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Original Research

Intermittent Exercise Triggers Synthesis of CYP19 Aromatase as a Key Enzym for Estrogen Formation In Sprague Dawley Rat Bone Innovarectomy

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Abstract

Menopause is a permanent cessation of menstrual cycle due to reduced secretion of the hormone estrogen which can result in osteoporosis. Osteoporosis is characterized by the process of bone resorption faster than the process of bone formation, resulting in a decrease in bone mineral density and bone microarchitecture damage resulting in bones becoming brittle and easily fracturing. Physical exercise is a holistic intervention to prevent osteoporosis due to menopause. This study is a pure experimental study using a post-test only control group design research design. The subjects of the study were the 12-week-old Sprague Dawley rat. The number of groups is 5 with the number of rats per 6 animals. There was a significant difference between the ovarectomy group of rats and the ovarectomy group of mice and were given intermittent exercise treatment of CYP19 aromatase expression. Discussion: Muscle contractions that occur due to intermittent exercise treatment can produce large amounts of IL-6 and IL-6 mRNA which can stimulate estrogen production. Local estrogen production can reduce bone resorption and increase bone formation and bone density Intermittent exercise can trigger the process of the synthesis of the CYP19 aromatase enzyme in ovarectomy rat femur bones

INTRODUCTION

Menopause is defined as a state of permanent cessation of menstrual cycles due to reduced secretion of ovarian hormones that occur naturally or are caused by surgery, chemotherapy or radiation (Nelson, 2005). Women at menopause will experience symptoms of hot flushes or sensations of heat in the body radiating to the face, vaginal dryness, sleep disorders, cognitive changes, mood changes (Al-Azzawi, 2009), and those associated with long-term health problems that may be affected by the disease coronary heart disease and osteoporosis (Cutson and Meuleman, 2000).

Osteoporosis is a progressive chronic disease characterized by a process of bone resorption faster than the process of bone formation, resulting in decreased bone mineral density and bone microarchitecture damage resulting in bones becoming brittle and easily fracturing (Rizolli et al., 2008). The World Health Organization (WHO)

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defines osteoporosis as a disease characterized by reduced bone mass and bone tissue microarchitecture abnormalities due to increased bone fragility and the risk of bone fractures (Conference report, 1991). Osteoporosis or loss of bone mass in menopausal women and / or after ovarectomy is mainly due to estrogen deficiency (Doren, 2002).

Hormone replacement therapy is believed to reduce menopausal symptoms and prevent osteoporosis, cardiovascular disease and Alzheimer's type dementia at menopause (Hanafiah, 1999). However, prolonged administration of hormone replacement therapy to women will increase the risk of developing breast cancer (Ross, 1997), the risk of getting endometrial cancer (Grady, et al. 1995) and the risk of ovarian cancer (Rodriguez, et al. 2001). Giving estrogen alone or a combination of estrogen and progestin increases the risk of stroke by 40%. Progestin supplemented estrogen also increases the risk of coronary disease, pulmonary embolism and breast cancer (Women's Health Clinical Research Center, 2006).

Controversy and the many complications resulting from hormone replacement therapy at menopause have led to the development of a holistic and natural approach to managing menopause including regulating diet, physical exercise or sports, skills in regulating body and brain rhythms, supplementation and herbal (McKee and Werber, 2005).

Research on the effect of exercise in overcoming menopausal symptoms has been carried out among them, stating that physical exercise or exercise is useful to prevent osteoporosis, obesity, depression and cardiovascular disease (Slaven and Lee, (1997). The effect of exercise on osteoporosis among others, stated that running sports during 9 weeks has an impact on increasing bone mineral content and bone mineral density of vertebrae and tibia in adult mice (Yeh et al., 1993). Isometric exercise performed by rotating movements without displacement or weight gain can have an impact on increasing bone formation with increased ability bone reconstructs or improves bone shape for bone strength requirements (Yeh et al., 2001). Running sports with a slope of 11% and a speed of 21 meters / minute performed as many as 40 sets of intervals 1 minute running and 1 minute rest for 4 weeks can prevent the process of bone loss in ovarectomy rats by suppressing the process of bone resorption (Wong et al., 2006). Running sports can prevent the total decrease in strength of the femur, tibia and humerus bones so that it is useful to eliminate the effects of ovarectomy on bone (Peng et al. (2009). Running sports contribute to the degree of protection against cartilage degeneration in mice after ovarectomy (Chang et al., 2010).

