Bone regeneration in a critical sized segmental defect in the Estrogen-deficient Rat fibula using rhBMP2-ACS implant

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Directed by Professor Hyung Jun Kim

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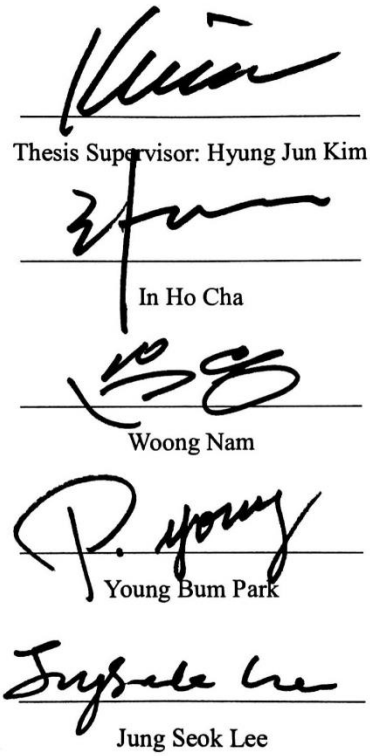

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ABSTRACT

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Menopause of female causes a series of hormone variation leading to consequence physiological and psychological changes including impeding bone regeneration after surgery. The use of recombinant human bone morphogenetic protein-2 (rhBMP-2) has been introduced to clinic to enhance bone remodeling procedure, but the effect of localized administration of rhBMP-2 under estrogen deficient condition is not clear. The aim of this study was to investigate the effect of rhBMP-2 with absorbable collagen sponge carrier (ACS) implant on bone regeneration in estrogen deficient rat fibula with critical sized defect. Eighty rats were included in the research and randomly divided into Sham group and ovariectomy group (OVX). Seven weeks after ovariectomy surgery, a

7-mm complete defect was created to bilateral fibula of all rats. On the left side a 5*5*8 mm ACS soaked with 10μg rhBMP-2 was placed, a same size ACS was placed on the right side but soaked with saline. Micro-CT, histologic, and molecular evaluation were performed after sacrifice at four and eight weeks. New bone formation degree in rhBMP-2 administrated Sham groups was significantly higher than other groups followed by OVX-BMP groups. Bone structure was better formed in groups treated with rhBMP-2. The expression of osteoprotegerin (OPG) was increased in rhBMP groups with decreased expression of receptor activator of nuclear factor kappa-B ligand (RANKL). The findings concluded that the localized administration of rhBMP-2 could enhance the process of bone remodeling and accelerate the degree of bone healing.

Key words: Bone morphogenetic protein-2, Bone regeneration, Estrogen, Osteoporosis, Defect

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1. Introduction

Postmenopausal osteoporosis is a disorder which commonly happens to female around their 40 to 50s resulting from hemostasis functional decline (Lopez-Otin et al. 2013). The disease may cause a progressive loss of bone tissue resulting in fracture, slow healing and other morbidity (Malluche, H.H. et al. 2007). Bone remodeling is a dynamic process that contains resorption of existing bone by osteoclast (OC) and formation of new bone by osteoblast (OB). The balance in postmenopausal patient is broken since the cessation of the ovarian function leads to a more active bone resorption by impaired regulation of proinflammatory cytokines from bone marrow (Augat, P et al. 2005). Among

all the factors that may influence bone biopsy, Recombinant human bone morphogenetic protein-2 (rhBMP-2) has been introduced to clinic for bone regenerative function (Murray, S. S. et al, 2016). rhBMP-2 is a type of protein belongs to transforming growth factor-beta (TGF-β) superfamily extracted from bone matrix and has been proved by the U.S. Food and Drug Administration that affects bone induction, maintenance and repair (Kwak et al. 2018). However, the effect of localized administration of rhBMP-2 under estrogen deficient condition on bone regeneration and osteogenesis is less clear.

OPG/RANKL/RANK system is one of the most important systems regulating bone formation/resorption. Osteoprotegerin (OPG), TNF receptor superfamily, is proved to promote bone formation by binding nuclear factor kappa-B ligand (RANKL), thus inhibits it from activating the receptor RANK, which is a key regulator of OC formation ,function and survival (Brendan BF et al, 2007). Estrogen prevents bone loss by reducing the production of RANKL, combined with the binding of OPG and RANK, the function and life span of OC is decreased. The importance of this system on regulating bone biopsy has been proved by several studies. Kong YY et al. discovered that RANK lacking rats showed severe osteopetrosis (Kong YY et al. 1999) while Mizuno A et al. found that OPG knockout rats presented general osteoporosis due to increased bone resorption (Mizuno et al. 1998). The relationship of rhBMP-2 with OPG/RANKL system would be studied in this research.

