Effect of Vibration on Lumbar Bone of OVX Rats compared with Risedronate-dosed Rat

Chang Yong Ko, Tae Woo Lee, Dae Gon Woo, Bum Seok Namgung, Han Sung Kim*
Department of Biomedical Engineering.
Institute of Medical Engineering, Yonsei University
Wonju, South Korea
hanskim@yonsei.ac.kr

Beob Yi Lee
Department of Anatomy. College of Medicine,
Kunkuk University
Chungju, South Korea

***Abstract*— Many different prevention and treatment regimens have been developed to resolve the growing problem of the osteoporosis. Some researchers showed positive effects of whole body vibration (WBV) on osteoporotic trabecular bones of animals. In the present study, the correlation between the morphological and mechanical characteristics and the treatments (interventions and whole body vibration) was investigated and analyzed. The rats were randomized into 3 groups: CON, DRUG and WBV 45Hz. The DRUG group was administered the risedronate (Actonel, 0.58mg/Kg, 5days/week). The WBV 45Hz group was vibrated on a vibration-plate (magnitude : 1mmpeak-peak, frequency : 45Hz) for 30 minutes every day in 5 days every week. The 4th lumbar on rats was scanned by In-vivo Micro-CT at the week 0 (just before treatment) and the week 8 (after treatment). Structural parameters and moduli of the 4th lumbar, based on two dimensional (2D) scan image data, were investigated and analyzed. The change of quantity and mechanical strength of trabecular bone on WBV 45Hz and DRUG groups were lower than that of CON group. There was no significant deference between WBV 45Hz and DRUG group. The results showed that WBV beneficially affected osteoporotic bones.**

***Keywords*— Whole body vibration, Risedronate, In-vivo Micro-CT, Mechanical properties Introduction**

I. INTRODUCTION

Osteoporosis, a disease characterized by the progressive loss and weakened strength of bone tissue, is one of the most common complications related with aging, especially post-menopause. Osteoporosis is accompanied with fractures that occur at the spine and many other sites[1-2]. Social cost for treatment and prvention of osteoporotic fractures increased in the world[1-2]. For treatment and prevention of osteoporosis, interventions such as estrogen and bishosphonates are mainly used in these days. The risedronate can improve biomechanical properties and decrease the risk of vertebral fracture related with osteoporosis[3-5]. However, the intervention treatment could be accompanied with adverse side effects[6-10]. Some researchers showed that vibration was an effective method to prevent a fracture related with osteoporosis[11-17]. However, there were few studies for detecting and tracking the effect of vibration on osteoporotic bone of living animals.

In the present study, not only morphologies but also mechanical characteristics in the lumbar vertebra of rats were detected and tracked to investigate the effect of whole body vibration (WBV) by using finite element (FE) analysis, based on acquired images from In-vivo micro computed tomography (In-vivo Micro-CT, Skyscan-1076, Skyscan, Belgium).

II. METHOD

All procedures were in accordance with approved National Institutes of Health (NIH) guidelines, under a protocol approved by the Yonsei University School of Animal Care Committee.

For this study, 8 virgin Sprague-Dawley (SD) rats, 14-week-old, weighing approximately 250g were housed in an individually ventilated cage (IVC) under standard condition (room temperature 23°±2°C, humidity 50%±10%). The period for day and night was alternated every 12 hours. 8 rats were

Figure 1. Vibration test machine to whole body of rats.

TABLE I. STRUCTURAL PARAMETERS

	BV/TV(%)*		Tb.Th(μm)		Tb.Sp(μm)*†		Tb.N*		SMI*†	
	WEEK 0	*WEEK 8*	*WEEK 0*	*WEEK 8*	*WEEK 0*	*WEEK 8*	*WEEK 0*	*WEEK 8*	*WEEK 0*	*WEEK 8*
CON	45.12 ±2.07	18.89 ±5.45	145.62 ±2.04	136.14 ±5.23	176.95 ±5.62	307.96 ±56.77	3.10 ±0.13	1.38 ±0.38	1.02 ±0.17	2.45 ±0.23
DRUG	45.22	30.17	148.75	142.81	184.61	239.98	3.04	2.11	0.96	1.82
WBV 45Hz	34.28 ±0.52	23.16 ±4.02	134.83 ±0.39	134.08 ±6.15	191.87 ±1.95	250.21 ±1.08	2.54 ±0.03	1.72 ±0.22	1.74 ±0.04	2.27 ±0.22

