Effects of Physical Exercise on Indicators of Inflammation Risk of the Gaster in a Male Wistar Rat Aging Model Created with D-galactose Induction

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Abstract

Background: Physical exercise is a non-pharmacological treatment for various diseases. Aging is associated with deteriorating physiological function, and elderly individuals generally have inflammation or infection in the digestive tract. This study aimed to examine the effects of mild and moderate physical exercise intensities on the indicators of inflammation risk of the gaster in a male Wistar rat aging model created with D-galactose induction. Methods: This experimental research study had a post-test-only group design. The study included 3-month-old male Wistar rats weighing 200–300 g. The total of 24 rats were equally divided into four groups (saline,+light-intensity physical exercise, and D-galactose+moderate-intensity physical exercise)D-galactose, D-galactose. D-galactose was continuously administered at 300 mg/mL/kg body weight. The study period was four weeks. The number of fibrocytes, mucosal thickness, and the number and size of mucosal glands were analyzed. Results: D-galactose induction triggered aging. Physical exercise had an effect on weight gain and decreased the number of fibrocytes. However, there were no effects on mucosal thickness and the number and size of mucosal glands. Conclusions: Physical exercise of mild/moderate intensity had an effect on the number of fibrocytes but did not have impact on the mucosal thickness or the number and size of mucosal glands.

Keywords: aging, D-galactose, exercises, inflammation

Introduction

Physical exercise is considered as a non-pharmacological treatment for various diseases.1 Regular physical exercise protects against immune senescence and may rejuvenate the aging immune system, reduce inflammatory cytokines.5-5 Regular physical exercise can also inhibit the symptoms of decreased gastrointestinal function as one of the effects of aging.6 Aging is a natural process that can increase the sus-ceptibility of organisms to environmental exposures and diseases.7 The multifactorial processes in aging include changes in metabolic homeostasis, inflammation and/or redox processes in cells and tissues, and the theory of oxidative stress (free radicals) that refers to an increase in the level of reactive oxygen species (ROS) as the primary process of cell aging.8

In elderly individuals, the digestive tract shows morphological changes. For example, in the mucosa, an increase in collagen deposits leads to an increase in the thickness of the mucosa and a decrease in the number/size of mucosal glands.9 The chronic administration of D-galactose in accelerating aging, influencing age-related cognitive decline.10 The present study aimed to examine the effects of mild and moderate physical exercise intensities on the indicators of inflammation risk of the gaster in a male Wistar rat aging model created with D-galactose induction.

Methods

Research design. This experimental research study had a post-test-only group design. This study included 3-month-old male Wistar rats (weight, 200–300 g) obtained from the Indonesian Islamic University Yogyakarta. The total of 24 rats were kept in cages, and the temperature and humidity were set within ±25 °C and 60%, respectively. They were fed standard AD-II, and they had access to boil water ad libitum. They were divided into four groups (K1, K2, K3, and K4), and each group had six rats. The rats in the K1 group received saline injection and did not perform any physical exercise. Those in the K2 group received D-galactose and did not perform any physical exercise. Those in the K3 group received D-galactose and performed light-intensity physical exercise. Those in the K4 group received D-galactose and performed moderate-intensity physical exercise. The research procedure was approved by the Ethics Committee of the Integrated Research and Testing Laboratory of Universitas Gadjah Mada Yogyakarta (certificate number: 00088/04/ LPPT/VIII/2017).
Preparation and treatment of experimental animals. Induction was performed by injecting D-galactose (300 mg/ml/kg body weight) intra-peritoneally for 28 days. The animals were weighed, and signs of aging were evaluated daily.11 With regard to treadmill exercise, the rats were gradually adapted to the physical exercise protocol for seven days. The rats that passed the acclimatization procedure were subjected to a physical exercise test protocol to estimate the VO2 max. and a physical training protocol for 4 weeks (28 days) at a frequency of 4 times a week for 40 minutes each, consisting of 5 minutes of warmup at 20% of the maximum speed, 30 minutes of core training, and 5 minutes of cooldown at 20% of the maximum speed.12,13