The usage of BMP-2 is sometimes controversial since it may be associated with complications as edemas, undesired ectopic bone formation, delayed bone

formation and possibly increased cancer risk (Karsenty, G. 2006). The possible reason for these side effects is the overdose or burst release. There are many researchers trying to find out a controlled delivery and releasing system to avoid such problems. Carriers like absorbable collagen sponge (ACS), biphasic calcium phosphate composite (BCPC), biphasic calcium phosphate (BCP), β-tricalcium phosphate (β-TCP) have been developed to deliver BMPs (Kim et al. 2016; Lazard et al. 2011; Lee et al. 2016). Among these carriers ACS is most commonly used in clinic with desirable biocompatibility, strength, shaping and remodeling period .

The aim of this study is to investigate the effect of rhBMP-2 with absorbable collagen sponge carrier (ACS) implant in critical sized defect estrogen-deficient rat fibula in bone regeneration. Along with the whether the administration of rhBMP-2 would affect OPG/RANKL system.

2. Materials and Methods

2.1. Animals

Forty 10-week-old female Sprague-Dawley rats (250-300g) were used in this study. All the laboratory animals were raised and managed by the Department of Laboratory Animal Resources in Avison Biomedical Research Center at Yonsei University College of Medicine. The animal breeding facility was kept at the temperature of 20℃ ± 5℃ and relative humidity of 50% ± 10%, and the standard laboratory diet was fed to the animals. Water and food was freely supplied, and a 12-hour light-dark cycle was maintained. The experiment began following one week of acclimation period for the laboratory animals. These methods are in compliance with the ARRIVE guidelines.

2.2. Experiment design

The rats were first randomly divided into two groups: OVX group (n=20): animals underwent ovariectomy surgery and SHAM group (n=20): animals underwent same surgery procedure only without removal of ovary. Six weeks after surgery, all the animals received bilateral fibula critical sized defect of 7 mm. On the left side fibula, a 5*5*8 mm ACS loaded with 10ug rhBMP-2 (0.1 mg/mL, 100uL) was placed into operative site. On the right side fibula, a same size ACS soaked with saline was placed. Animals were euthanized after 4 and 8

weeks. The fibula were analyzed radiographically and histomorphometrically. Based on implants and time of sacrifice, final grouping was set as following: OVX-S-4w, OVX-B-4w, SHAM-S-4w, SHAM-B-4w, OVX-S-8w, OVX-B-8w, SHAM-S-8w, SHAM-B-8w (Table. 1). Each group contains 5 rats with 10 operative sites.

Table 1. Study design and grouping method of the experiment

	Time	Implants
SHAM	4wks	ACS+Saline
		ACS+BMP
	8wks	ACS+Saline
		ACS+BMP
OVX	4wks	ACS+Saline
		ACS+BMP
	8wks	ACS+Saline
		ACS+BMP

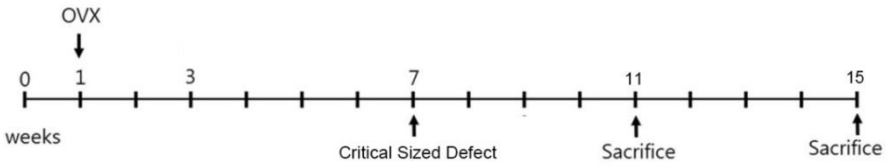

Figure 1. Time flow of treatment administrated to experimental animal.

2.3. Surgical Procedure

2.3.1 Ovariectomy Procedure

The rats were general anesthetized by Zoletil (Tiletamine and zolazepam, Virbac Laboratories, Carros, France; 15 ㎎/㎏, SC) and Rompun (xylazine, Bayer, Leverkusen, Germany; 5 ㎎/㎏, IM). After the removal of later-dorsal area fur, the operation sites were sterilized with 10% betadine solution. A 1.5 cm incision was made and ovaries were exposed. In OVX group, ovaries were carefully removed by electrocauterization. Ovaries in SHAM group remain untouched. The wound was sutured layer by layer. After the surgery, animals were kept for 2 weeks for recovery.

2.3.2 Critical sized defect creation procedure

The rats were general anesthetized by Zoletil (Tiletamine and zolazepam, Virbac Laboratories, Carros, France; 15 ㎎/㎏, SC) and Rompun (xylazine, Bayer, Leverkusen, Germany; 5 ㎎/㎏, IM). An incision was performed in the both lateral side of the lower limb followed by fascia and muscle dissection. A 7mm-long defect was created in the middle part of fibula with bone saw. After the creation of defect, in BMP groups, a 5*5*8mm ACS (Rapiderm Pad; Dalim

Tissen, Korea) loaded with 10ug rhBMP-2 (CowellBMP; Cowell Medi, Korea) was placed between defect edges; in saline groups, the same size ACS was placed but soaked with saline. The incision was then sutured layer by layer (Figure 2).

