Effect of Endurance Treadmill Training on mIGF-1 Expression and PAX7 Satellite Cell in Rat Muscle Tissues

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Abstract This study examined the effects of endurance exercise training on muscle Insulin Growth Factor-1 (mIGF-1) and PAX7 transcription factor of satellite cell activation in an anterior tibialis muscle of the rat. The rat was subjected to the treadmill, divided into three groups, each containing 6 rats. Groups of high, moderate and low doses of exercise received 28, 17, and 12 m/s of treadmill speeds respectively. Treadmill exercise training conducted over 5 days a week within 9 weeks. At the end of 9 weeks of the experiment, the rat was sacrificed, tibialis anterior muscle tissues were removed and then subjected to immunohistochemistry examination. There were significant differences in the intensity of training in mIGF-1 and PAX7 in muscle tissues (P < 0.05). These results suggested that endurance exercise training which high, moderate and low intensity able to increase mIGF-1 and PAX7 that associates with satellite cell development of muscle tissues.

Introduction

Exercise inherently alternative medicine to overcome the problem of chronic diseases. However, exercise will work well if the dose of exercise to meet the needs of the body. Mechanical stimulus due to exercise can increase the mass of skeletal muscle tissue as well as the capacity of the systemic body function. However, exercise intensity to exhaustive will increase the disruption of body homeostasis, likewise exercise intensity is too light, body system unable to enter adaptation level (Sugiharto, 2012). Satellite cells own the function of maintaining growth reserves in muscle cells (Patrick et al., 2000). Endurance and resistance exercises have the potential ability to increase the number of satellite cells (Neil & Henning 2006 & Shefer et al., 2010).

Endurance exercise with moderate intensity for 13 weeks increases the satellite cell per muscle fiber in both male and female and young and old rat. However, Sophie (2014) study did not find any increase in satellite cells with endurance exercise. The satellite cell is characterized by PAX7 as the cell cycle activator and is responsible for the activation and regulator pool reserve of the satellite cell. Muscle regeneration is due to pressure or injury, mIGF1

Is responsible for activating the satellite cell (Anastasssios et al., 2007). However, whether endurance exercise with the lightest and the highest intensity may increase co-expression between mIGF1 and PAX7, need further examination.

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Material and methods

Male rats of Wistar strain weighing around 170-180 g were purchased from De’ Wistar Bandung West Java. The experiments were performed in accordance with guidelines and approval (No. 775-KEP-UB) of the institutional requirements concerning the care and handling of animals according to Guiding Principles for the Care and Use of Animals for Scientific Purposes in the Institutional Animal Care and Use Committee (IACUC). The rat was subjected to the treadmill, divided into three groups, each containing 6 rats. Groups of a high, moderate and low dose of exercise received 28, 17, and 12 m/s of treadmill speed respectively. Treadmill exercise training conducted over 5 days a week within 9 weeks. The rats were randomly divided into 4 groups. Group-1: High-intensity treadmill exercise program. Group-2: Moderate intensity treadmill exercise program. Group-3: Low-intensity treadmill exercise program, and group-4: received no treatment.

Treadmill running

Animals were familiarized with treadmill apparatus CIS Ideas (Industry of Electronic & Software Indonesia) to minimize stress and then divided into two groups: exercised and non-exercised. The procedures followed are described in detail elsewhere [7]. The group ran for 1 hour at 17 m/min for two weeks, whereas the control group remained in the treadmill without any training.

Immunohistochemistry

Rabbit Anti-mIGF-1 Polyclonal Antibody (bs-0014R) and PAX7 Polyclonal Antibody (bs-2413R) was purchased from BIOS USA. Immunohistochemistry staining kit was purchased from Histofine Simple Stain Rat MAX PO (Tsukiji, Chuo-ku, Tokyo, JAPAN). The left tibial anterior muscle was fixed in 10% formalin tissues. After dehydration, they were embedded, sectioned, and stained with anti-mIGF-1 and anti-PAX7 polyclonal antibody. For microscopic histological evaluation, formalin-fixed tissues were embedded in paraffin and 5 mm sections. Staining specificity was confirmed using appropriate negative control. Samples were observed under the Nikon microscope at 40x magnification and captured and analyzed using the OlyVIA ver. 2.2 software. Histological assessment of muscle tissue was performed by a single pathologist in a blinded fashion.

