ATLANTIS PRESS

Advances in Social Science, Education and Humanities Research (ASSEHR), volume 98

1st International Conference Postgraduate School Universitas Airlangga:

Implementation of Climate Change Agreement to Meet Sustainable Development Goals (ICPSUAS 2017)

ACTIVATION OF FAK EXPRESSION IN INFLUENCING BONE DENSITY THROUGH EXERCISE TRAINING FOR DECREASING THE OSTEOPOROTIC RISK

Nurul Mahmudati[1)], Fazat Fairuzia[2)], and Hawin Nurdiana[3)]

[1)]Biology Department [2)] Chemisry Laboratory [3)] Medical Faculty

Muhammadiyah Malang University

Malang, Indonesia

nurul.mahmudati@yahoo.com

***Abstract*—Mechanical stimulation mechanism influences bone density by in vitro and in vivo both on human and animal. Stimulation can be done by various methods, such as fluid flow, shear stress, tension, low power ultrasound, and exercise training. It is however, until today the mechanical stimulation in influencing bone density after exercise training up is not clear yet. This research aims to proof the effect of exercise training on increasing FAK expression and its role in influencing bone density. The research design employs "The Randomized Posttest only Control Group Design". The experimental unit is female rat (*Rattus nurvegus)*, in the age of 2.5 months with six replying and total number are twelve. FAK and *bone alkalin phosfatase* (BALP) test was measured by using ELISA method. The data was analyzed by using *T test* and *Pearson correlation.* The result of this research shows that FAK expression of the exercise training group is higher (853,76) than the control group (726,25) and BLAP of the exercise training were higher (3470,12) than control (4197,00) α 0,01. Based on Pearson correlation analysis shows that increasing of FAK has possitive correlation with bone marker BALP. The conclusion of this research is exercise training can increase FAK expression and has positives correlation with BALP.**

Key words : Excercise training, FAK expression , bone density, osteoporotic risk.

I. INTRODUCTION

Up to now, the mechanical stimulation mechanism in inflicting signal upon the osteoblast activity in bone is not clear yet. One of hypotheses is through the phenomenon which is known as mechanotransduction. Mechanotransduction comprises of the changing of biophysical activity into cellular and molecular activity (biochemical). The mechanotransduction in bone covering 4 phases, these are: mechanocoupling, biochemical coupling, transmission signal from censor cell to the effectors cell and effectors cell's response. The whole mechanism is responsible to the dynamic of balance between bone forming and bone resorption (remodeling) [1]. Furthermore, [2] the mechanical stimulation can influence transcription factor, as well as influence the gene expression which responsible upon the development and sustainment of the tissue. In vitro shows that mechanical stimulation increase osteoblast proliferation in bone cell progenitor bone marrow mesenchymal stem cell (MSCs)); however the mechanism of increasing proliferation and differentiation osteoblast has not clear yet.

Focal adhesion complex (FAC) engages the integrin and binds with the ECM (extra cellular matrix) takes part in responding signal from the outside cell into the cell, in order to create response from the cell. Focal adhesion that takes part in transduction signal is named focal adhesion kinase (FAK). FAK is a protein tyrosine kinase in which engaged with β integrin in integrin receptor in all types of cell. Additionally, the binding between integrin and ECM will activate the FAK and will take part as mediator signaling in which organized the development, morphogenesis, and defense [3].

Furthermore, the mechanical stretch model influences the FAK signaling through the advancing of FAK phosphorylation and further it can influence tenogenic differentiation[3-4]. The similar model of mechanical stimulation is given to the culture rat mesenchymal stem cell (rMSC). The research [5] shows that FAK-ERK signaling is necessarily needed for proliferating in progenitor osteoblasts cell. ERK (extra cellular signal regulated kinase) is a protein kinase from mitogen activated protein kinase (MAPK), ERK takes part in controlling cell in proliferation, differentiation [6], in the case of osteoblast cell, ERK influence c-bfa (core binding factor) gene, proliferation and cell adhesion [7]. Nevertheless, the way of transduction signaling integrin-ERK to the gene expression after exercise training is not clear yet.