Running sports with a slope of 11% and a speed of 20 meters / minute carried out as many as 40 sets of intervals 1 minute running and 1 minute rest for 4 weeks and administration of Genistein can prevent the process of bone loss in ovarectomy rats (Nakajima et al., 2001). Regular exercise with moderate zone intensity can increase serum estrogen levels in postmenopausal women (Agustingsih, 2006). The effect of exercise in overcoming the symptoms of menopause, probably due to the effect of exercise on estrogen and estrogen levels is most likely derived from extragonadal aromatization, this is given in women after menopause all estrogen and almost all androgens are made locally in the peripheral tissues of DHEA (dehydroepiandrostenedion) (Liben, 2006).

Research relating to the effect of exercise on the formation of extragonadal estrogens has also been carried out among them, stating that the expression of CYP19 aromatase in adipose tissue is higher due to regular exercise in ovarectomy rats (Bebasari, 2010). CYP19 aromatase expression in the adrenal cortex is higher due to regular

exercise for 8 weeks in ovarectomy mice (Asnawati, 2010). Exercise prevents the accumulation of fat in the liver and increases the expression of α (alpha) estrogen receptors in rats that are ovarectomized or not ovarectomized and whether or not estrogen therapy is given (Hao et al., 2010). Has not been studied about the effect of intermittent exercise on the expression of CYP19 aromatase in the femur bone.

METHODS

This research is a pure experimental study using a post-test only control group design research design. This study was conducted to determine the effect of intermittent exercise on the expression of CYP19 aromatase, in ovarectomy femur bones. The research variables included independent variables namely intermittent exercise which is a treatment of running sports with a slope of 11% and a speed of 21 meters / minute conducted 40 times intervals 1 minute running and 1 minute resting, a total time of 80 minutes per day as much as 5x in one week for 4 weeks. The dependent variable was the level of CYP19 aromatase expression which was the percentage of cells from the observation of 10 field views of fragments of femur bone tissue after immunohistochemical staining using CYP19 primery antibody kit using 400x magnification in brown.

The subjects of this study were female Sprague Dawley rats aged 12 weeks or ± 3 months with a body weight of 140-186 grams. Rat animals were obtained from the Experimental Animal Maintenance Unit (UPHP) of the Integrated Research and Testing Laboratory (LPPT) Unit IV of the Gadjah Mada University (UGM) in Yogyakarta. The number of groups was 5 with the number of rats in each group being 4 and added 2 for each so that each group became 6.

The research materials used were catgut, cotton, xylazine and ketamine HCL sewing threads for rat anesthesia, 70% alcohol, povidone iodine, gloves, small pots as samples to be examined, saline, 1.5 ml microtube, microhematocrit tubes, buffers 10% formalin, 5% formic acid as a decalcification agent, calcium obtained by mixing and heating CaCO3 powder in CMC. Standard rat feed in the form of AD II pellets with water content of 12% crude protein 15%, crude fat 3-7%, crude fiber 6%, ash 7%, calcium 0.9-1.1%, phosphorus 0.6-0, 9%, antibiotics and coccidiostats. Preparation ingredients are alcohol with various concentrations (30%, 40%, 50%, 60%, 70%, 80%, 90%, 96% and 100%) for dehydration and rehydration, xytol, phosphate buffer saline (PBS) , buffer citrate. Immunohistochemical staining of rabbit serum, primary antioby, bioetynilated secondary antibodies, streptavidine peroxidase, diaminobenziydine (DAB), hematoxylin mayer, Mounting with E.Z Mount goat and Canada Balsam.