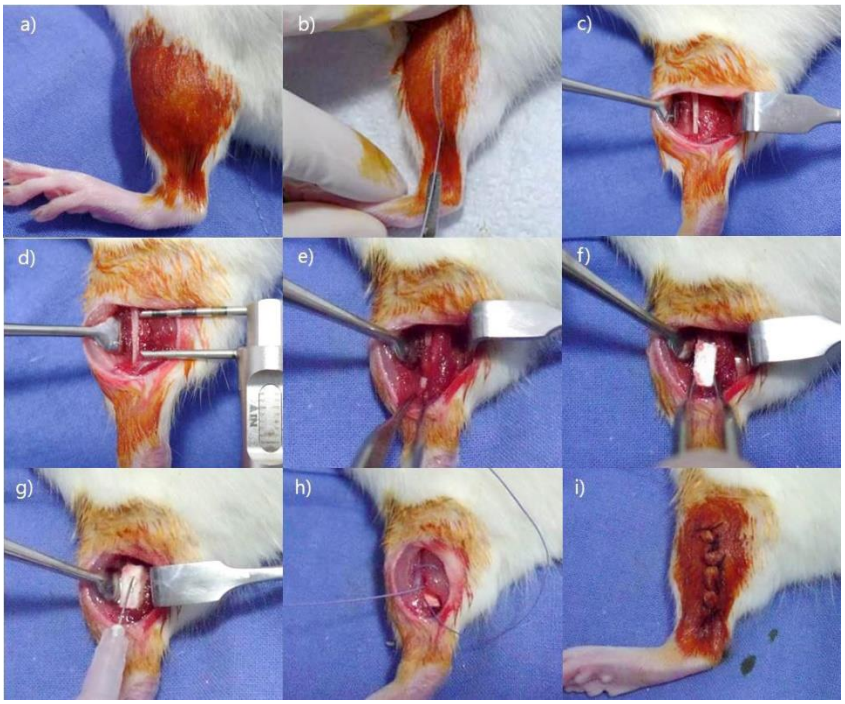

Figure 2. Critical defect creation and implant placement.

a) Operation field sterilization; b) 1.5cm skin incision; c) Fibula exposure; d) Caliper measuring 7mm; e) Defect creation; f) ACS with rhBMP-2 placement; g) ACS with saline placement; h) Layer by layer suturing; i) Postoperative dressing.

2.3.3 Postoperative treatment and sacrifice

A broad-spectrum antibiotic (0.05 mL/day; Baytril, Korea) was used by intramuscular injection for infection control, meloxicam powder (0.75 mg/day; Mobic; Boehringer Ingelheim) was administered via water intake for pain reduction.

The rats were sacrificed by CO2 inhalation by group at 4 or 8 weeks after surgery. The femur, tibia and fibula were harvested and evaluated by radiographic and histological assessment.

2.4. Evaluation methods

2.4.1 Plain radiographic evaluation.

Plain X-ray exam were performed (voltage, 60 kV; 70 mA; 0.08 s). Each digital image was assessed for the gross profile of the operative site.

2.4.2 Micro-computed tomography (Micro-CT) analysis

The harvested specimens were evaluated by Micro-CT (SkyScan; Kontich, Belgium) with the source voltage and current set to 130 kV and 60 mA and rotation step size set to 0.3°. Images were acquired with an image pixel size of 22mm, a 1.0-mm aluminum filter to optimize contrast, exposure time is set to

500 ms. In the defect area, the region of interest (ROI) of newly formed bone was set to 4 mm width and 3 mm length in sagittal plane.

Following parameters were analyzed: Percent volume (BV/TV), Specific surface (BS/BV), Trabecular pattern factor (Tb.Pf), Trabecular thickness (Tb.Th), Trabecular number (Tb.N), Trabecular separation (Tb.Sp), Structure model index (SMI) and Degree of anisotropy (DA).

The degree of newly formed bone was assessed as following: None healing: No newly formed bone was observed; Grade 1 healing: the length of new bone was shorter than 50% of the defect; Grade 2 healing: the length of new bone was longer than 50% of the defect but not complete; Complete healing: bone bridge formed completely between the edges of the defect.

2.4.3 Histology and histomorphometry analysis

The specimens were fixed in 10% formalin after harvested. A 10% ethylenediaminetetraacetic acid (EDTA; Chelator Cal, USA) solution was used for decalcification for 4 weeks. Samples were then dehydrated by alcohol (70%, 95%, 100%). The samples were then stirred and embedded in dimethylsulfoxide (Paraplast Plus; Leica Biosystems Richmond, Inc., Richmond, IL, USA). 5 um serial sections were created along the longitudinal axis of the fibula. The selected sections were ground and attached to an acrylic slide. Hematoxylin and eosin (HE) staining was performed and the bone formation was observed by microscopy.

2.4.4 Immunohistochemistry

The sections were used to identify the expression of OPG and RANKL using antibodies (ab239670 for anti-RANKL antibodies; ab73400 for anti-OPG antibodies; Abcam) in the defect area. Antigen retrieval for RANKL and OPG was performed by incubation with 10 mM sodium citrate buffer (pH 6.0) for 30 min at 95℃. The sections were then incubated with the primary antibody at a 1:100 dilution at 37℃ for 2 hours. Samples were then incubated with secondary antibody (ab64264; Abcam). Detection was performed with DAB (Abcam). Hematoxylin was used as background staining for 30–120 s to facilitate visualization. The immunohistochemical reactivity for RANKL and OPG was evaluated by a scoring system of: − (absent staining), + (weak staining; <25% of cells), ++ (moderate staining; <50% of cells), and +++ (strong staining; >50% of cells).