II. MATERIAL AND METHODS

A. Material and Methods

This research is an experimental research. It employed The Randomized Posttest only Control Group Design Gibbon et al (1997). The experimental unit in this research was white rat strain Wistar, female in the age of 2,5 months. The independent variable was exercise training on moderate intensity, and the dependent variables were FAK expression and bone density that is through Bone Alkaline Phosfatase (BLAP).

The treatment was exercise training in moderate intensity for 45 minutes (independent variable). The dependent variable is 1 expression FAK and bone density through *Bone Alkaline Phosfatase* (BALP). This research was conducted at Laboratory of Physiology University of Gadjah Mada for exercise training and the examination on FAK and BALP was conducted in the Laboratory of Physiology Sciences, Brawijaya University in Malang.

B. *Research procedure*

Firstly, it conservates the sample from age 2,5 months till 3 months. Second, aacclimatization the control group and experimental group for 2 weeks in order to adapt to the research environment. For the experimental group, the rats were introduced to the treadmill by running on a treadmill. This was an intended procedure, so that when the study was conducted the sample had adapted to the treadmill. The exercise training was conducted in moderate intensity treatment. Each treadmill took place the for 5 times in a week. The exercise treatment was done in 45 minutes and took a rest for 5 minutes in the middle of the treatment after 25 minutes running.

After the sample had 2 months of treatment, the sample put into sleep in order to take their upper leg part (femur). The upper leg part from the muscle tissue which attached to the bone was cleaned and disposed. After taking the bone density measurement, only the bone part remained. The protein isolation and SDS-PAGE performed after the bones were cleaned. Then, examine the expression of FAK and BALP employed ELISA Method.

III. RESULT AND DISCUSSION

The Data obtained from the examination of the expression of FAK and BAL P employed ELISA Method. The data expression of FAK and BALP are presented in Tables 1 and 2.

TABLE 1. THE AVERAGE OF FOKAL ADESION KINASE EXPRESSION IN POSTTEST EXERCISE TRAINING

Treatment	*Control*	*Exercise trining*
Average µ/ml	726.550	853.760

TABLE 2. THE AVERAGE OF BONE ALKALIN PHOSFATASE EXPRESSION IN POSTTEST PHYSICAL TRAINING

Treatment	*Control*	*Exercise training*
Average *µ/ml*	3470.12	4197

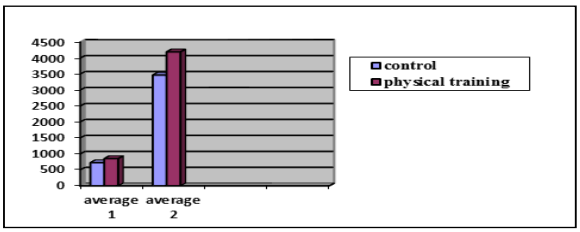

Fig. 1. FAK Expretion and BALP After exercise Training

The average number in Table 1 and Table 2 contribute the data expression of FAK and BALP in exercise training. They were higher than the control group. In α 0,01. The Pearson correlation test showed that a positive correlation between the increasing expression FAK and BALP as the marker of bone density.

The average number in Table 1 and Table 2 contribute the data expression of FAK and BALP. The result in physical training was higher than the control group. Based on the result, there is an increasing expression of FAK and BALP extent sig 0:01. The Pearson correlation test shows that a positive correlation between the increasing expression FAK and BALP. They are the marker of bone density.

Based on the results of the test the physical training affects to the increasing expression of FAK and BALP. The inenhancement of FAK expression has positive correlation with inenhancement of the expression of BALP. The increasing of BLAP provides the information that an increase in bone formation activity is because of physical training. Bone formation on physical training occurs because the cell proliferation process. It then follows the process of differentiation which leads to the formation of bone matrix. The results were not different from the results of research [9] that an increase on the expression of genes responsible for the differentiation of osteoblast gene RUNX2. It leads the process of osteoblast differentiation that leads to bone formation in cultured osteoblast cells by mechanical stress. Although mechanical stress can induce osteoblast differentiation and osteogenesis however osteoblast activity enhancement mechanisms are not known yet [9].