Statistical analysis

The results were expressed in ± standard deviation. Statistical analysis was carried out by using one-way ANOVA as in standard statistical software package for the social science (SPSS).

Results and discussion

The results were clearly illustrated in all categories of intensity training. The increasing of mIGF-1 expression was stimulated by the myogenic process for adaptation. Histologic changes in wistar rats in 3 difference treatments of exercise treadmill for 9 weeks were identified by immune-histochemical staining. Muscle adaptation due to exercise is indicated by the increase of muscle fibers, which was indicated as a sign of active regeneration of muscle tissues. The control group showed low mIGF-1 expression among the other groups (Figure 1), whereas the low-intensity exercise group (12 m/s) showed an average expression of almost similar with a control group. This phenomenon suggests that the low exercise intensity did not cause of fiber changes muscle. In addition, muscle fibers can adapt to changing demands by changing size or Regeneration of skeletal muscles is regulated by mIGF-1. During the process of hypertrophy, regeneration and muscle development, mIGF-1 plays an autocrine and paracrine in mitogenic and miogenic processes. High and moderate intensity presents a considerable population of mIGF-1 expression by 116.66 and 79.5 ± per group, respectively (Figure 1), whereas low treatment and control did not increase significantly.
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Figure 1. The effects of treadmill endurance training on mIGF-1 in tibialis anterior Wistar rat. mIGF-1 in striated muscle tissue. A: Rat were given high-intensity training; B: Rat were given moderate intensity; C: Rat were given low intensity, D: Rat given no training and E: mIGF-1 expression level between the group. Original magnification × 400 (Scale bar = 20 μm). **P < 0.001.

To determine the effect of satellite cell activity, we observed the PAX7 expression. PAX7 is a common transcription factor in a satellite cell adjacent to the cell nucleus of the sarcolemma and is essential in the myogenic factor and regeneration of the satellite cell itself. Moderate and low exercises increased PAX7 expression, whereas high-intensity exercise decreased significantly than other groups. These results indicate that the exercise does have a different effect on the response of PAX7 satellite cell on anterior tibial muscle fiber tissues.

Several studies have been conducted to unravel the potential for endurance exercise against satellite cell expression. Sophie et al. (2014) reported exercises did not increase the reserves of satellite cells but increased the cells that were active due to the reconstruction of muscle fibers, whereas Shefer et al. (2010) revealed an increase in satellite cells per muscle fiber with moderate intensity exercises. The present study shows that all intensity treatment of endurance exercise increases satellite cell expression.

In the present study, a general body weight of all groups was high at the beginning or end of the exercises. The body weight before the treadmill treatment of the control group showed highest number among all groups, followed by low-intensity treatment group and moderate group, whereas high-intensity treatment groups showed the lowest one. The different results of each treadmill treatment can be explained by the difference in predominant energy when exercising. The predominant differences in energy are characterized by differences in exercise intensity.

Based on the results, there was a difference in mIGF-1 expression between exercises performed (Figure 1). Also, it described that mIGF-1 expression in low intensity group was smaller than the control group. The expression of mIGF-1
in the control group was higher than moderate and low exercises because it never received physical stress from induction exercises stimulate the increase of energy metabolism. In low-intensity training, mIGF-1 expression was still lower than other exercise groups. According to Eliakim, (2010), exercise has a little impact on mIGF-1. The decreasing of growth hormone binding protein (GHBP) is followed by the low receptor of tissues and then activates liver to produce GHBP, so that the increasing of mIGF-1 receptors binding capacity would occur exercise adaptation. The levels of mIGF-1 (Anastasssios et al., 2007) GH work marker in the liver rather than the second messenger by GH. The high and moderate intensity of treadmill treatment able to increase mIGF-1 expression. mIGF-1 expression of the high-intensity group has the highest value compared to all groups (Figure 1). This result supported with Anastasssios et al., (2007) that local overexpression of mIGF-1 has been shown to regenerate myofiber and hypertrophy and increase levels of myogenic regulatory factors, and contractile mRNA proteins.

