

Simvastatin Toxicity Induces Mitochondrial Dysfunction in Rat Skeletal Muscle

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Abstract

Background: Statins are the class of drugs that are widely used for lowering LDL cholesterol and as primary and secondary prevention to cardiovascular disease. However, the widespread use of statins is constrained by the presence of toxicity or intolerance, which affects drug control rates. The toxicity or intolerance of statins ranges from 10-15%. The most common statin toxicity is statin-associated muscle symptoms (SAMS). The underlying mechanisms of SAMS involve the disruption of mitochondrial biogenesis, potential membrane changes, reduced number of mitochondria, and changes in protein oxidative activity due to the accumulation of ROS in cells and tissues. The disruption of mitochondrial biogenesis can be marked by a decrease of peroxisome proliferator-activated receptor co-activator gamma (PGC-1 α). This study aimed to determine the effect of simvastatin on skeletal muscle PGC-1 α .

Methods: Sixteen female Wistar rats (8-10 weeks of age) were randomized into 2 groups: (1) control group (n=8), and (2) simvastatin group(n=8). For 30 days, the simvastatin group was exposed to simvastatin at a dose of 10 mg/kg/day. Meanwhile, the control group animals only received 0.5% methyl cellulose. Gastrocnemius muscles were collected and PGC-1 α levels were evaluated by using ELISA Kit.

Results: Following 30 days of treatment, a significantly lower level of skeletal muscle PGC-1 α was observed in the simvastatin group compared to the control group ($p = .026$).

Conclusion: Our finding indicates that administration of simvastatin at a dose of 10 mg/kg/day for 30 days may decrease skeletal muscle PGC-1 α leading to mitochondrial dysfunction in rat skeletal muscle.

Keywords: Statin; Toxicity; Mitochondrial dysfunction; peroxisome proliferator-activated receptor co-activator gamma; Skeletal muscle

Introduction

Statins are the class of drugs that are widely used for lowering LDL cholesterol and as primary and secondary prevention to cardiovascular disease ⁽¹⁾. The widespread use of statins is restricted by the presence of toxicity or the associated intolerance, which influences the rates of

drug monitoring. Statin toxicity or intolerance varies from 10 to 15%. In other studies, toxicity can approach 30 % ⁽²⁾. Statin-associated muscle symptoms have been the most common statin toxicity (SAMS). The underlying mechanisms of SAMS involve the disruption of mitochondrial biogenesis, potential membrane changes, reduced number of mitochondria, and changes in protein oxidative activity due to the accumulation of ROS in cells and tissues. The disruption of mitochondrial biogenesis can be marked by a decrease of peroxisome proliferator-activated receptor co-activator gamma

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(PGC-1 α). This study aimed to determine the effect of simvastatin on skeletal muscle PGC-1 α .

Material And Method

Animal

Sixteen female Wistar rats (8-10 weeks of age), were housed in proportion 3 rats per cage with a 12h light/dark cycle. The sample size was determined by using *the Research Equation Method*, where E is the total number of animals – total number of groups. The value of E should lie between 10 and 20. Any sample size, which keeps E between 10 and 20, should be considered as adequate. The total number of animals included in this study were 16 rats. The total numbers of groups in this study were 2 groups. ⁽³⁾

Rats were randomized into 2 groups: (1) control group (n=8), (2) simvastatin group (n=8). For 30 days, the simvastatin group was exposed to simvastatin at a dose of 10 mg/kg/day. Simvastatin (Kimia Farma, Indonesia) was suspended in 0.5% methyl cellulose and administered via oral gavage at a dose of 5.0 ml/kg. Meanwhile, control group animals received 0.5% methyl cellulose by oral gavage at the same relative volume for 30 days. Food consumption was monitored daily and rat body mass was measured every week. On day 30, 24 hours following the last simvastatin or vehicle treatment, animals were sacrificed by intraperitoneal injection of ketamine (0.05 ml.kg⁻¹) dan xylazine (0.01 ml.kg⁻¹) followed by cervical dislocation. Gastrocnemius tissue was collected and stored immediately at -20°C for further analysis.

