The Effect of Osteocyte Signalling on Osteocyte Apoptosis

Dwi Setiani Sumardiko[1], Purwo Sri Rejeki[2,3], Gadis Meinar Sari[2]
[1]*Master of Basic Science in Medicine (Physiology), Department of Physiology, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen. Prof. Dr. Moestopo No. 47*
[2]*Department of Physiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*
[3]*Sport Health Science Master Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*
dwisetiani90@yahoo.co.id

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Abstract: Background: Physical exercise has been known that is one of the ways to evoke mechanical stimulation that can cause the change of the "strain". The "strain" itself allows the converted mechanical signal to be a biochemistry that can produce a biological response to bone cells. Objective: To show what the effect of physical exercise of sub maximal intensity through "osteocyte signaling" towards "osteocyte apoptosis". Methods: The researcher believes that the randomized posttest only control group design can solve the problem of this study. Moreover, 20 male white rats (experimental sample) were separated by the researcher into the control group and a group that did physical exercise of sub maximal intensity. The exercise was swimming (exercise: load 6% and intensity 80% three times a week). Results: These show that a group that did physical exercise of submaximal intensity (8.60 ± 3.27) lower than the control group (28.60 ± 6.20), and in terms of an independent sample test (osteocyte apoptosis), they show that there is a significant difference between the two groups (p = 0.000). Conclusion: a group has "osteocyte signaling" can obstruct the group (male white rats) has osteocyte apoptosis.

1 INTRODUCTION

The advancement of technology and science in the modern era, besides having a positive impact, has also had a negative one on human health in that it has made people tend to reduce physical activity (sport) (Lau et al, 2006). It is known that nationally almost half of the Indonesian population aged > 10 years (48.2%) do physical activity less (Riskesdas 2007). Decrease in physical activity due to technological advances is one of the factors causing some chronic diseases, one of which is osteoporosis (WHO, 2002). Osteoporosis is a condition in which bone or bone density decreases and this increases bone fragility with the higher risk of fractures resulting in increased morbidity and mortality (Siddapur, 2015).

Tom Lloyd (2003) reported that physical activity is an important lifestyle factor in promoting bone formation. Exercise is essential for bone density in both men and women (Kemmler et al, 2002), Yamazaki, 2004). Good bone density is needed to reduce the risk of fractures in old age (Janz et al, 2001; Warden, 2006). Physical exercise is recommended to prevent fractures, one form of which is swimming. Swimming activity will train the upper and lower back muscles that can form muscle mass to support bone endurance. The basic principle of muscle strengthening is one of overloading: "*the overload principle*" (Fox, 1993). Weight training in water can reduce the risk of fractures because load pressure can stimulate increased bone density (Sidik, 2007). Research on the effect of physical exercise of submaximal intensity through osteocyte signalling in osteocyte apoptosis needs to be done to determine the role of physical exercise in bone density.

2 MATERIALS AND METHODS

The research is classified as a type of laboratory experimental research using the research design of the randomized post-test only control group design. The experimental unit in this study used white male rats aged 8-10 weeks weighing about 100-200 grams, 10 heads per group (two groups), so a total of 20 tails. Physical exercise of submaximal intensity

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in question is exercise in the form of swimming with moderate intensity (load 6% weight). Rat training time was 80% of the maximum time achieved by every rat. Exercise was given once a day with a frequency of three times a week for two weeks. Groups that were not treated with physical exercise remain conditioned in an environment exposed to water. The osteocyte apoptosis index was calculated in one field of view, up to as many as five fields of view with the 'DeadEnd™ Colormetric TUNEL System.

3 RESULTS

The results were obtained in the form of an osteocyte apoptosis index. For the each variable group in the analysis with descriptive statistics, Shapiro-Wilk normality test, homogeneity test and different test with independent-sample t test. All data was processed by using computer program SPSS 20.0 for Windows with significance level of 0.05. Result of normality test using Shapiro-Wilk and in each group showed ($p > 0.05$) is (K0) $p = 0{,}767$ and (K1) $p = 0{,}948$ meaning data of apoptosis index normal distribution. The homogeneity test obtained p value = 0,057 ($p < 0.05$), which means variance in the osteocytes apoptosis index in each group is homogeneous.

