The effects of regular exercise on the number of osteoclasts in ovariectomized Sprague dawley rat mandible

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ABSTRACT

Objective: This study aimed to examine the effect of regular exercise on the number of osteoclasts in ovariectomized Sprague Dawley rat mandible.

Methods: Eight female Sprague Dawley rats, 3 months of age, were ovariectomized and divided into control (untreated) and treatment group (regular exercise). The exercise conducted by the rats ran on a specially designed rat treadmill for 12 weeks, 5 times per week, at a speed of 10-18 m/min for 60 minutes per day. At the end of the treatment, the mandible was taken and cleaned out from the tissue for making the histological preparations with Hematoxylin and Eosin (HE) stain. The number of osteoclasts counted from each histological preparation on 10 visual fields and the obtained data were analyzed using independent t-test.

Result: The number of osteoclast was lower significantly in ovariectomized rats compared to control group (P < 0.001).

Conclusion: Regular exercise can decrease the number of osteoclasts in ovariectomized Sprague Dawley rat mandibles.

1. Introduction

Menopause happens because the ovaries are no longer able to secrete progesterone and estrogen in an adequate amount. Decrease in estrogen production causes the decrease of bone strength and density in the whole body including the mandible [1,2,3]. Mandible will continually change as a response from the mechanical pressure and metabolic needs from phosphate and calcium ions and also the hormonal change including decrease of estrogen in menopause [4]. Mandibular deviations caused by the decrease of estrogen levels are low mandible density, resorption of residual ridge alveolar, diminished mandibular cortex, and denture disappearance [5].

Estrogen is important for mineral homeostasis in bones in order to keep the balance between the formation and the resorption of bones both in men and women. Estrogen can suppress the function and production of osteoclasts [6,7], decrease bone resorption, and increase osteoblasts activities [8]. Decrease of estrogen levels in women during menopause increase osteoclasts activities so the bone resorption increases and bone density decreases [9,10].

Estrogen synthesis in adipose tissue, skin, osteoblasts, and endothelial cells of blood vessels, aortic smooth muscle cells, and brain is the main source of estrogen after menopause. Estrogen synthesized from these tissues is from aromatization of androstenedione performed by CYP19 aromatase enzyme [11]. Estrone (estrogen) produced in adipose tissue can reduce bone resorption after menopause [12]. According to the research by Sasano et al. there is aromatase expression in osteoblasts and chondrocytes which plays an important role for bone homeostasis after menopause [13]. Mutation of the gene which codes aromatase enzyme or mutation of estrogen receptor will cause a change in histomorphometry, failure of epiphyseal fusion, osteopenia, and bone growth delay [14].
The risk of cardiovascular disease, breast cancer, obesity, osteoporosis, and also poor sleep quality in women during menopause can be overcome with exercise [15,16]. The effect of exercise on bone density has not been able to be explained certainly. According to Sternfeld and Marcus exercise could affect the peak of bone and the rate of bone mass disappearance [17]. Chen et al. states that running as exercise in 14-month-old rats could increase bone formation and decrease bone resorption by increasing osteoblast formation and decreasing the number of osteoclasts [18]. Research by Tartabian and Saei indicates that exercise could increase serum estradiol levels which are presumed from the activation of estrogen production outside the ovaries [19]. The aim of this study is to examine the effect of regular exercise on the number of osteoclasts in ovariectomized Sprague Dawley rat mandibles.

2. Materials and Methods

2.1 Animals and treatment

This study has obtained an Ethical Clearance Letter from the Ethics and Advocacy Unit Faculty of Dentistry Gadjah Mada University by the decree number of Ref:322/KEP/FKG UGM/EC/2012. Subjects are 8 female Sprague Dawley rats 3 months of age which were ovariectomized (weighing 150-200 grams) and given food and drink according to their needs. Subjects were divided into two groups: control group which did not do exercise; and treatment group which did regular exercise. Both of the rat groups were ovariectomized to make a conditions similar to menopause in humans.

2.2 Ovariectomy procedure

Ovariectomy was conducted after the rats were anesthetized, the fur of the skin where the surgical area was shaved, and the operation area was sterilized and incisized layer by layer until the ovaries were found. The ovaries were cut on both sides and then the surgical operation was stitched. The rats were disregarded for 1 week so the wound would heal by continuing to give food and drink according to their needs. Treatment group was treated with the exercise according to the protocol of Hao et al. [20]. Two weeks before the treatment, the rats were adapted to the treadmill by increasing the duration and speed until the rats were able to run at a speeds of 10-18 m/minute for 60 minutes. Treatment of exercise was conducted after the period of adaptation, rats ran on a specially designed rat treadmill for 12 weeks, 5 times per week, speed 10-18 m/minute for 60 minutes per day.