CON : CONTROL, WBV : Whole Body Vibration, mean±standard deviation

* Statistically significant by ANOVA between CON and WBV 45Hz groups within $p<0.5$

† Statistically significant by ANOVA among groups within $p<0.5$

randomized into 3 groups (CON : 5, Drug : 1, and WBV 45 : 2).

All experiment groups were ovariectomized (OVX) to induce osteoporosis. DRUG group was administered risdronate (Actonel, HANDOK Pharmaceuticals Co., Ltd.) orally by catheter at 0.58mg/Kg for 5days/week. For 30 minutes/day for 5 days/week, the rats in the WBV groups were stood in a customized cage and were stimulated on a vibration test machine (Fig. 1, , 45 Hz, $1mm_{peak\text{-}peak}$). The frequency and peak-to-peak acceleration were calibrated by a vibration meter (SHOWA SOKKI, JAPAN) before stimulation.

The 4th lumbar vertabrae of rats were scanned by In-vivo Micro-CT at week 0 (just before treatment) and week 8 (after treatment). To minimize quantity of radiation exposure for rats, 35μm resolution and the shutter was used.

Three dimensional (3D) structural parameters were calculated by acquired images from In-vivo Micro-CT. The volume of interest (VOI) selected a 4.9mm length of trabecular, locating 1.4mm below the anular epiphyses (Fig. 2.). In the 3D analysis, the bone volume fraction (BV/TV, %) was calculated. Trabecular thickness (Tb.Th, μm), trabecular separation (Tb.Sp, μm) and trabecular number (Tb.N, 1/mm) were measured directly on 3D model. The relative prevalence of rods and plates in the trabecular bone can be measure by using the structure model index (SMI). The SMI value is 0 for an ideal plate-like and 3 for a rod-like trabecular structure. Woo et el.(2003) showed that the results of FE analysis were accurate until 84μm×84μm×84μm cubic block[18]. Voxel models with 70μm×70μm×70μm cubic blocks for FE analysis were generated from acquired 2D cross-section images by using BIONIX 3.3 (CANTiBio Co., Korea). The bone volume fraction of FE models was similar to that of structural parameters. The material properties of rat bone were assigned the value of Kinney et al.(2000), 12.5Gpa (Young`s modulus) and 0.3 υ (Possion`s ratio), and all FE models were assumed to be isotropy[19]. To investigate mechanical characteristics in the whole vertebral bone model of the 4th lumbar vertebrae, simulated compression tests of 3D FE-models were performed. Displacement boundary conditions were applied to the FE models to simulate a compression test.

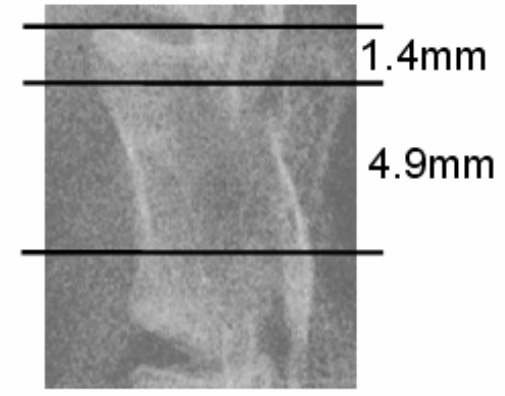


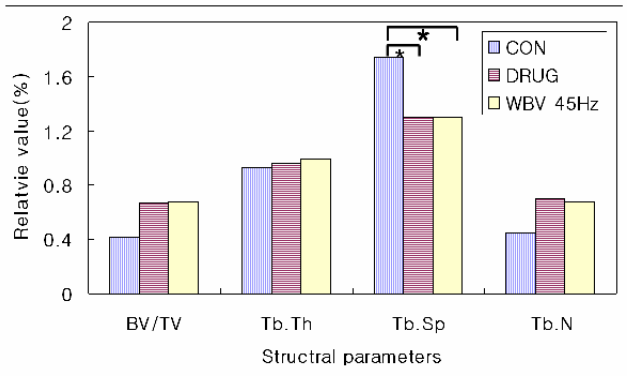
Figure 2. Range of VOI(volume of interest)