Histopathological examination of the gaster. After completion of the physical exercise protocol, the rats were killed and the gaster was assessed. For extraction of the gaster, the rats were anesthetized with an injection of HCl ketamine (40 mg/kg body weight). The gaster was removed and placed into PBS for cleaning. Subsequently, it was placed into a PBS + 10% formalin solution until hematoxylin/eosin staining and immunohistochemical staining. The results were observed by using a light microscope at magnifications of 40× and 400×, with five fields of view each. Each field of view was photographed by using Optilab software, and the results were stored in a computer. Furthermore, the thickness of the mucosa and size of the mucosal glands were analyzed using ImageJ software.

Results

Body weight. The weight of the rats was measured prior to D-galactose induction and at termination. The final body weight was generally higher than the initial body weight (Table 1). The normality test results before and after treatments showed a normal distribution. One-way ANOVA showed that there were significant differences among the groups (Table 1). According to paired t-test results, there was a significant difference before and after treatments (Table 2). Additionally, there were significant differences in weight gain between K1 and K2 and between K1 and K4 (Table 3).

Thickness of the mucosa. The normality test results showed that the thickness of the gaster mucosa was normally distributed. One-way ANOVA indicated no significant differences for all the groups (Table 4).

Number of cells producing collagen (fibrocytes). The normality test results showed that the fibrocyte data for the gaster were normally distributed. One-way ANOVA indicated significant differences between the groups (p = 0.038) (Table 4). Furthermore, an LSD post-hoc test was performed to compare among the groups. There were significant differences between K1 and K2 (p = 0.042), between K1 and K3 (p = 0.015), and between K1 and K4 (p = 0.011) (Table 5).

Size of the mucosal glands. The normality test results showed that the data were not normally distributed. Furthermore, the non-parametric test and Kruskal–Wallis test showed no significant difference for the groups (Table 4). The size of the mucosal glands was observed by using a light microscope at 400× magnification, and the size was measured by using ImageJ software. The p-value was obtained with the Kruskal–Wallis test. The number of mucosal glands was investigated by using a light microscope at 400× magnification. The p-value was obtained with the Kruskal–Wallis test.

| Table 1. Initial and final body weights of the experimental animals |
| --- |
| Group | n | The mean of Initial body weight (g) ± SD | The mean of final body weight (g) ± SD | p* |
| K1 | 6 | 237.50 ± 1.87 | 326.67 ± 18.62 | 0.002 |
| K2 | 6 | 243.50 ± 1.87 | 270.00 ± 28.81 | |
| K3 | 6 | 249.50 ± 1.87 | 299.17 ± 54.26 | |
| K4 | 6 | 255.50 ± 1.87 | 255.83 ± 26.91 | |

*One-way ANOVA, p < 0.05

| Table 2. Difference between the initial and final body weights of the experimental animals (n = 24) |
| --- |
| Mean (SD) | Difference (SD) | 95% CI | p* |
| Initial body weight | 246.50 (7.07) | 41.42 (46.21) | 21.90 - 60.93 | <0.001 |
| Final body weight | 287.92 (42.78) | 41.42 (46.21) | 21.90 - 60.93 | <0.001 |

*Paired t-test, p < 0.05
**Table 3.** Difference in weight gain among the groups of experimental animals

|                           | The mean weight gain (SD) | 95% CI       | \( p^* \) |
|---------------------------|---------------------------|--------------|-----------|
| Weight gain in K1 - weight gain in K2 | 62.67 (45.13)            | 15.31 – 110.03 | 0.019     |
| Weight gain in K1 - weight gain in K4 | 88.83 (38.00)            | 48.95 – 128.71 | 0.002     |

\( ^* \text{Paired t-test, } p < 0.05 \)