2.4.5 Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) and Kruskal-Wallis test. Bonferroni test was used for post-hoc analysis. $P<0.05$.

3. Results

3.1. Osteoporosis phenotype was created by OVX surgery

Femur samples were evaluated by Micro CT to assess bone quality and quantity at 4-week sacrifice (Fig. 3). Samples from SHAM and OVX group without rhBMP-2 administration were used. Comparing bone quality of distal femur, trabecular bone structure in OVX group was significantly inferior to SHAM group with loose bone mass (Fig 3 A-F). Quantified result indicated that SHAM group showed higher trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.SP) than OVX group, however, bone volume/total volume (BT/TV) was higher in OVX group (Fig. 3G). The findings proved that the estrogen deficient induced osteoporosis model was set.

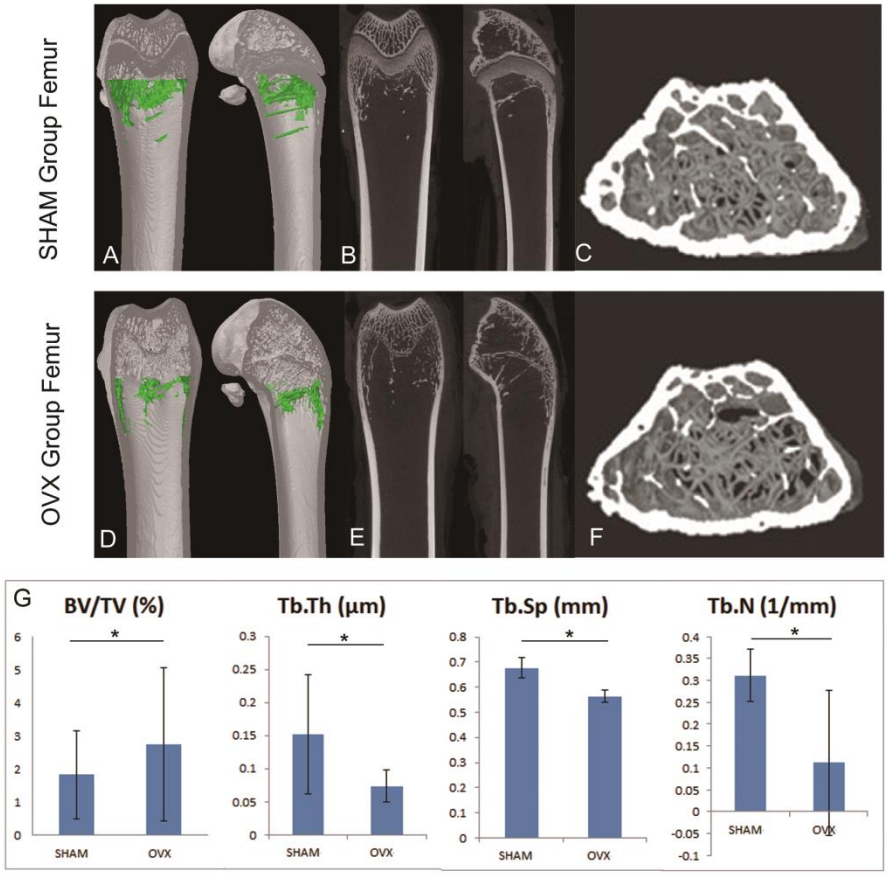

Figure 3. Ovariectomy (OVX) surgery produces a bone osteoporosis condition. The micro-CT sections of distal femur showed that the gross amount of trabecular structure in sham group (A,B,C) was superior than that of OVX groups (D,E,F). Quantification of BV/TV, Tb.Th, Tb.SP, Tb.N are compared between the two groups (G) BV/TV, bone volume/total volume; Tb.Th, trabecular thickness; Tb.Sp,trabecular separation; Tb.N, trabecular number. Values are presented as mean ± standard deviation. *P < 0.05.

3.2. rhBMP-2 promotes bone healing procedure

The bone remodeling degree was set according to the length of new bone formation (Fig. 4 A-D).After fibula samples were harvested, the gross profiles of new bone formation were observed. At 4 week, in group SHAM-B, 90% (n=9) samples formed a complete new bridging bone and 10% (n=1) reached grade II healing degree which is significantly higher than other groups. No other group samples formed complete defect repair. As for OVX-B group, 60% samples (n=6) reached grade II healing degree and 20% (n=2) reached grade I, however, 20% (n=2) showed no sign of new bone forming. The healing ratio in OVX-S group is that 70% (n=7) samples reached grade I healing with 30% (n=3) showed none sign of healing (Fig. 4E). At 8 week, the length of newly formed bone to the defect was significant higher in SHAM-B group than other three groups with 90% (n=9) formed complete bone repair and 10% (n=1) formed Grade II healing rate. OVX-B group showed higher healing degree than SHAM-S and OVX-S group with 70% (n=7) formed Grade II healing degree (Fig. 4F). The administration of rhBMP-2 in both OVX and SHAM group demonstrated enhanced healing procedure compared with those treated with saline.