For the elastic characteristic of micro-FE analysis, structural modulus was measured by applying a compressive displacement of 0.5% strain. All FE analyses were performed by using the FE software (ABAQUS 6.4, HKS Inc, U.S.A).

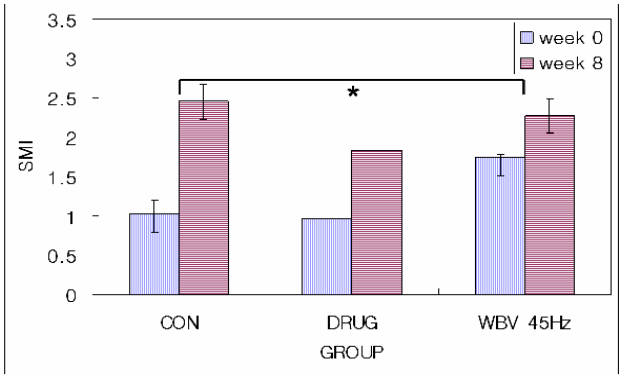
The effect of whole body vibration was investigated using analysis of variance (ANOVA) based unbalanced single factor between structural parameters or structural moduli and groups. In addition, Duncan`s Multiple Range Test (Duncan`s MRT) was used to compare average values of structural parameters for each group.

III. RESULT

To detect and track for change of morphological characteristics on rat lumbar trabecular bone, structural parameters were calculated from images which were acquired by In-vivo Micro-CT (Table 1, Fig. 3.). For BV/TV, Tb.Sp, Tb.N and SMI, there were significant difference between CON and WBV 45Hz groups by ANOVA ($p<0.05$). However, In Tb.Th, there was no significant difference between CON and WBV 45Hz groups. The results showed smaller loss of quantity and the least change of structure in WBV 45Hz groups than that of CON group. In structural parameters, there were no significant differences between DRUG and WBV 45Hz groups by Duncan`s MRT. The changes of 3D architecture from 0 week to 8 week in a identical rat were shown in Fig. 4. To detect and track the change of mechanical characteristics on rat lumbar trabecular bone, structural moduli were measured by FE analysis (Fig. 5). The relative values of structural modulus,



(a) BV/TV, Tb.Th, Tb.Sh and Tb.N



(b) SMI

Figure 3. Structural parameters (a) relative value, normalized to the values for the week 0, and (B) SMI. * significant difference between groups by Duncan`s MRT method within $p<0.05$.

normalized to the values for the week 0, were shown in Fig. 6. The relative values of structural moduli in groups of 45Hz were significant bigger than that of CON group by ANOVA ($p<0.05$).

However, There were no significant differences in structural modulus between DRUG and WBV 45Hz groups by Duncan`s MRT.

IV. DISCSSION

In the present study, the effect of whole body vibration (based on the mechanical stimulation), on osteoporotic bone were evaluated by the biomechanical method, combined with In-vivo micro-CT and micro FE analysis. In vivo Micro-CT system make it possible to perform longitudinal studies in identical small animals (rats). The biomechanical method was non-invasive or non-destructive and was able to detect and track changes of morphologies and mechanical characteristics in the lumbar vertebrae of identical rats and to reduce the sacrifice of animals needed, and to accurately measure morphological changes of the bone. In the result, the biomechanical method would cut the cost of experiment and