The tools used in research; special treadmills for rats (Gama-tread 2006), minor surgical instruments for ovarectomy (scapel, scissors, sewing thread, sewing needles, needle holders and tweezers), injection syringes, digital scales, mouse cages with rat eating and drinking equipment and digital cameras . Histological preparations, slices of rat femur bone tissue, namely microtome, staining jar, microwave, poly-lysine coated slides microscope, coverslip, timer and SSA tool; homogenizer to destroy rat bone tissue. To observe preparations that have been colored using a light microscope and micrographic tools.

RESULTS

The results of this study relate to the parameters measured in this study, namely the expression of CYP19 aromatase using a sample of rat femur bone tissue. Observation and evaluation of CYP19 aromatase expression was carried out on immunohistochemical preparations with CY19 aromatase antibodies that would

show a cytoplasm of cells that were brown or positively painted (expressing CYP19 aromatase) and cell cytoplasm which did not express CYP19 aromatase in dark blue or purple. Each preparation was observed and examined as many as 10 visual fields. The percentage of CYP19 aromatase expression can be done by counting the number of cells that express CYP19 aromatase and cells that do not express CYP19 aromatase in each slice. The percentage of CYP19 aromatase expression was done by counting the number of cells expressing CYP19 aromatase and those not expressing CYP19 aromatase in each field of view as the total number of cells. Calculate the percentage of CYP19 aromatase expression by counting the number of cells expressing CYP19 aromatase multiplied by 100% divided by the total number of cells. An example of a description of CYP19 aromatase expression in a preparation is shown in Figure 1.

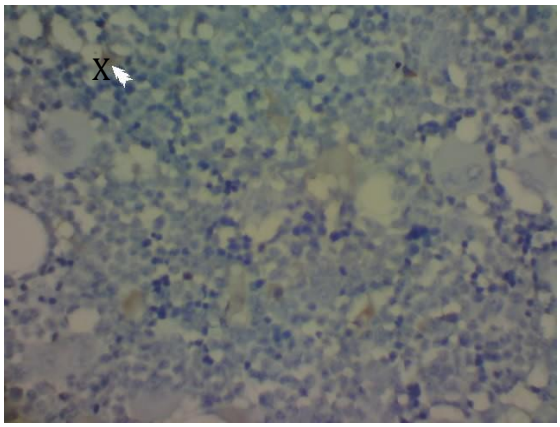

Figure 1
Picture of CYP19 Aromatase Expression on IHC painting on observation of Olympus light microscope at 400x magnification
Description: X. Cells that express CYP19 aromatase

The average description of CYP19 aromatase expression, osteoclast counts and calcium levels in ovarianectomized femur bones of 12 samples with observations of each sample of 10 visual fields grouped into five treatment groups, are shown in Table 1.

Table 1
Mean ± standard deviation of parameters for all treatment groups

Parameter	Mean ± standard deviation of all parameters in all treatment groups	
	X1	X2
Ekspresi CYP19 aromatase	0.15±0.36[a]	7.57±3.34[ab]

Information:
a, b = the same letter in one line shows that there are significant differences between treatment groups
X1 = Ovarectomy group of rats
X2 = Group of rats that were ovarekotmi and treated with intermittent exercise

The mean levels of CYP19 aromatase expression showed different values in the group of ovarectomy rats and the group of rats that were ovarectomized and treated with intermittent exercise. The ovarectomy group of rats obtained a mean CYP19 expression level of 0.15 standard deviations of 0.36. In the group of ovarectomized rats and treated with intermittent exercise, the mean CYP19 expression level was 7.57 with a standard deviation of 3.34.

Statistical analysis of the effects of intergroups on CYP19 aromatase expression was analyzed using the Mann-Whiteney test. Mann-Whiteney test results between the ovarectomy group of rats and the ovarectomy group of rats treated with intermittent exercise related to their effect on CYP19 aromatase expression obtained mean levels of 3.50 and mean levels of 9.50, with a p value of 0.003 ($p \leq 0.05$). This shows a significant difference between the ovarectomy group of rats and the ovarectomy group of rats who were given intermittent exercise treatment related to their effect on the expression of CYP19 aromatase, which means that the intermittent exercise treatment in ovarectomy rats gave a significant increase in the level of CYP19 aromatase expression

in ovarectomy femur bone with a difference in mean level of 6.00.

