Exercise Training Induced ERK1/2 Expression in Bone

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Abstract. Osteoporosis is a bone metabolic disorder that can decrease quality of life. It is the reason why this study on the factors on bone metabolism urgently conducted. The research was conducted to describe the role of exercise training on bone metabolism on rat ovariectomized by using cell and molecular approach. This research was purely experiment research that was carried out to prove that exercise training can increase extra cellular signal regulated kinase (ERK1/2) and it correlation with calcium. Design of the research “The Randomized Posttest only. Control Group Design”. The unit of experimental was female Sprague Dawly Rat, 2,5 months and divided in three group A) Ovariectomized (O); B) O + exercise training (ET) and C) Ovariectomized calcium supplement (CS). Exercise training as independent variable and ERK1/2 expression was dependent variable. ERK1/2 was measured by using ELISA, while and calcium level measured by using SSA. The data was analysis by using ANOVA. The result of this research showed that ERK1/2 expression of the exercise training were higher (1,861) than the control group (1,031) α 0.005 and calcium level were not different in all group. It can be concluded that exercise training can decrease ERK1/2 expression but no change on level of calcium.

Key words: Exercise training, ERK1/2 expression, bone density, osteoporotic risk.

Introduction

1.1 Background of Study
One of the successive standards on building health in Indonesia is the enhancement of life expectancy. Indonesian’s life expectancy was 69.1 years of male and 73.06 years (BPS,2017) (1). Nevertheless, the elderly can cause problem which is need to be concerned, that is the emergence of some degenerative diseases including osteoporosis which can lead to the decreasing of quality of life. Homeostasis of bone require balance of bone formation and bone resorption activity [2]. Osteoporosis is characterized by an in balance in normal bone cell remodeling and result bone lost, decrease of strength and altered bone porosities [3] and increase risk skeletal fracture most often in hip, wrist, vertebrae [4]. Exercise especially exercise training can prevent bone loose and improve bone mass [4, 5]. However, the increasing bone density mechanism by exercise training not well under stood

Bone metabolism involves many factor, but estrogen is the one of the factors that is very potential in bone metabolism on male and female. Low level of estrogen can cause osteoporosis in postmenopausal women [6]. The action of estrogen is known as genomic action and non genomic action. The non genomic action through activation protein kinase that it MAPK (mitogen activated protein kinase) this case ERK (extra cellular signal
regulated kinases) 1/2 [7]. ERK is a protein kinase from mitogen activated protein kinase (MAPK), ERK1/2 takes part in controlling cell in proliferation and differentiation [8]), in this case of osteoblast, ERK influence c-bfa (core binding factor a) gene, proliferation and cell adhesion [9]. The other factor that is important enough in bone metabolism is mechanical loading. Mechanical stimulation on osteoblast culture can increase proliferation cell through activation of estrogen receptor (ER) α and also via ERK activation. [10].

The mechanical stimulation on many cell types include osteoblast can activate ERK 1/2. It influenced proliferation and differentiation of osteoblast. However mechanical stimulation by exercise training can activate ERK1/2 expression in menopause has not been reported.

Based upon theoretical review, thus this research is going to unfold the role of mechanical stimulation through the exercise training upon ERK 1/2 expressions on ovariectomized white female rat.

2. Research method
2.1 Types of Research

This research was an experimental research and designed by using The Randomized Posttest Only Control Group Design. The treatment performed the exercise training with moderate intensity on white female rat.

2.2 Experiment Unit

In this research, white female rat (Rattus norvegicus Strain Sprague Dayli) and the age was about 2.5 months as the experiment unit. The white rat comes from Research and Testing Unit (LPPT) University of Gajah Mada (UGM), Yogyakara, and the amount of total unit experiment were 18 rats.

2.3 Research Variables

The independent variable (ovariectomized with no exercise) was controlled by the exercise training in moderate intensity for 80 mins (1 min exercise training and 1 min rest) and (exercise training calcium supplements). The dependent variable was set as the expression of ERK1/2 and levels of bone calcium.