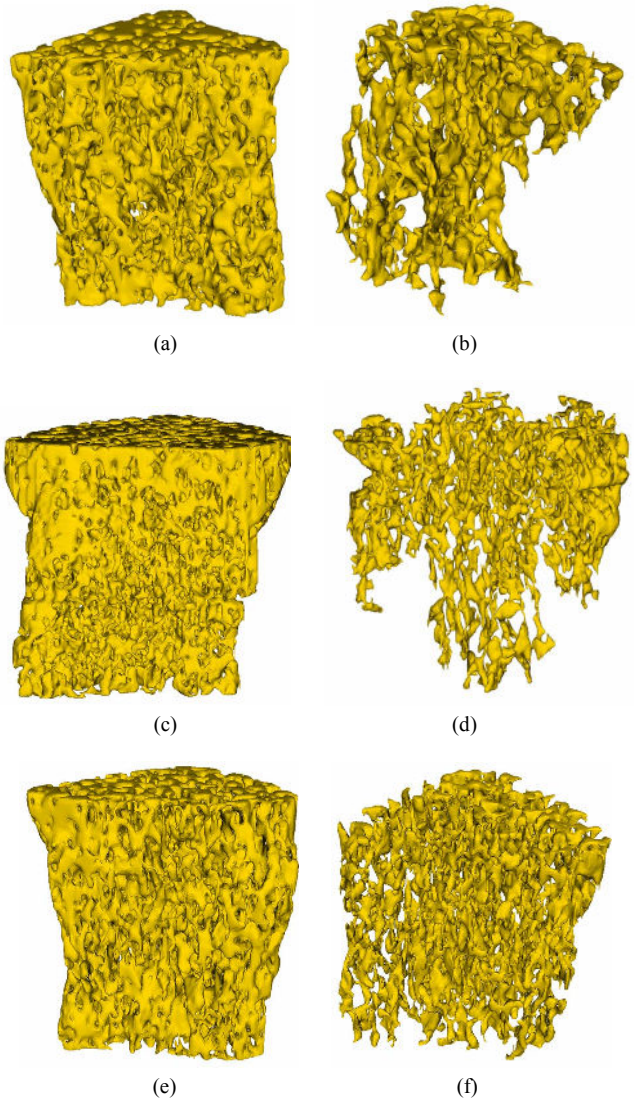


Figure 4. Changes of 3D structure among groups at 0 week (left) and 8 weeks (right) in identical rats, (a) and (b) CON group, (c) and (d) DRUG group and (e) and (f) WBV 45 Hz group.

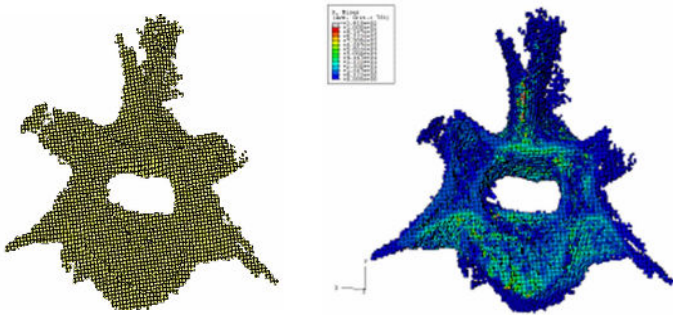


Figure 5. 3D models in the 4th lumbar of the rat. (a) 3D FE model and (b) simulated result of FE model (top view)

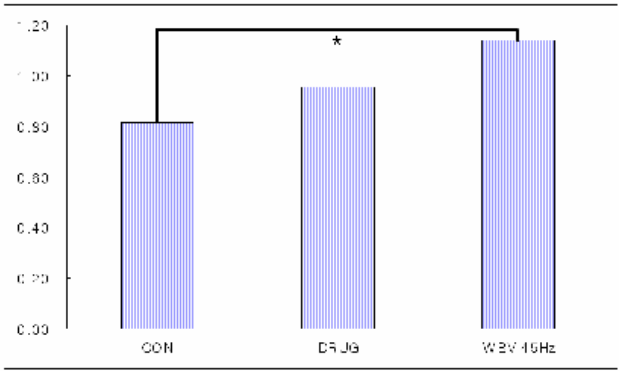


Figure 6. Relative value of structural moduli normalized to the values for the week 0. * significant difference between groups by Duncan`s MRT within $p<0.05$.

ethical issue.

