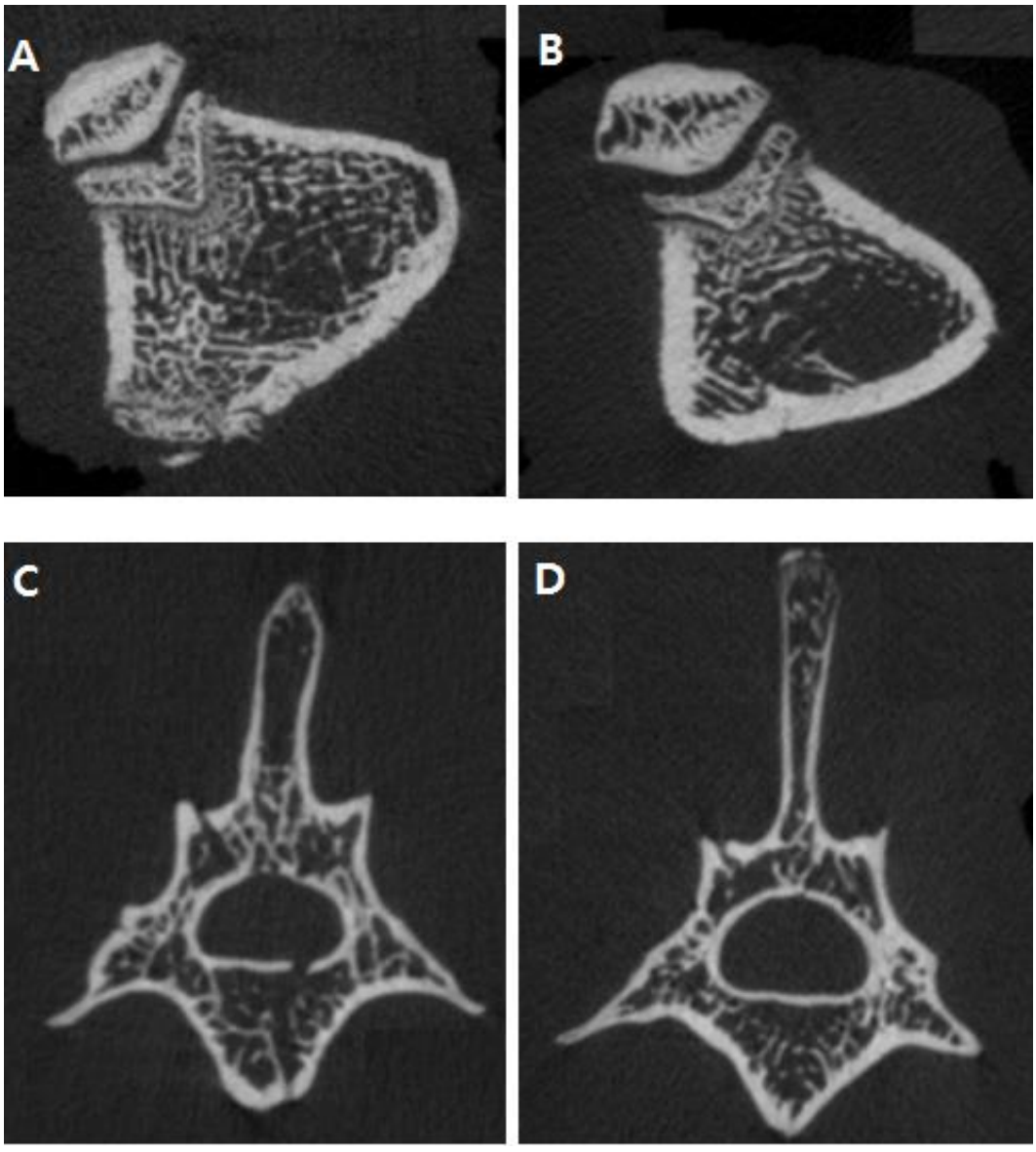

Figure 4. Micro CT axial images of femur and lumbar

A, B shows femur of the sham and OCX group rats at 12 weeks post-operatively

C, D shows lumbar of the sham and OCX group rats at 12 weeks post-operatively

나. Bone Volume

Group 1 are orchiectomized rats with posterior spinal fusion using an autologous bone graft from the tailbone, Group 2 are orchiectomized rats with posterior spinal fusion using hydroxyapatite and beta tricalcium phosphate, and Group 3 is additional bone morphogenic protein 2 usage compared to prepared rats in group 2. (Figure 1.) Group 1, 2, and 3 showed new bone formation volume as 32.9±6.22 mm3, 19.2±3.71 mm3, and 243.5±46.35 mm3, respectively. (Table 5) Each group showed a statistically significant difference in bone volume formation.