2.3 Sample collection and histological procedure

At the end of the treatment, the rats from both groups were sacrificed and the mandible was taken and cleaned out from the tissue. The mandibles which had been cleaned then fixated in 10% buffered formalin solution for 24 hours. Then, decalcification was conducted with formic acid for 4 days. The following processes were gradual-dehydration with alcohol, and infiltration with liquid paraffin and embedding in paraffin blocks. The paraffin blocks were sliced with a microtome as thick as 3μ and 6 slices from each mandible cut were taken and attached on the object glass and then deparaffinization and Hematoxylin and Eosin staining.

2.4 Osteoclast Count and data analysis

The histological preparations observed with a light microscope with 400x magnification and number of osteoclasts counted on 10 visual fields. Data obtained were analyzed using independent t-test.

3. Result

![Figure 1. The Average number of osteoclasts in 10 visual fields](image)

The result of study in the effect of regular exercise on the number of osteoclasts in ovariectomized Sprague Dawley rat mandibles as shown in figure 1.

There is a difference in the average number of osteoclasts between the control group and the treatment group, with the number of osteoclast in the treatment group is less than the control group.
There is a significant difference in the number of osteoclasts between the control group and the treatment group with a significant value of $P=0.000$ ($p<0.005$) analyzed using independent t-test.

Figure 2. Osteoclast cells (indicated by arrows) in Hematoxylin Eosin (HE) staining. A) control group; B) treatment group. Observation was conducted with 400x magnification.

4. Discussion

Bone, including the mandible, is an organ that is dynamic and changes continually. Normally, the speed of the formation and bone resorption (remodeling) is equal so the bone mass is constant [7]. The cells that regulate this remodeling process are osteoblasts and osteoclasts. Osteoblasts have a role in bone formation and osteoclasts have a role in bone resorption. Bone remodeling is a complex process. A number of hormones and other factors also regulate the proliferation and differentiation of osteoblasts and osteoclasts [21].

The activity of osteoclasts in reorganizing cells is affected by various factors such as hormones and cytokines. The disturbance of those factors can cause disorder in osteoclast functions [22]. One of the hormones which has a role in the bone remodeling process is estrogen. Some research indicates that the decrease of estrogen due to menopause can disturb the balance of the bone remodeling process because the bone resorption is faster than the bone formation due to the increase in the number of osteoclasts [23]. Increase in the number and activity of osteoclasts causes an increase in bone mass loss so the bone becomes porous and breaks easily [24].

Physical activity or exercise is recommended to increase the bone mass in children and to decrease the loss of bone mass in menopausal women so that osteoporosis is prevented [25]. A research by Douchi et al. [26] states that exercise can increase bone density in menopausal women.

In this study, the number of osteoclasts in the treatment group is lower than the control group. Our finding is in accordance with the research by Kohrt et al. [27] that exercise in early menopause can increase the density of the backbone and femur. Exercise can increase the bone density by increasing the serum estrogen level so that the number of osteoclasts can be suppressed. Muscle contraction during exercise will trigger Hiphotalamus-Pituitary-Adrenal (HPA) axis and stimulate secretion of androstenedione, dehydroepiandrosterone, and dehydroepianandrosterone sulfate from adrenal that will be changed into estrogen in the tissue by aromatase enzyme so the estrogen production in the tissue increases [28,29].

Estrogen can suppress the number of osteoclasts by preventing differentiation and stimulating osteoclasts apoptosis. Estrogen triggered osteoclasts apoptosis by inhibiting the binding between Receptor Activator of NF-kB (RANK) with its ligand RANKL, because the bond between RANK with RANKL activated a signal for the development and osteoclast maturation [30,31]. Nakamura et al. [32] states that estrogen can prevent the loss of bone mass because there are estrogen receptors in osteoclasts that affect osteoclast lifespan. Chen et al. [33], in their research, found that estrogen in cell culture can suppress osteoclast formation which is signaled by the decrease in the expression of Vitronectin Receptor (VNR) as a protein signaling in osteoclast differentiation. Estrogen directly induces osteoclast apoptosis which is signaled by chromatin condensation, DNA fragmentation,
and changes in osteoclast form under fluorescence microscope [34].

Treadmill exercise in young rats will stimulate bone formation and suppress bone resorption, increase the level of 1,25-dihydroxyvitamin D3, and decrease the level of parathyroid hormone so the bone mass increases [35]. Physical activity will alter the motility and permeability of the intestines, therefore calcium absorption increases and the bone density increases [36].

5. Conclusion

Regular exercise can decrease the number of osteoclasts in ovariectomized Sprague-Dawley rat mandibles.

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Conflict of Interest

The authors report no conflicts of interest

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