The vibration test with magnitude $1mm_{peak-peak}$, and frequency of 45Hz performed to rats in WBV 45Hz group. DRUG group was administered risdronate orally by catheter at 0.58mg/Kg. In the lumbar trabecular bone on OVX rats in WBV 45Hz group, there was smaller loss than that in CON group. The results supported that whole body vibration was able to prevent loss of osteoporotic trabeculae bone. The relative values of structural modulus in 45Hz group significantly increased, compared with CON group. The results indicated that whole body vibration was able to prevent weaken of osteoporosis of trabecular bone. However, there were no significant difference in the loss of quantity and relative values of structural modulus between WBV 45Hz and DRUG groups. These results indicated that the effect of whole body vibration was similar to that of risedronate. The previous studies showed that the risedronate could improve biomechanical properties of osteoporotic bones and decrease the risk of vertebral fracture[3-5]. In these results, the vibration with 45Hz and $1mm_{peak-peak}$ was likely to have a beneficial effect on osteoporotic bones quality as well as quantity which was also investigated by Flieger et al.(1998), Oxlund et al.(2003), Rubin et al.(1995, 2001a,b, 2002), and Verschueren et al.(2004)[11-17].

The previous researchers investigated the effect of vibration on osteoporotic bones which were close to vibration-plate. However, the present study was investigated for changes of lumbar bones which were far from vibration-plate. These results were similar to Rubin et al. (2003)[20].

In conclusion, whole body vibration will be likely to treat and prevent osteoporosis, and partly replace interventions such as estrogen and bishosphonates.

ACKNOWLEDGMENT

This research was supported by the Program for the Training of Graduate Students in Regional Innovation which was conducted by the Ministry of Commerce Industry and Energy of the Korean Government.

REFERENCES

[1] M. Stevenson, M. Lloyd Jones, E De Nigris, N. Brewer, S. Davis and J. Oakley, "A systematic review and economic evaluation of alendronate, etidronate, risedronate, raloxifene and teriparatide for the prevention and treatment of postmenopausal osteoporosis", Health Technology Assessment, 9(22), 2005

[2] NIH Consensus statement, "Osteoporosis prevention, diagnosis, and Therapy", NATIONAL INSTITUTES OF HEALTH, 17(1), 2000

[3] R. D. Wasnich, D. Paul and PD. Miller, "Antifracture efficacy of antiresorptive agents are related to changes in bone density", J Clin Endocrinol Metab, 85, 231-236, 2000

[4] J. Y. Reginster, H. W. Minne, O. H. Sorensen , M. Hooper, C. Roux, M. L. Brandi, B. Lund, D. Ethgen , S. Pack, I. Roumagac and R. Eastell, "Randomized trial of the effects of risedronate on vertebral fractures in women with established postmenopausal osteoporosis", Osteoporosis Int, 11, 83-91, 2000

[5] I. Masako, N. Akifumi, A. Kiyoshi, U. Masataka, H. Kuniaki and K. Masahiro, "Effects of risedronate on trabecular microstructure and biomechanical properties in ovariectomized rat tibia", Osteoporos Int., 16(9), 1042-1048, 2005

[6] D. Grady, B. Ettinger , E. Moscarelli , L. Plouffe Jr, S. Sarkar, A. Ciaccia. and S. Cummings., "Safety and Adverse Effects Associated with Raloxifene: Multiple Outcomes of Raloxifene Evaluation", OBSTETRICS & GYNECOLOGY, 104(4), pp. 837~844, 2004

[7] R. C. Steven, E. Stephen, A. K. Kathryn, G. Deborah, J. P. Trevor, A. C. Jane, N. Larry, N. Thomas, H. B. Nina, M. Monica, E. L. Marc, B. Dennis, E. G. Joan, C. Alberto. and J. V. Craig, "The Effect of Raloxifence of Risk of Breast Cancer in Postmenopausal Women : Results From the MORE Randomized Trial", JAMA, 281(23), pp. 2189~2124, 2006