Measurement of PGC-1 α Concentration

The tissue samples (90-100 mg) were homogenized with 1000 μ L ice-cold PBS using ultra-turrax homogenizer. Homogenates were centrifuged for 5

minutes at 5,000 g at 4°C. Supernatants were removed and aliquots were stored at -20°C. Protein contents of the homogenates were quantified using a Thermo fisher Bradford Assay. Quantitative measurement of PGC-1 α in tissue homogenate samples was performed by using a commercial enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's instructions. Absorbance from each sample was measured in duplicate using a microplate reader at a wavelength of 450 nm. PGC-1 α concentration data (ng/mg) were presented as a ratio between PGC-1 α concentration (ng/mL) and total protein content of homogenates (mg/mL).

Statistical Analysis

All measurement data are expressed as mean \pm standard error mean (SEM). To determine the effect of simvastatin on skeletal muscle PGC-1 α , an independent t-test was performed to identify the differences of skeletal muscle PGC-1 α between the control and simvastatin groups. A P value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical software SPSS version 23.0

Result and Discussion

Table 1 summarizes the changes in the anthropometric profiles in both groups. No significant differences in body weight changes were observed between the control and simvastatin groups. However, the increase in BMI was significantly lower in the simvastatin group compared to the control group ($p = .030$).

Our finding is similar to the study conducted by Seshadri *et al.* (2019) which reported that simvastatin 20 mg kg⁻¹day⁻¹ did not provide a significant change in body weight in high-sucrose diet rats following 30 days of administration. However, when a longer duration of simvastatin treatment (i.e up to 80 days) was applied, significant weight loss was observed.⁽⁴⁾

Table 1. Anthropometric Changes in Control and Simvastatin Group

| Variables | Control (n=8) | Simvastatin Group (n=8) | p value |
|-----------------------|-------------------|-------------------------|---------|
| % Body Weight Changes | 12.34 \pm 6.37 | 9.77 \pm 9.00 | .162 |
| % BMI Changes | 21.12 \pm 18.26 | 13.86 \pm 22.17 | .030* |

The main finding of the present study (**Figure 1**) revealed a significantly lower level of skeletal muscle PGC-1 α in the simvastatin group compared to the control group ($p = .026$). This is similar to the study by Goodman *et al.*(2015) which found a decrease in PGC-1 α levels in the soleus muscles of mice given simvastatin 60 mg kg⁻¹day⁻¹ and simvastatin 80 mg kg⁻¹day⁻¹ for 2 weeks.⁽⁵⁾ Besides, Boutbir *et al.* (2012) showed that there was a decrease in skeletal muscle PGC-1 α level in rats treated with atorvastatin 10 mg/Kg BW compared to the control group. In addition, mice received simvastatin 5 mg kg⁻¹day⁻¹ for 3 weeks showed worsened muscle dysfunction and impaired mitochondrial respiration in both oxidative and glycolytic muscle fiber types ⁽⁷⁾. Statin-induced reduction in PGC-1 α can explain that statin-induced myotoxicity can be associated with the occurrence of mitochondrial dysfunction.

Mitochondrial dysfunction is a condition characterized by impaired mitochondrial biogenesis, changes in membrane potential, reduced number of mitochondria, and changes in protein oxidative activity due to accumulation of ROS in cells and tissues. Mitochondrial dysfunction is also defined as a decrease in the ability of the mitochondria to synthesize high energy compounds such as adenosine 5 'triphosphate.