Cell proliferative activity lately conducted more intensive to study the regeneration of cells whether they are favorable for repair cells to the detrimental as in cancer. Cell proliferation involves supramolecular called extracellular matrix (ECM) and integrin. Cells undergoes proliferation and attaches to the ECM. The cling to the ECM is mediated by integrins. Integrin extracellular ECM molecule will connect to intracellular cytoskeleton and integrins are also associated with the growth factor of receptors to regulate the proliferation and differentiation of defense cells [10]. Signaling through the interaction of proteins and cytoskeleton is important for the maintenance of cell functions, among others proliferation [11]. It requires mechanical stimulation. Transmission of mechanical signals into the cell through a transmembrane protein α actinin via integrin. Other molecules is another focal adhesion which is the mechanosesensor organelle, and conecting ECM to the actin cytoskeleton which there is a conversion of signal mechanics become the signal for the chemical in the cell [1]. The lately research shows that FAK has an important role on the proliferation of various cell types, including osteoblasts. FAK-ERK signaling is required on mechanical stimulation cyclic model stretching for induced cell proliferation in cultured rat osteoblasts mesemchimal stem cell (rMSC) [5]. The Results of research is not different from the provision of mechanical stimulation due to the exercise training.

Exercise training in this study shows increasing FAK expression followed by the increasing of BLAP. So, the mechanism of increased bone density after exercise training is possible due to the activation of FAK. It is because of mechanical stimulation in exercise training.

IV Conclusion

The conclusion of this research is the increase of exercise training can increase the FAK expression. It has positives correlation with BALP.

V Acknowledgement

This Research was funded by DPPM DIKTI Research Grant .

References

[1] C. H**ua**ng dan R. Ogawa, "Mechanotranduction in bone reapir and regeneration," FASEB J 24: pp. 3625-3632, 2010.

[2] A. Mammoto, E. Mammoto, D.E. Ingber, "Mechanosensitive mechanism in transcriptional regulation. Journal of Cell Science 125: pp. 3061-3073, 2012.

[3] B. Xu, G. Song, Y. Ju, "Effect of focal adheaion kinase on the regulation of realignment and tenogenic differentiation of hMSC by mechanical stretch. Connective Tissue Research 52 (5): pp. 373-379, 2011.

[4] B. Xu, G. Song, Y. Ju, X. Li, S. Watanabe, "Rho A/ROCK cytoskelataldinamis, and focal adhesion kinase are require for mechanical stretch induce tenogenic differentiation of Hmsc", J Cell Physiol 227: pp. 2722-2729, 2012.

[5] L. Yuan, Q. Luo, L. Yang, G.B. Song, "Role of fak-erk 1/2 signaling pathway in proliferation of rat bone morrow mesenchymal stem cells stimulated by cyclic stretching, Journal of Medical and Bliological Engineering 33(2): pp. 229-238, 2012.

[6] Y. Li et.al, "Differentiation-dependent association of phosphorylated extracellular sighromatin of osteoblast-related gene", integrin-dependent force transmission to the extracelullar matrix by alfa Actinin Triggers Adhesion Maturation PNAS: pp. 1-10, 2013.

[7] C.F. Lai et.al, "ERK is essential for growth, differentiation, integrin expression, and cell function in human osteoblastic cells, 2001.

[8] Gibbon, Barry, Herman & Joan, " True and Quasi experimental design. Practical research and assasement, Research and evaluation 5(14), 1997.

[9] K.S. Kang, S.J. Lee, H. Lee, W. Moon, D.W. Cho, " Effect of combined mechanical stimulation on proliferation and diffrentiation of preosteoblast, Experimental and Molecular Medicine 43: pp. 367-373, 2011.

[10] R.O. Hynes, "The extracellular matrix: not just pretty fibrils. Science, 2009.

[11] J.W. Lee, R. Juliano, "Mitogenic signal tranduction by integrin-growth factor receptor- mediated pathway. Mol Cell 17(2): pp. 188-202, 2004.