Table 5. The bone volume (mm^3) and fusion rate, score of posterior spinal fusion groups

Group 1	Group 2	Group 3	*p* - value
32.9±6.22	19.2±3.71	243.5±46.35	0.000*
3/8 (37.5%)	1/8 (12.5%)	8/8 (100%)	0.000*
2.3±1	1.8±0.7	3.9±0.4	0.000*

*- statistically significant (*p*-value < 0.05)

다. Sagittal section observation

Micro CT revealed low power magnification of a sagittal section through the fusion mass from a rat. Figure 5D, E, F. was reconstructed from Group 1, 2, 3 fusion mass. Bone was fused between inter lamina, but not enough bone formation was observed in Figure 5D. There is robust bone formation and fusion in Figure 5F. Continuity of cortical bone in fusion mass also well observed in Group 1, 3. (Figure 5D, F.) There is space between fusion mass, and continuity of cortical bone was absent in Group 2. (Figure 5E.)

3. Manual palpation result

Group 1, 2, and 3 had fusion rates of 37.5%, 12.5%, and 100%, respectively. The fusion scores were 2.3±1, 1.8±0.7, and 3.9±0.4, respectively. There was statistical significance (Table 5) between group 1 and group 3, group 2 and group 3. There was no statistically significant difference between group 1 and group 2 in fusion rate and fusion score. ($p = 0.442, 0.382$)