2.4 Research Sites

This research was conducted at Laboratory of Physiology University of Gadjah Mada (UGM), Yogyakarta for exercise training and examination of calcium. ERK1/2 expression determination was conducted in the Laboratory of Physiology University of Brawijaya, Malang.

2.5 Research Procedure

Pre research procedure in this research were:
1. Maintenance of the animal from the holding age of 2.5 months until 3 months.
2. Ovariectomized performed after the division of the group of experimental animals as experimental unit at the age of 3 months.
3. Acclimatization of experimental animals for 2 weeks for adapting to the good environmental conditions on the control group and the treatment group as well.
4. After acclimatization the rat was perfume exercise training in moderate intensity 5 time per week for two month
5. The run was done within 80 minutes by means of one minute run 1minute break.
6. The part of the upper leg in rat was taken by way off after being treated for two months.
7. The protein isolation and SDS-PAGE performed after the bones were cleaned.
8. Then, examine the expression of ERK1/2 by using ELISA, and bone calcium levels by AAS.

3. Data and Analysis

4. Discussion

Based on the results of ANOVA showed that the expression of ERK1/2 are higher in exercise training compare to control. The result showed that these research was not different with in vitro loading and in vivo loading although different in model of mechanical stimulation. According to Gusmau [11], 20 mins after treatment of LPUS (low power ultra sound) as a mechanical stimulation model in the intact-tibia increased synthesis of FAK and ERK1/2 activation. Yuan [12] showed that a cyclic stretching at a 1-Hz frequency, amplitude 10% for 15 were increase proliferation and c-fos expressions compared to control also induced focal adhesion kinase (FAK) activation in rMSCs, followed by rapid extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation for up to 1 h, while no significant difference was found in total FAK (t-FAK) or total ERK1/2. Futhermore, inhibitor of FAK abolished proliferation, c-fos expression and activation of ERK. According to Thomson [13] mechanical force can induce Mitogen Activated Protein Kinase (MAPK) cascade. MAPK are serine threonin protein kinase essential in differentiation proliferation and cell survival. Liu [14] showed that Fluid Shear Stress (FSS) not only increased ALP activity and expression of ALP but also enhanced the phosforelation of ERK1/2 Runx 2 and FAK.

ERK1/2 is a group of MAPK and ERK1/2 is required for osteogenic mesenchimal stem cell differentiation [15]. Matshusita in 2009 [16] showed that ERK1/2 are essential for osteoblast differentiation and inhibit chondrogrnic differentiation in the pericondrium. Increase MAPK signaling promotr promote differentiation of mesenchimal stem cell in to osteoblast and inhibit condrogenic differentiation Increased MAPK signaling promotes differentiation of mesenchymal cells into osteoblasts and inhibits condrogenic differentiation (see figure 2). ERK1/2 activation has been well known as necessary for cell proliferation, which is partly involved in G 1 and G2/M progression [17]. Furthermore, they said that the ERK1/2 cascade activated by mitogenic stimuli is critical for cell proliferation and survive and is required for normal progression in to mitosis.
FAK-ERK signaling is required on mechanical stimulation cyclic model stretching for cultured rat osteoblasts mesenchimal stem discuss cell proliferation in cell (rMSC). Exercise training also activation of FAK (Focal adhesion kinase) [18]. Increasing ERK1/2 after exercise training showed the role of exercise training on decrease risk of osteoporosis because ERK1/2 will activation proliferation and differentiation of osteoblast for increasing bone formation.

This research has not been able to describe the increase of bone density with increased levels of marks of the bone calcium. Marking of increased bone density testing is required by the biochemical markers of increased bone density (bone marker) such as levels of osteocalcin and bone alkaline phosphatase.

5. Conclusion

The conclusion of this study is the physical training at the age of menopause affects the expression of ERK1 / 2, which have a role in the proliferation and differentiation of osteoblasts.

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