DISCUSSION

This research is a pure experimental study using a post-test only control group design research design. The study was conducted using 30 experimental female white mice (Rattus Norwegicus) Sprague Dawley strain aged 12 weeks (3 months). Some of the reasons why using mice as experimental animals in this study are because they have the same analogy as humans, have conditions or information that can be transferred / transmitted, have genetic similarities that can be applied to all living things, have a common background behind knowledge related to biological processes, available and affordable in terms of cost or price, easy and able to adapt to research treatments and have ethical and social code implications (Davidson et al., 1987). The existence of comfort and relevance or can be compared with conditions in humans (Rodger et al., 1993). The advantages of using mice as experimental animals include cheap or inexpensive, easy to maintain and are commonly publicly used rats for research (O'Brien et al., 1997).

Rats are used as experimental animals aged 12 weeks (3 months) with the consideration that mice reach the maturity of sexual organs at 2.5 months (Jee & Yao, 2001) or 3 months (Kalu, 1991), so according to the research design that makes rats in menopausal conditions induced by ovarectomy surgery. This is because age is closely related to bone mass and bone strength in both humans and mice (Martin, 2002). Twelve rats were divided into two treatment groups, each group consisted of six samples which included the X1 treatment group namely the ovarectomized rat group and were given a standard feed-drink which was at the same time a negative control group from the X2 treatment group namely the ovarectomy rat group, fed with standard drinks and treated with intermittent exercise.

The reason for ovarectomy is because the ovarectomy mouse model is well known to be used in postmenopausal osteoporosis research. After ovarectomy the process of bone resorption exceeds the process of bone formation resulting in loss of bone mass (Wronski et al. 1989). Ovarectomy was agreed to have the same effect of histological changes on rat bone and human bone so that this could provide benefits related to information about bone mass loss in menopausal humans (Kalu, 1991). Ovarectomy is a method used to produce menopausal conditions artificially (Wronski & Yen, 1992). Ovarectomy is a potential factor that can induce the process of bone matrix deminaralization in mice (Cesnjaj et al. 1991). The ability of bones to repair damage due to ovarectomy in mice is the same as the ability of bones in humans (Abee et al. 1993).

Osteoporosis is a chronic progressive disease characterized by bone resorption process faster than bone formation process, resulting in decreased bone mineral density and bone microarchitecture damage resulting in bones becoming brittle and easily fracturing (Rizolli, et al., 2008). Osteoporosis or loss of bone mass in menopausal women and / or after ovarectomy is mainly due to estrogen deficiency (Doren, 2002). The research sample used is femur bone tissue, this is based on consideration that the strength of mechanical stress in the femur bone in the neck is a very sensitive indicator of bone loss due to ovarectomy, orchidectomy and immobilization (Peng et al., 1994). It also relates to osteoporosis predilection areas that often occur in the femur, vertebrae, pelvis and forearm bones (Sherwood, 2001).

The results of statistical analysis using the Mann-Whitney test obtained p value of 0.003 ($p \leq 0.05$), which means that intermittent exercise treatment in ovarectomy rats had a significant effect on increasing the expression of CYP19 aromatase in the ovaryectomy of the rat

bone. An increase in the expression of CYP19 aromatase in ovarectomy rat femur bone in this study, is in line with the concept that regular exercise with a moderate zone can increase serum estrogen levels in postmenopausal women (Agustiningsih, 2006). Regular exercise is likely to reduce the degree of perimenopausal estrogen deficiency, at least activating estrogen production which does not originate in the ovaries or extragonadal regions (Asnar, 2005).