**Table 4.** The mean and SD of mucosal thickness (mm), number of fibrocyte (%), number of mucosal glands, and size of mucosal glands (mm) Gaster Groups Mean mucosal thickness

| Group of Gasters | n | The mean of mucosal thickness (mm) | \( p \) |
|------------------|---|----------------------------------|-------|
| The thickness of the gaster mucosal |   |                                   |       |
| K1               | 6 | 0.03 ± 0.004                     | 0.265*|
| K2               | 6 | 0.03 ± 0.008                     |       |
| K3               | 6 | 0.03 ± 0.007                     |       |
| K4               | 6 | 0.03 ± 0.009                     |       |
| The number of fibrocyte gaster mucosa |   |                                   |       |
| K1               | 6 | 51.38 ± 19.48                    | 0.038*|
| K2               | 6 | 28.96 ± 17.48                    |       |
| K3               | 6 | 24.05 ± 17.01                    |       |
| K4               | 6 | 22.46 ± 17.22                    |       |
| The number of mucosal glands |   |                                   |       |
| K1               | 6 | 88.50 ± 41.50                    | 0.524**|
| K2               | 6 | 96.17 ± 35.67                    |       |
| K3               | 6 | 72.00 ± 23.48                    |       |
| K4               | 6 | 78.17 ± 13.45                    |       |
| The size of mucosal glands |   |                                   |       |
| K1               | 6 | 0.01 ± 0.00                      | 1.000**|
| K2               | 6 | 0.01 ± 0.00                      |       |
| K3               | 6 | 0.01 ± 0.00                      |       |
| K4               | 6 | 0.01 ± 0.00                      |       |

\( ^* \text{One way ANOVA, } p < 0.05 \)

\( ^{**} \text{Kruskal–Wallis test} \)

**Table 5.** Comparison of the number of fibrocytes among groups.

| Gaster     | Mean number of fibrocytes | 95% CI       | \( p \) |
|------------|---------------------------|--------------|-------|
| K1 vs. K2  | 22.41                     | 0.94 – 43.88 | 0.04* |
| K1 vs. K3  | 27.33                     | 5.86 – 48.80 | 0.02* |
| K1 vs. K4  | 28.91                     | 7.44 – 50.38 | 0.01* |
| K2 vs. K3  | 4.92                      | -16.55 – 26.39 | 0.64 |
| K2 vs. K4  | 6.50                      | -14.97 – 27.97 | 0.54 |
| K3 vs. K4  | 1.58                      | -19.89 – 23.06 | 0.88 |

The \( p \)-value was obtained with the LSD post-hoc test. \( ^* p < 0.05 \)

**Discussion**

The present study found that physical exercise altered weight and decreased the number of fibrocytes in older rats. However, physical exercise did not have any influence on the thickness of the mucosa or the number and size of the mucosal glands. Exercise training may increase vagal nerve activity, which is important for reducing inflammation.\(^{14}\) The efferent vagal nerve stimulates the parasympathetic nerve that can inhibit the production of pro-inflammatory cytokines and protect against systemic inflammation.\(^{15}\) In addition, efferent vagal nerve stimulation can slow the heart rate and stimulate gastric motility.\(^{16}\)
The present study used a rat aging model created with D-galactose administered at a dose of 300 mg/mL/kg body weight for four weeks. D-galactose is a physiological nutrient and a reducing sugar that reacts with the free amino group of amino acids in proteins forming advanced glycation end products through non-enzymatic glycation. D-galactose also contributes to the generation of ROS via metabolism of D-galactose. Increased ROS coupled with the destruction of antioxidants will cause oxidative damage to mitochondria, resulting in intracellular damage and cause decreased physical function, resulting in aging. The results obtained in the preliminary test showed an increase in malondialdehyde (MDA) by as much as 3–5 times the normal value, elongation of the QT interval on ECG, proteinuria, and an increase in the serum creatinine level. These results are in accordance with the findings of previous study mentioning that there was an increase in MDA in old age.