Various hypotheses explain that myotoxicity due to statins may result in mitochondrial dysfunction. Inhibition of HMG-CoA reductase caused by statins also results in the decrease in several intermediates of the mevalonic pathway, such as dolichol, prenylated protein, and electron transport chain protein, heme A and ubiquinone (coenzyme Q10, CoQ10), which close the bonds between complex I and II of the electron transport chain in mitochondria. ⁽⁸⁻¹⁰⁾

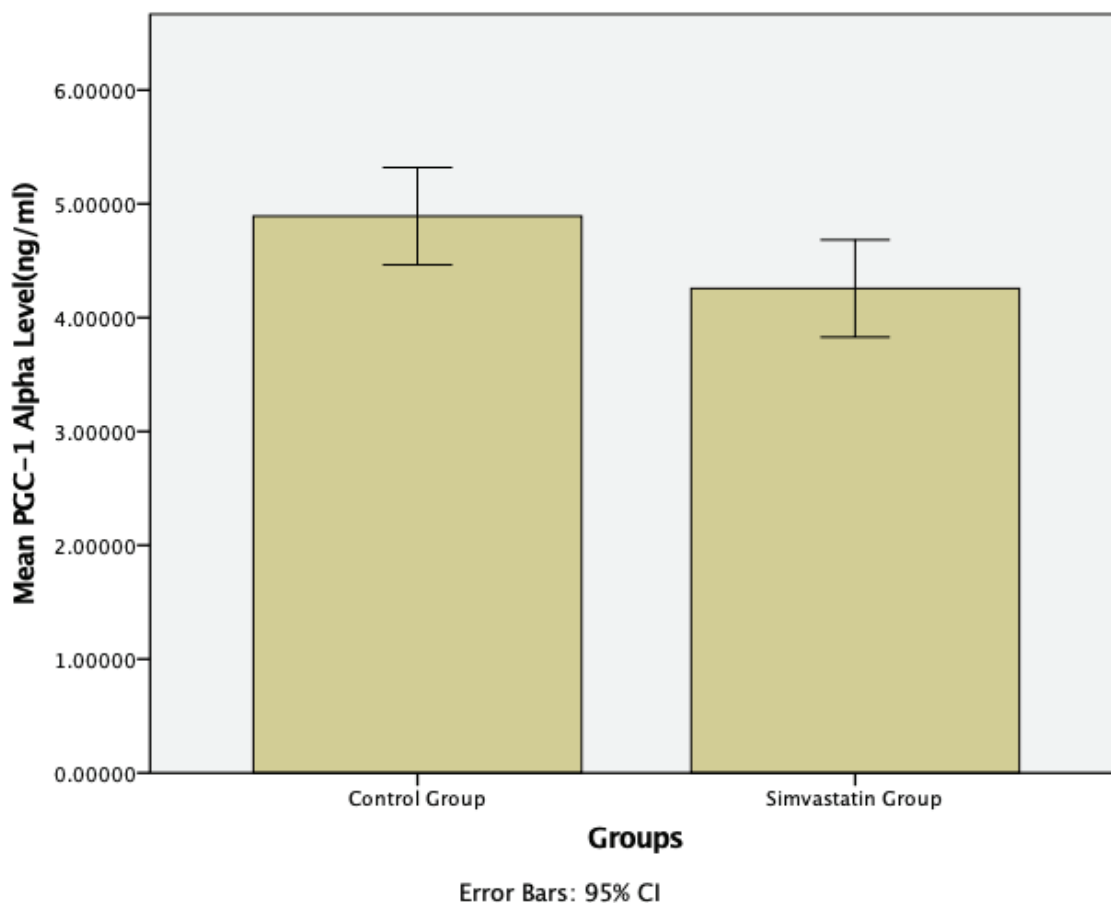


Figure 1. The Differences of Skeletal Muscle PGC-1 α Levels between Simvastatin and Control Group

Conclusion

In conclusion, administration of simvastatin at a dose of 10 mg/kg/day for 30 days may decrease skeletal muscle PGC-1 α leading to mitochondrial dysfunction in rat skeletal muscle.

Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Clearance: The Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University has confirmed the proposal and research protocol (No.458/UN4.6.4.5.31/PP36/2020).

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