3.3 rhBMP-2 effects bone marrow structure formation

Bone volume was significant higher in groups treated with rhBMP-2 but the difference was not significant between 4-week and 8-week in either group (Fig. 4G). Trabecular thickness was almost equivalent between groups at 8-week but higher than their 4-week results except for SHAM-B group (Fig. 2H). Trabecular spacing was significantly increased in OVX-S group whereas decreased in OVX-B group from 4-weeks to 8-week (Fig. 4I). Trabecular number was much higher in groups treated with rhBMP-2 (Fig. 4J). Structural model index (SMI) was significantly lower in SHAM-B group than other groups and a significant decrease could be observed between 4-week and 8-week (Fig. 4K). Degree of Anisotropy (DA) was significant lower in OVX groups than SHAM groups at 4 week, however, at 8-week the DA of OVX-S group was significant higher than that of 4-week (Fig 4L).

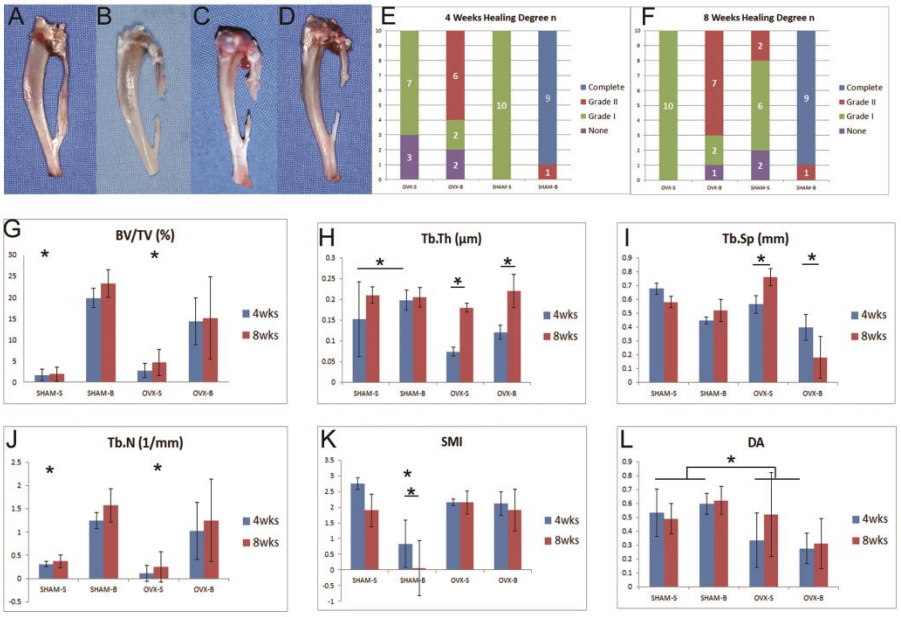

Figure 4. Bone healing and bone mass structure with or without estrogen deficiency are affected by the administration of rhBMP-2. Degree of bone healing was classified by complete healing (A); Grade II healing (B); Grade I healing (C); none healing (D). Healing degree were higher in groups with the usage of rhBMP-2 in both 4-week (E) and 8-week (F). Quantification of BV/TV (G), Tb.Th (H), Tb.SP (I), Tb.N (J), SMI (K) and DA (L) are compared. BV/TV, bone volume/total volume; Tb.Th, trabecular thickness; Tb.Sp,trabecular separation; Tb.N, trabecular number; SMI, structure model index; DA, degree of anisotropy . Values are presented as mean ± standard deviation. *P < 0.05.

3.4 The administration of rhBMP-2 leads to increased osteoblast differentiation and bone regeneration

The histology result of SHAM-S group showed little new bone formation at 4-week, no sign of osteoblast can be found (Fig. 5A). OVX-S group also had no new bone formation in the defect area osteoblasts cannot be observed (Fig. 5B). In SHAM-B group, osteoblast and osteocytes were observed and new bone started to reform (Fig. 5C). In OVX-B group, osteoblast and osteocytes were also found but new bone formation was limited (Fig. 5D). Other than SHAM-B group, ACS implant were not fully resorbed and surrounded by soft tissue (Fig. 5A,B,D)

Among 8-week groups, SHAM-S group new bone formation, osteoblasts can be found (Fig. 5E). OVX-S group demonstrated ACS remnants but few osteoblasts can be observed (Fig. 5F). In SHAM-B group, a bony union of the defect can be found as long as bone marrow structure (Fig. 5G). The OVX-B group showed new bone formation but the structure of trabecular was irregular (Fig. 5H). The ACS implant in OVX groups remain not fully resorbed (Fig. 5F,H).