More specifically in line with the concept that estrogen biosynthesis by extragonadal tissue; adipose, bone, mammary and brain tissue are highly dependent on the presence of circulating steroid C19 precursors (Labrie et al. 1997). Synthesis of the hormone estrogen can occur in osteoblasts or bones (Bruch et al. 1992, Bayard et al. 1995). Estrogen biosynthesis by extragonadal tissue; adipose, bone, mammary and brain tissue are highly dependent on the presence of circulating steroid C19 precursors (Labrie et al. 1997). CYP19 aromatase is a key enzyme in the process of estrogen synthesis in peripheral / extragonadal tissue, especially in bone tissue (Watanabe, et al. 2004). This enzyme is expressed in response to catalyzing aromatization processes for transformation from C19 androgens to C18 estrogens such as estradiol to estron transformation (Simpson et al. 1994; Conley & Hinshelwood, 2001; Kamat et al. 2002; Bulun et al. 2005). This CYP19 aromatase parameter is certainly appropriate as an indicator for assessing the effect of a combination of measured exercise exercise and calcium supplementation as an effort to prevent or manage osteoporosis during menopause and / or after ovarectomy which is mainly caused by estrogen deficiency (Doren, 2002). The CYP19 aromatase enzyme is expressed as a response in catalyzing the aromatization process for the transformation from androgen or testosterone to estrogen and from androstenediol to estron (Simpson et al. 1994;).

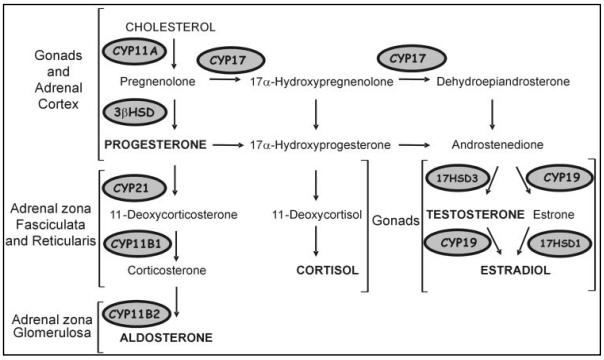

Figure 2
The Role of Aromatase in the Biosynthesis of Steroid Hormones in the Adrenal and Gonad Glands (Payne and Hales, 2004)

The mechanism of the occurrence of CYP19 aromatase expression in ovarectomy rat femur bones due to intermittent exercise in this study is most likely because the muscle contractions that occur due to intermittent exercise treatment can produce large amounts of IL-6 and IL-6 mRNA. Muscle biopsy after exercise shows an increase in IL-6, this indicates that muscle contraction is a stimulus for IL-6 production by muscles (Toft et al., 2002). Interleukin-6 is thought to work like a hormone, carrying out metabolic control by increasing energy supply during exercise. With increased glucose uptake by muscles, hepatic glycogenolysis must be activated. The content of muscle glycogen affects the release of IL-6 during exercise (Steinacker, 2004)., States that the expression of aromatase in bone osteoblasts can be triggered by several cytokines including IL-6 (Pedersen, 2004). Increased aromatase activity in bone osteoblast cells triggers an increase in local estrogen production which can decrease bone resorption and increase bone formation and bone density will be maintained because of the role of estrogen produced by the activation of aromatase induced by IL-6 (Purohit et al., 1992) This is in accordance with the theory that cytokines, especially IL-6 can increase aromatase expression (Shozu & Simpson, 1998). Running sports with a slope of 11% and a speed of 21 meters / minute

conducted as many as 40 sets of intervals 1 minute running and 1 minute rest for 4 weeks can prevent bone loss in ovarectomy rats by suppressing the process of bone resorption (Wong et al., 2006). Running sports with a slope of 11% and a speed of 20 meters / minute carried out as many as 40 sets of intervals 1 minute running and 1 minute rest for 4 weeks and administration of Genistein can prevent the process of bone loss in ovarectomy rats (Nakajima et al., 2001).

CONCLUSION

The level of CYP19 aromatase expression of femur bone tissue in rats that are ovarectomized and given intermittent exercise treatment is higher than the level of CYP19 aromatase expression in rats that are only ovarectomized. Intermittent exercise can trigger the process of the synthesis of the CYP19 aromatase enzyme as a key enzyme for the formation of the hormone estrogen in the ovaryectomy of the femur bone.

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CONFLICTS OF INTEREST

Neither of the authors have any conflicts of interests that would bias the findings presented here.

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