[8] C. Schairer, J. Lubin, R. Troisi, S. Sturgeon, L. Brinton, and R. Hoover, "Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk", JAMA, 283(4), pp. 485-491, 2000

[9] V. L. James, J. M. Pamela, H. L. Jay, E. S. Mark, T. Rebecca, H. Patricia, S. Arthur and S. Catherine, "Menopausal hormone replac`ement therapy and risk of ovarian cancer.", JAMA, 288(3), pp. 334~341, 2002

[10] J. Porthouse, S. Cockayne, K. Christine, S. Lucy, S. Elizabeth, A. Terry, B. Mike, B. Yvonne, D. Jo, F. Roger, I. Cynthia, P. Suezann, S. Anne, W. Ian and J. T. David, "Randomised controlled trial of calcium and supplementation with cholecalciferol(vitamin D3) for prevention of fractures in primary care", BMJ, 330, pp. 1003~1008, 2005

[11] B. S. Oxlund, G.. Ørotft, T.T. Andreassen, and H. Oxlund "Low-intensity, high frequency vibration appears to prevent the decrease in strength of the

femur and tibia associated with ovariectomy of adult rats", BONE, 32, pp. 69-77, 2003

[12] C. Rubin, C. Li, Y. Syn, C. Fritton, K.McLeod, "Non-invasive stimulation of trabeucular bone formation via low magnitude, high frequency strain", 41st Orthop Res Soc, 20, pp. 548, 1995

[13] C. Rubin, D.W. Sommerfeldt, S. Judex and Y. X. Qin, "Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli", DDT, 6, pp. 848-858, 2001a

[14] C. T. Rubin, S. Turner, S. Bain, "Low mechanical signals strengthen long bone", Nature, 412, pp. 603-604, 2001b

[15] C. Rubin, A.S. Turner, R. Müller, E. Mittra, K. Mcleod, W. Lin and Y.X. Qin, "Quantity and Quality of Trabecular Bone in the Femur Are Enhanced by a Strongly Anabolic, Noninvasive Mechanical Interventi on.", Journal of bone and mineral research, 17,pp. 349-357, 2002

[16] J. Flieger, Th.Karachalios, L. Khaldi, P.Raptou and G..Lyritis, "Mechanical Stimulation in the Form of Vibration Prevents Postmenopausal Bone Loss in Ovariectomized Rats", Calcif Tissue Int, 63, pp. 510-515, 1998

[17] M. P. Verschueren, M. Roelants, C. Delecluse, S. Swinneu, D. Vanderschueren and S. Boonen, "Effect of 6-Month Whole Body Vibration Training on Hip Densisy, Muscle Strength, and Postural Control in Postmenopausal Women : A Randomized Constrolled Pilot Study", Journal of Bone and Mineral Research, 19, pp. 352~359, 2004

[18] D. G. Woo, H. S. Kim and G. R. Tack, "A study on the mechanical characteristics of vertebral trabecular bones using the micro-FE models", AP Biomech 2004, 1, pp. 37-38, 2004

[19] J. H. Kinney, D.L. Haupt, M. Balooch, A.J.C. Ladd, J.T. Ryaby and N.E. Lane, "Three-Dimensional Morphometry of the L6 Vertebra in the Ovariectomized Rat Model of Osteoporosis: Biomechanical Implications", Journal of Bone and Mineral Research, 15(10), pp. 1981~1991, 2000

[20] C. Rubin, M. Pope, J. C. Fritton, M. Magnusson, T. Hansson and K. McLeod, "Transmissibility of 15-Hertz to 35Hertz Vibrations to the Human Hip and Lumbar Spine : Determining the Physiologic Mechanical Stimuli to Skeletal Regions at Greatest Risk of Fracture Because of Osteoporosis", Spine, 28(23), pp. 2621-2627, 2003