The number of osteoblast was quantified. The number of osteoblast was significant higher in SHAM-B group than other groups both in 4-week and 8-week period; OVX-S group presented least amount of osteoblast. The result of OVX-B group was significant higher than OVX-S group (Fig. 5I).

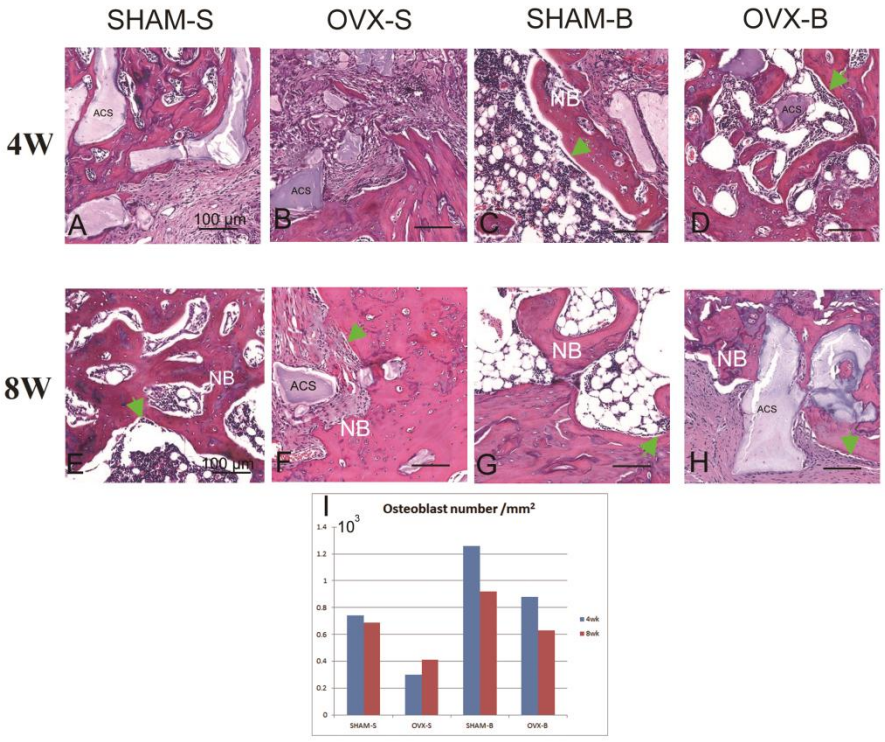

Figure 5. The bone regeneration degree is affected by the administration of rhBMP-2 with. 4-week groups (A-D) and 8-week groups (E-H) are studied by hematoxylin and eosin staining. The number of osteoblast is quantified (I). Green arrow: osteoblast.

3.5 rhBMP-2 effects expression of OPG and RANKL

Immunohistochemical results of SHAM-S group were used as baseline. At 4-week, a moderate expression of RANKL was observed along with weak expression of OPG in SHAM-S group (Fig. 6A,E); the RNAKL and OPG expression in OVX-S was both weak (Fig. 6B,F); in groups treated with rhBMP-2, SHAM-B group showed a moderate to strong expression of RANKL whereas OPG expression was weak to moderate (Fig. 6C,G); the RANKL expression in OVX-B group was moderate while OPG expression was weak to moderate (Fig. 6D,H).

Among 8-week groups, SHAM-S group showed moderate RANKL expression and weak OPG expression (Fig. 6I,M); weak to moderate expression of RANKL was observed in OVX-S groups with weak OPG expression (Fig. 6J,N); in SHAM-B group, both RANKL and OPG showed weak expression (Fig. 6K,O); both weak expression of RANKL and OPG was also observed in OVX-B group (Fig. 6L,P).