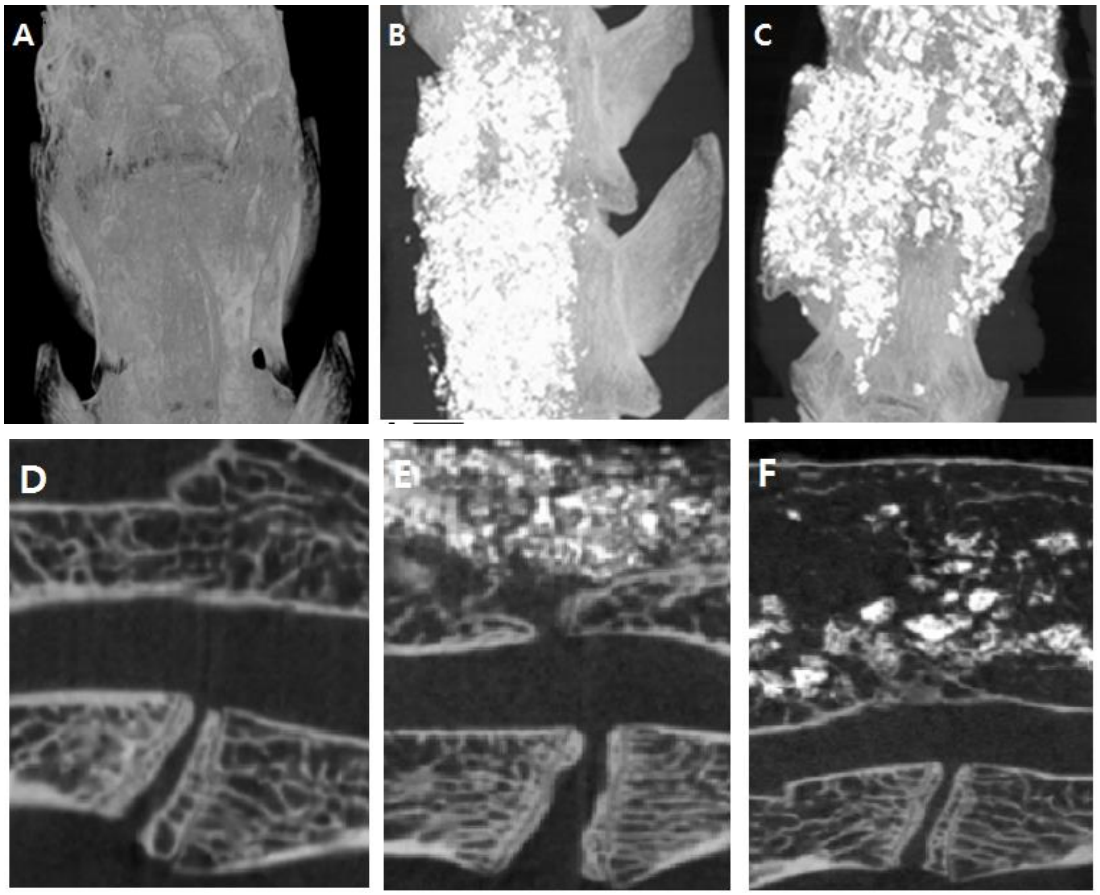

Figure 5. Bone formation of posterior spinal fusion groups

A, D. shows posterior spinal fusion with autobone from tail

B, E. shows posterior spinal fusion with HA/b-TCP

C, F. shows posterior spinal fusion with HA/b-TCP + BMP-2

IV. Discussion

For successful posterior lumbar fusion surgery, attention should be paid to the loss of bone parenchyma as a result of osteoporosis, which can result in instrument malpositioning and fusion failure.[21,22,23] We believe that this emphasizes the importance of evaluating the BMD of not only the lumbar bones but also materials for spinal fusion surgery.

Sex hormones are recognized as important factors in the maintenance of bone mass and architecture.[24,25] Increased bone resorption is observed in women after menopause, whereas in males, the decline in gonadal function with age is far more progressive.[7,26] The use of recombinant human bone morphogenic protein-2 to achieve posterolateral lumbar fusion and anterior lumbar fusion, and to treat open tibial fractures has already reported.[10,11,12,13]

We took this into account by analyzing the BMD of lumbar bones in a male osteoporosis rat model that involved orchiectomy to induce hypogonadism and found a way to overcome the above mentioned problem by using BMP-2.

Consistent with our previous study,[27] the present study showed osteopenia in the bilateral OCX model, making it possible to examine spinal fusion in osteopenic male rats. Because we used an autograft from the tailbone rather than the iliac bone and there were no dropouts, the results were statistically robust. The tailbone was used because excessive bilateral removal of the iliac bone can result in massive bleeding and morbidity.

Sex hormones have a key role in bone homeostasis in male. In case of estrogens, that regulate both osteoclast and osteoblast activity of men with expression of estrogen receptors.[28,29,30,31,32] Also, serum estrogens are related positively with spinal bone mineral density in healthy older men.[33,34]

The bilateral orchiectomy diminished functional activity of the testes, that may result in diminished sex hormone biosynthesis. Lack of sex hormone biosynthesis change bone metabolism and make osteopenic condition. To reveal mechanism of hypogonadal

osteopenia, we investigated the serum bone turnover makers.

In case of female osteoporosis, following estrogen deficiency after surgical menopause, the concentrations of the markers of turnover, including both the markers of resorption such as CTX and markers of bone formation such as osteocalcin and bone specific ALP, increase significantly.[34] In case of male osteoporosis, biochemical markers of osteoclast and osteoblast activity increase progressively after hypogondal condition with treatment of GnRH agonist.[36] In prostate cancer, GnRH agonists increase parathyroid hormone-mediated osteoclast activation,[37] suggesting that changes in skeletal sensitivity to parathyroid hormone play an important r ole in the pathogenesis of hypogonadal bone loss.

Likewise previous reports,[38,39] Our study showed increasing serum bone markers (serum osteocalcin, CTX, ALP, P1NP) in the OCX group 12 weeks post-operatively and revealed that osteoporotic changes occurring in young male rats with castration were related to high bone turnover.(Table 2, Figure 3.)