According to the results of this study, D-galactose administration at a dose of 300 mg/mL/kg body weight for four weeks increased body weight in the experimental animals. This is in line with the findings of of a previous study mentioning that an animal’s weight increases with age. The body weight was higher in the D-galactose-induced experimental animals than in the D-galactose-induced and physical-exercise-given experimental animals. The results indicate that weight gain in the experimental animals performing physical exercise was less than that in the experimental animals not performing physical exercise. Moreover, weight gain in the experimental animals performing moderate-intensity exercise was lower than that in the experimental animals performing light-intensity physical exercise. In addition, regular physical exercise can reduce fat mass and adipose tissue; thus, excessive fat in the elderly can be reduced with regular physical exercise. These results are consistent with the findings of a previous study mentioning that weight loss occurred in obese women who performed moderate-intensity exercise for 12 weeks.

Aging affects all organs in the body, including the gastrointestinal tract. The gastrointestinal tract wall consists of three major tissue layers, and each layer consists of various cells. The composition of the intestinal wall and its cell components are generally consistent. Changes in cells during aging can affect the function of the gastrointestinal tract. In addition, immune system-related damage to the intestine may increase the incidences of infection and inflammation. Mucosal resistance plays an important role in maintaining mucosal integrity. Under normal circumstances, mucosal integrity is maintained by defense mechanisms that involve pre-epithelial, epithelial, and post-epithelial components. The important factors in mucosal resistance are mucus and bicarbonate secretions, mucosal blood flow, prostanoids, and some growth factors. In aging, there are decreases in mucus, level of nitric oxide, and sensory nerve damage that responds to luminal acid, which can cause damage to mucosal defenses.

In this study, D-galactose induction resulted in aging and was associated with inflammatory risk in the gastrointestinal tract in the form of fibrocytes (cells producing collagen). These results are consistent with the findings of another study mentioning that aging is associated with partial atrophy in the gastric part of the mucosal basal region, which is replaced by connective tissue involving collagen fibers. An increase in this connective tissue is associated with the replacement of glandular cells. Atrophy is caused by an increase in apoptosis preceded by a decrease in mucosal blood flow of about 60%, leading to severe hypoxia in cells that later become apoptotic which way similarly reported earlier. These authors mentioned that aging is associated with the presence of collagen deposits in glands located at the bottom of the lamina propria.

In addition, the results showed the effects of physical exercise of mild and moderate intensities on the number of fibrocytes. The number of fibrocytes was lower in rats that performed moderate/mild-intensity physical exercise than in those that did not perform physical exercise, indicating that physical exercise can indeed reduce the indicators of inflammation risk. These results are consistent with the findings of the studies by another studies mentioning that physical exercise can prevent or reduce the indicators of inflammation risk, but through different pathways, including inhibition of the production of tumor necrosis factor-α (TNF-α); reduction of C-reactive protein (CRP), IL-6, and IL-18; and reduction of oxidative stress through the antioxidant defense system. Besides activating the vagal nerve, regular and measurable physical exercise can improve the function of mitochondria, which can increase energy production for the needs of active cells.

This study also found that the number of fibrocytes was higher in the K1 group (rats without D-galactose induction) than in the K2 group (rats with D-galactose induction but without physical exercise). This may be related to weight gain, as the increase in body weight was higher in the K1 group than in the K2 group. Others researchers argued that obese adipose tissue can produce pro-inflammatory cytokines among which are TNF-α, IL-6, leptin, visfatin, resistin, angiotensin II and plasminogen activator inhibitor 1 that can cause systemic inflammation. Thus, an increase in weight associated with an increase in adipose tissue will trigger the production of pro-inflammatory cytokines that can cause an increase in the risk indicators of inflammation, such as an increase in the number of fibrocytes.

The cell proliferation ability is one of the most important factors for maintaining mucosal integrity and for healing mucosal lesions. In the present study, there...
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Conclusions

Physical exercise of mild and moderate intensities had an effect on the number of fibrocytes but did not have effects on the thickness of the mucosa or the number and size of mucosal glands in our rat aging model created with D-galactose induction.

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Conflict of Interest Statement

None declared.

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