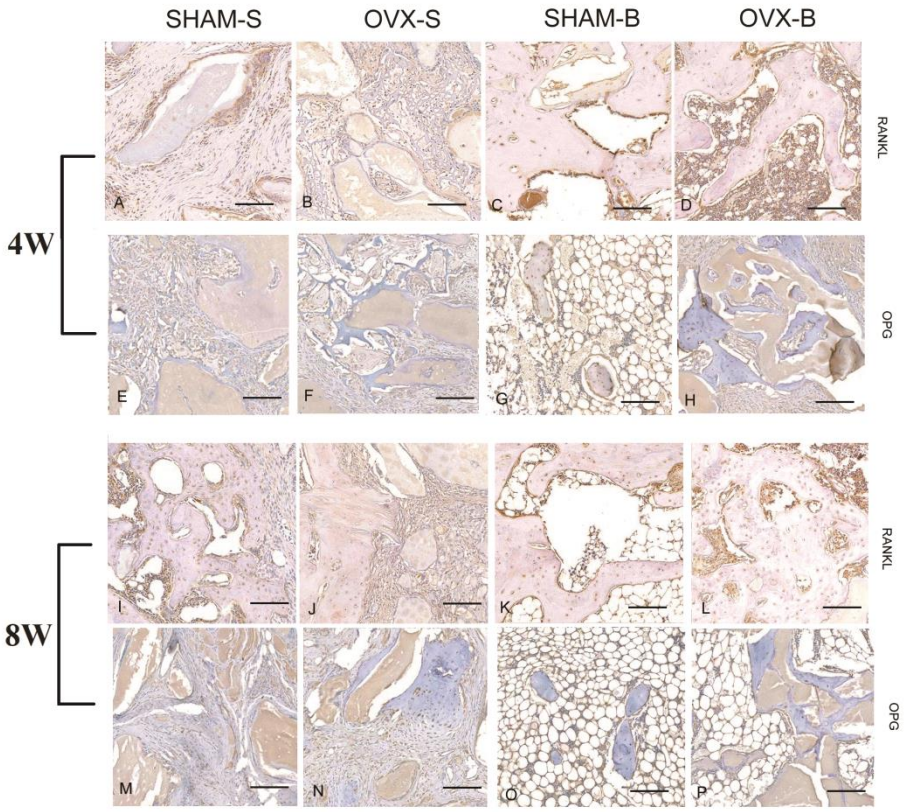

Figure 6. The administration of rhBMP-2 affects the expression of OPG and RANKL. Tissue sections were stained by RANKL (A-D, I-L)and OPG(E-H, M-P) antibody. A scoring system of − (absent staining), + (weak staining; <25% of cells), ++ (moderate staining; <50% of cells), and +++ (strong staining; >50% of cells). OPG, Osteoprotegerin; RANKL, Receptor activator of nuclear factor kappa-B ligand.

4. Discussion

In this research, rh-BMP2 was used to study the effect on bone regeneration in estrogen deficient animal model. A 7 mm critical sized defect on rat fibula was created since it had been proved that is was difficult for bone spontaneous healing a defect more than 6 mm (Pacifici. 1996) thus the bone repair procedure could only be induced by external factors, in this study, rhBMP-2. SHAM-S group was regarded as control group to evaluate the effect of target growth factor on osteoinductive function (Park et al. 2013).

To study the effect of rhBMP-2 on estrogen deficient induced osteoporosis condition, ovariectomy surgery was performed to animals to create an estrogen deficient model (Khajuria et al. 2012). According to recent research, the bone osteoporosis can be observed within 4 weeks, in this study, a period of 6 weeks were waited after ovariectomy surgery before creating defect in order to assure the onset of osteoporosis (Huang et al. 2016).

BMP is generated mainly by bone marrow plays an important role in development and regeneration of bone and had been studied by many researchers by various animal models. From last decade, BMP had been used in clinic in different skeleton surgeries. The use of BMP alone is less efficient because of its short half-life and rapid exposure (Rodan et al. 2000; Rodriguez et al. 2004; Roodman. 1996). In this study, an absorbable collagen sponge (ACS) was used as the carrier. ACS compared with other carriers had following advantages: firstly, it was effective on maintain BMP and could provide a

proper osteoconductive effect; on the other hand, it was X-ray transmitting which was convenient for later examination; thirdly the shape of the carrier was easy to modify. (Sarban et al. 2009; Nam et al. 2017). In this study the ACS remained not fully resorbed until 8 weeks except for SHAM-B group, this finding indicates that the release of rhBMP-2 is controlled. Bone regeneration of SHAM-B group showed no difference between 4-week and 8-week, which indicates that the bone repair procedure had mostly finished at the time of 4 weeks.

When comparing SHAM groups with OVX groups, the trabecular pattern factor showed significant difference which indicated that the estrogen deficiency successfully created osteoporosis condition. Along with the bone healing process, in 8-week groups, the length of new bone formation to repair the defect was increased compared with 4-week. However, based on Micro-CT results, the quantity of newly formed bone in OVX-B group did not increased significantly based on percent bone volume and trabecular number. The difference between SHAM-B and OVX-B group on percentage bone volume and trabecular thickness was significant, indicating the bone quantity was significantly different. This finding indicated that the healing speed in OVX groups was much slower than SHAM groups. In osteoporosis model, the callus area after fracture was proved to decrease because the pathological condition had a critical effect on the differentiation of mesenchymal stem cells (MSC) (Shields et al. 2006).

MSC, also known as progenitor cell, plays an important role in bone

fracture healing. It has been reported that under the osteoporosis condition, the proliferation rate of MSCs are lower and the cell line of differentiation is more toward adipocyte other than osteoblast (Teitelbaum et al. 2003). If osteoblastogenesis differentiation failed to be formed by MSCs, the function of rhBMP-2 would also decrease dramatically (Maureen et al. 2013). In this study, the OVX groups demonstrated much lower bone formation rate than SHAM group may partly because of the lower rate of osteoblastogenesis differentiation. On the other hand, both SHAM-B group and OVX-B group showed better new bone formation than those without administration of BMP. This finding could prove that the use of rhBMP-2 promotes bone regeneration in osteoporosis condition and the effect of ostioinductive.