When we observing the bone marker tracking, Serum levels of CTX and bone-specific and total ALP showed an abrupt decrease in Group 4 at POD 1 and 4 week, serum P1NP increased gradually, and serum osteocalcin showed a gradual increase and decrease. (Table 3) Previously, Clarke et al[40] reported these dissassociation in the levels of the markers of bone formation 4 weeks after orchiectomy. Levels of serum bone specific alkaline phosphatase decreased immediately after orchiectomy, but osteocalcin increased. This report could not find its meaning. These changes in bone turnover are difficult to find its accurate meaning but would be related with complete shut down the synthesis of testosterone and activation of parathyroid hormone-mediated osteoclast.

The micro-computed tomographic study showed a statistically significant difference in BMD between Group 4 (OCX group) and Group 5 (sham group). (Table 2) Micro CT axial images showed increased vacancy and low density of the trabecular bone in the femur and vertebral body. (Figure 4.) These results confirmed the osteopenic environment in our posterior spinal fusion study.

Each group also showed a statistically significant difference in bone volume formation. Groups 1, 2, and 3 showed new bone formation volume as 32.9±6.22 mm^3, 19.2±3.71 mm^3, and 243.5±46.35 mm^3, respectively. Using BMP-2, Group 3 showed a large amount of new bone formation, as compared to the other groups. (Table 5, Figure 5A, B, C.)

Sectional images also demonstrated that the fusion masses in Group 3 contain abundant bone bridging and remodeling between lumbar vertebra. This was less evident in Group 1 where bone formation was present but did not completely bridge the adjacent lumbar vertebra. The Group 2 did not show any evidence of new bone formation at the fusion sites. (Figure 5D, E, F.) In the manual palpation comparison, there was a statistically significant difference between Group 1 and Group 3 and Group 2 and Group3. There was no statistically significant difference between Group 1 and Group 2 in fusion rate or score ($p = 0.442, 0.382$), which suggests that in the osteopenic condition, usage of autobone alone is sometimes not sufficient for posterior spinal fusion material. (Table 5, Figure 5.) Group 3 also showed that BMP-2 usage is good for not only new bone formation but also the rate and score of posterior spinal fusion status.

The bone regeneration requires three critical elements, which are osteogenic, osteoinductive, osteoconductive factors. An osteogenic potential that is capable of directly providing cells to the newly forming bone. Osteoinductive factors are able to cause the osteoblastic differentiation of osteoprogenitor stem cells, and osteoconductive scaffold that utilizes neovascularization and supports the ingrowth of bone.

The ideal bone graft material contains all of these three potentials and without a risk of transmission of diseases. Autogenous bone grafts possess each of these three essential properties; therefore, they have been considered as the first choice for graft material in patients undergoing spinal fusion.[41]

However, in situations where a balance of bone metabolism is malfunction, there is also a limitation of the use of autogenous bone graft for spinal fusion. Consequently, spinal surgeons often treat osteoporotic patients who are associated with higher rates of instrumentation failure.[42]

Hydroxyapatite (HA) have its osteoconductive properties and desirable characteristics as a bone graft biomaterial. Bone morphogenetic proteins (BMPs) can induce the formation of new bone in numerous orthopedic and dental applications in which loss of bone is the main issue. The combination of BMP with a biomaterial that can carry and deliver proteins has been demonstrated to maximize the therapeutic effects of BMPs.[43] For this reason, our experiment showed superior spinal fusion result in Group 3 than Group 1, 2.

There are several limitations of our study. One is the absence of a histologic examination of fusion mass. Through histologic observation, we could identify micro-structure changes in osteopenic male bones and differences in fusion status when BMP-2 was applied in posterior spinal fusion. In order to overcome this, we observed the sagittal section of low power magnification on fusion mass. Using micro CT reconstruction image, we can identify continuity of cortical bone and new bone fusion status. Second limitation is tail bone as autobone material. In clinical condition, autobone is usually grafted from iliac bone and we prefer cancellous bone. On the other hand, tail bone composed with cortical bone rather than cancellous bone. For reducing mortality on posterior lumbar fusion, it was inevitable. In addition, all male rats were housed under the same conditions, and it would be helpful to measure the blood levels of calcium and vitamin D in order to understand the nutritional aspects of bone formation and resorption. Although the fusion operation performed after 1 week underwent orchiectomy, at that time, it was not confirmed that the osteoporosis is caused, there is also limit. The authors assume that osteopenia is in progress in accordance with the decrease of sex hormones after resection of the testes, and the status of the rats underwent spinal fusion surgery when we use the word osteopenia, not osteoporosis. Clinically, even if not present the reduction of severe bone density at the time of the spinal fusion surgery in older men, in this situation a reduced sex hormones, We implemented the state of spinal fusion surgery is thought to be the mean of the experiment in that. Further studies are needed to reveal the intracellular mechanisms of spinal fusion process with BMP-2 in osteopenic conditions, using DNA and RNA quantification techniques.