The measurement of the osteocyte apoptotic index in the control group (K0) obtained mean and standard deviation higher than the submaximal physical exercise group (K1). After the independent samples test, $p = 0{,}000$ ($p < 0.05$) was obtained, meaning that there was significant difference between the control group and the submaximal intensity exercise group. Histological examination results are as follows:

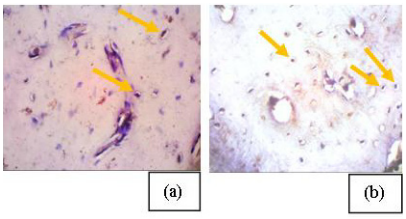

Figure 1: Histologic picture of osteocyte apoptosis (arrow) with TUNEL staining (400 x enlargement). Group K1 (a) and control (b).

4 DISCUSSION

The result of the independent-sample t test showed that there was a significant difference in the control group with physical exercise of submaximal intensity with $p = 0{,}000$. The mean values in the submaximal exercise group had a higher mean than the control group, so it can be concluded that the submaximal physical exercise is better at inhibiting osteocyte apoptosis. Davis (2001), states that the shear stress due to physical exercise given to the experimental animals can cause changes in endothelial nitric oxide synthase (eNOS) expression. Increased eNOS expression is the expected result for nitric oxide (NO) production that plays a role in stimulating osteoblasts and suppressing osteoclasts in order to increase bone density (Becker, 2004).

NO is a toxic, short-lived compound, a freely diffusing free radical gas molecule that affects various biological functions (Venkatakrishnan, 2009). NO is a highly reactive molecule, making it difficult to measure directly. NO is endogenously produced in large quantities through enzymatic pathways. The formation of NO through enzymatic pathways is catalyzed by nitric oxide synthase (NOS) through a series of redox reactions accompanied by oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) presence. eNOS synthesizes NO by oxidizing L-arginine to L-citrulline by mono-oxygenation, this reaction forming cyclic guanosine monophosphate. NADPH is used for the formation of one NO molecule (Luiking, 2010).

The osteocyte apoptosis index in the control group showed a significant difference to compared with the group that did physical exercise of submaximal intensity group with $p = 0{,}000$. The results can be interpretation as that submaximal physical exercise has an influence to inhibiting the occurrence of osteocyte apoptosis for male white rats. Osteocytes (sensosensory cells) are the main sensors of load received by bone, through mechanical strains that flow in the lacunocanalicular system (Wang, 2010). Osteocytes are the most sensitive to fluid flow as it is caused by mechanical loads. Osteocytes will have apoptosis when there is no load. The loss of osteocytes will increase resorption, due to the loss of inhibitory cells. However, if the osteocytes receive a burden sensor, then this cell apoptosis does not occur and induces a signal that represses osteoclasts (Bergmann, 2011).

Mechanical loading of the bone (skeletal load) results in mechanical stress and strain or resultant tissue deformation resulting in bone formation

effects and decrease bone turn over, which reduces bone resorption (Bemben, 2000). Physical exercise can cause mechanical loads on bone that stimulate some physical signals so that osteocytes activate and stimulate bone formation by osteoblasts, increase osteoblast activity, and suppress osteoclast activity (Bergmann, 2011). Mechanical loading is one method of weight training with moderate intensity. The mechanical loading applied to the bone tissue will form an endogenous signal. The signals are detected by the mechanosensoric system then captured by the osteocytes to be converted into biochemical signals to regulate bone turnover (Yuliana, 2012).

5 CONCLUSIONS

The physical exercise of submaximal intensity make signalling osteocytes may inhibit osteocyte apoptosis of the male rats (rattus norvegicus)

6 SUGGESTION

There is a need for further research with physical exercise treatment with an intensity that is different to the comparison. There is also a need to do research using other biomarkers as a marker of bone density, as supporting data.

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