Estrogen deficiency increases bone resorption caused by increased osteoclast (OC) numbers and by increased OC activity (Manolagas 2000). The function of estrogen is associated with a number of cytokines including, on one side, IL-1, IL-6, TNF-α, M-CSF and so on which increases osteoclast formation and activity with lower level of estrogen (Watanabe et al. 2016). Another important pathway that affects the function of osteoclast is through OPG/RANKL/RANK system. The binding of RANKL to its receptor, RANK, potently stimulate all aspects of OC function while OPG is a soluble decoy receptor that neutralizes RANKL which decreases the function and activity of osteoclast.

Recent study claimed that estrogen may increase the production of OPG (Hofbauer et al. 2000) and RANKL (Carmen et al. 2017; Nirupama et al. 2000);

also, rhBMP-2 could influence the expression of RANKL (Usui M et al. 2008). OPG is mainly produced by osteoblast and inhibits the binding of RANKL to RANK thus decrease the function and activity of osteoclast (Brendan and Lianping, 2008). The cross effect of estrogen and rhBMP-2 on bone turnover was studied in this research. OVX-B group demonstrated a higher degree of bone regeneration than SHAM-S group; the expression of RANKL and OPG was also higher. This result may indicate that the function of rhBMP-2 increased OPG/RANKL ratio that positively regulate bone regeneration procedure.

Based on this study, rhBMP-2 was found to be effectively osteoinductive under osteoporosis condition, promoting bone healing degree. Since SHAM-B group formed a complete bone reunion compared with OVX-B group that no complete defect was found, it could be considered that osteoporosis triggered by estrogen deficiency may reduce the function of rhBMP-2, instead.

The study had some limitations. Firstly, although fibula had been shown as a proper site for critical sized defect research, it was mainly composed by cortical bone. Osteoporosis is mainly result in pathological change in cancellous bone. In this study, after the healing process, bone marrow formed with proper trabecular structure in SHAM-B group. A longer time study is needed to study the later bone remodeling procedure whether the newly formed cancellous structure would change to cortical bone. Secondly, in order to study the localized administration of rhBMP-2, the two ACS with and without rhBMP-2 were place a same rat left and right fibula respectively, it is possible that with

the transport of BMP through circulatory system, it may affect the result of the non-BMP side (Lee et al. 2018). In this study the difference between BMP site and Saline site was significant indicating an acceptable result.

5. Conclusion

The results of this study suggest that local administration of rhBMP-2 effectively enhances bone regeneration procedure in estrogen deficient osteoporosis pathological condition. The OPG/RANKL/RANK system was affected by both estrogen and the administration of rhBMP-2, however, the OPG/RANKL ratio was positively improved by rhBMP-2, suggesting that bone regeneration inducted by osteoinductive function of rhBMP-2 is stronger than estrogen deficiency related bone resoprtion.

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국문 요약

골다공증쥐 비골의 분절성 골 결손에서 rhBMP2 와 absorbale collagen sponge 를 이용한 골재생

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손준위

지도교수: 김형준

여성의 폐경은 일련의 호르몬 변화를 유발하여 수술 후 골재생을 방해하는 등 생리학적 및 심리적 변화를 초래합니다. 재조합 인간골형태형성 단백질(rhBMP-2)의 사용은 골리모델링 과정을 향상시키는 클리닉에 도입되었지만, 에스트로겐 결핍상태에서의 rhBMP-2 의 국소투여효과는 명확하지 않습니다. 본 연구의 목적은 에스트로겐이 결핍된 쥐 비골에 흡수성 콜라겐 스폰지(collagen sponge carrier, ACS) 삽입물이 rhBMP-2 의 골재생에 미치는 영향을 조사하여 심각한 크기의 결손이 있는 환자를 대상으로 하였습니다. 연구에 포함된 80 마리의 쥐를 무작위로 샴그룹과 난소절제그룹(OVX)으로 나누었고, 난소 절제수술 7 주 후에 모든 쥐의 양측 비골에 7mm 의 완전 결손이 생겼습니다. 왼쪽에는 5*5*8mm ACS 에 10μg rhBMP-2 를 담그고 같은 크기의 ACS 를 오른쪽에 놓고 식염수에 담근 후,

Micro-CT, 조직학적 및 분자적 평가는 4 주 및 8 주에 희생시킨 후에 수행되었습니다. rhBMP-2 를 투여 한 Sham 군에서 새로운 골형성 정도는 다른 군보다 OVX-BMP 군에서 유의하게 높았습니다. rhBMP-2 로 치료한 군에서 골구조가 더 잘 형성되었고 osteoprotegerin(OPG)의 발현은 핵인자 κ-B ligand(RANKL)의 수용체 활성화제의 발현이 감소된 rhBMP 군에서 증가하였습니다. 연구결과 rhBMP-2 의 국소투여가 골리모델링의 과정을 향상시키고 골치유의 정도를 가속시킬수 있다고 결론 지었습니다.

핵심되는 말: Bone morphogenetic protein-2, Bone regeneration, Estrogen, Osteoporosis, Defect