V. Conclusion

The effectiveness of autologous bone used for posterior spinal fusion is already well known. This study revealed that the combination of hydroxyapatite and BMP-2 is also effective for spinal bone fusion in male osteopenia. Therefore, the combination of hydroxyapatite and BMP-2 may play a significant role in spinal fusion in men with osteopenia.

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ABSTRACT (IN KOREAN)

남성 골감소증 백서 모델에서 자가골 대체제로 hydroxyapatite와 beta Tricalcium phosphate의 조합과 BMP-2의 사용의 효과

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목적: 본 연구를 통하여 남성 골감소증 백서 모델에서 자가골 대체제로 hydroxyapatite와 BMP-2 조합의 효과를 분석한다.

실험 방법: 본 총 41마리의 백서를 임의로 5개의 군으로 할당한다. 제 1군은고환절제술 시행 후 꼬리뼈를 이용한 자가골 척추 후방 유합술을 시행 하였다. 제 1군의 쥐를 9마리로, 나머지를 8마리로 하였다. 제 1군에서 한 마리의 쥐가 수술 후 생존하지 못하였다. 제 2군은 hydroxyapatite와 beta Tricalcium phosphate를 사용하여 척추 후방 유합술을 시행 하였다. 제 3군은 hydroxyapatite와 beta Tricalcium phosphate의 복합체와 BMP-2를 사용하여 척추 후방 유합술을 시행 하였

다. 제 4군은 고환 절제술만 시행 하였고, 후방 유합술은 시행 하지 않았다. 제 5군은 가장 수술 군이었다. 골대사 혈청 지표는 고환 절제 군과 가장 수술 군에서 수술 후 12주에 심장에서 채취하였다. 수술 후 12주에 백서를 희생하여 골편을 채취하여 분석 하였다.

결과: 고환절제 후 혈청 osteocalcin, alkaline phosphatase (ALP)와 collagen type 1 cross-linked C-telopeptide (CTX) 는 점차 감소하였고, 대조적으로, N-terminal type 1 procollagen (P1NP) 는 조금 상승하는 것이 보였으나 통계적으로 유의미하지는 않았다. 대퇴골과 요추 골밀도의 경우 통계적으로 유의미한 차이가 관찰되었다. 유합의 정도는 자가골 이식의 경우 중간에서 높은 정도, HA/b-TCP를 이식한 군에서는 낮거나 중간 정도, HA/b-TCP를 BMP-2와 같이 사용한 경우는 높은 정도로 관찰되었다.

결론: hydroxyapatite와 beta Tricalcium phosphate의 조합과 BMP-2의 사용은 척추 유합 수술에서 자가골 이식을 시행한 경우보다 더 효과적인 것을 관찰할 수 있었다. 그러므로, 골감소증 남성의 척추 유합수술에서 hydroxyapatite와 BMP-2의 사용은 자가골 이식 대체제로서 중요한 역할을 할 것으로 생각된다.

핵심되는 말: 골다공증, 고환절제술, 골밀도, 혈청 골 대사 지표

PUBLICATION LIST

Ryu SJ, Ryu DS, Kim JY, Park JY, Kim KH, Chin DK, et al. Bone Mineral Density Changes after Orchiectomy using a Scrotal Approach in Rats. Korean Journal of Spine 2015;12:55-9.

Ryu SJ, Ryu DS, Kim JY, Park JY, Kim KH, Chin DK, et al. Changes in Bone Metabolism in Young Castrated Male Rats. Yonsei Medical J 2016;57:1386-94.