The expression of mIGF-1 increased in moderate intensity group (Figure 1), but this expression lower than the high-intensity group. This condition can be caused by the difference in the intensity of the treadmill exercise. The expression of mIGF-1 increased significantly of all groups. The increasing of mIGF-1 expression in the tissues supported by myogenic factors, DNA templates for protein synthesis and lead to improvement and increased in the amount of muscle tissue. During intense exercise, most mIGF-1 are expressed by active muscle.

Muscle tension or injury, locally produced mIGF-1 because exercise causes the increasing of mIGF-1 levels. Furthermore, the expression of mIGF-1 can be regulated by a certain amount of GH and mIGF-1. Our results showed that the treatment of high intensity exercises able to increase significantly of mIGF-1 expression compared to control groups (Figure 1). The treatment of exercise in the low-intensity exercise group showed lower expression of mIGF-1 rather than moderate intensity and high intensity but has similar value to control group (Figure 1). These findings suggest that the expression of mIGF-1 increased with increasing intensity of exercise.

These results indicated that the variation of PAX7 number as transcription factors in the treatment groups. In the control group, the amount of PAX7 expression of left anterior tibial tissue increased compared to the control group (Figure 2). The treatment of high-intensity exercise group described low expression than the control group (Figure 2). In the moderate-intensity group, explaining an increase in satellite cells by the moderate intensity of treadmill exercises. The group with low-intensity treatment increased PAX7 expression in average (Figure 2). The cause of the difference in PAX7 expression between low and high-intensity groups was still unidentified. In other words, that even with low-intensity treadmill exercises, satellite cells remain less expressed. The high-intensity treatment in this study showed the lowest expression of all groups including the control group. This difference in expression can be explained by the activation process but also the restriction of satellite cell cycle activation. The results are supported to Tim et al., (2015), reported that PAX7 is a special marker transcription factor for satellite cell activity, also regulating the presence of satellite cells within the sarcolemma. So, the satellite cells regulate its own renewal. When the stimulus occurred during exercise, muscle with stress activates PAX7 to regulate the renewal of myoblasts. In the control group, satellite cell expression was greater than high treatment group, but there was no increase in the number of myoblasts in the muscles. Whereas, in the high intensity, moderate and low group, the satellite cell went through proliferation cycle of differentiation and maturation myoblast and self-renewal for its own reserves.

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The histologic analysis of the muscles explains the presence of co-expression between mIGF-1 and PAX7 at 24 hours after exercises, but mIGF-1 was not yet apparent in the myofiber, so the initial appearance of mIGF-1 was not dominant in the satellite cell compartment. However, mIGF-1 protein appears in the myofiber and satellite cell compartment during 72-120 hours after exercises. Therefore mIGF-1 is responsible for activation and proliferation of satellite cells into the terminal differentiation phases (Tim et al., 2015). Thus, the expression of mIGF-1 and PAX7 explained that mIGF-1 cooperate with satellite cells and confirms that mIGF-1 was responsible for regulating satellite cell. In this study, the treadmill endurance exercise was performed for as long as nine weeks, so the response of the muscle satellite cells was due to adaptation. The correlation values show the relationship between mIGF-1 and PAX7 expression with medium interpretation with a value of 0.654. The result of high treatment indicated the direction of negative correlation between mIGF-1 and PAX7. PAX7 expression in the control group was higher than the high-intensity group, so further analysis needed for future study.

**Conclusion**

The treadmill treatment increase mIGF-1 and PAX7 expressions intensity, but PAX7 shown lower in high intensity regardless still expressed dramatically in low and moderate intensities, and also in the control group. This data confirms that satellite cell will recycle their self after stimulates by the intensity of exercise, therefore, muscle regenerate rapidly.

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