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Hyperbaric oxygen effects towards SIRT1 level in Sprague dawley with endothelial dysfunction by high-cholesterol diet



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ABSTRACT

Introduction: Endothelial dysfunction is an early risk factor for cardiovascular disease and hypertension. Mechanisms that participate in endothelial dysfunction include the decrease of nitric oxide (NO) and the increase of endothelin-1 (ET-1). ATP-sensitive potassium channels (KATP channels) also participate in endothelial cells in the vascular system. This study aims to investigate whether energy / ATP / SIRT1 is involved in endothelial dysfunction after the administration of hyperbaric oxygen (HBO).

Methods: This study was an experimental study, using a randomized post test only control group design using thirty white rats (*Rattus norvegicus*) Sprague dawley strains. There were 3 groups of p1, p2, and p3, and each group consists of 10 rats with the normal control for p1, high cholesterol diet for p2, and high cholesterol diet with hyperbaric oxygen of 2.4 ATA with 98% O₂ for 3 sessions with the duration of 30 minutes / session, and air break for 5 minutes between

each session for the period of 10 days consecutively for p3. The level of SIRT 1 was tested by using Elisa method. We applied statistical methods of investigation.

Results: The SIRT1 from ELISA results tend to increase after the administration of hyperbaric oxygen (HBO). Decreased of SIRT1 occur at group was given by high-cholesterol diet (p1=0,188±0,017 ng/ml; p2=0,114±0,029 ng/ml; p3=0,252±0,027 ng/ml). Hypercholesterolemia and endothelial dysfunction affect energy metabolism and reduce ATP levels and nicotinamide adenine dinucleotide (SIRT1). HBO therapy increases ATP and nicotinamide adenine dinucleotide levels which affect the increase of silent mating type information regulation two homologs 1, resulting in the optimal endothelial cell function.

Conclusion: Hyperbaric oxygen can significantly affect the endothelial dysfunction due to a high-cholesterol diet, by increasing the levels of Sirtuin-1.

Keywords: Hyperbaric oxygen, endothelial dysfunction, SIRT1, high-cholesterol diet

Cite This Article: Setianingsih, H., Soetjipto, Sudiana, I.K., Suryokusumo, M.G. 2018. Hyperbaric oxygen effects towards SIRT1 level in Sprague dawley with endothelial dysfunction by high-cholesterol diet. *Bali Medical Journal* 7(2): 535-538. DOI:10.15562/bmj.v7i2.1154

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INTRODUCTION

In 2020, atherosclerosis is predicted to be a major cause of morbidity and mortality in a developing society due to unhealthy lifestyle changes.¹ Atherosclerosis causes coronary heart disease (CHD) and is the leading cause of death. In 2002, the World Health Organization (WHO) recorded more than 117 million people died from CHD, worldwide, the incidence of CHD is also predicted to continue to grow. Health survey results in 2001 showed that 3 out of 1000 Indonesians suffer from CHD. The burden of the country's economy becomes higher because most people with CHD are productive age.²

Atherosclerosis is a dynamic and progressive disease derived from a combination of endothelial dysfunction and inflammation. In atherosclerosis, a local chronic inflammatory process occurs in arteries that affects the cardiovascular system, the thickening of the arterial tunica (sclerosis) and the accumulation of lipids (athere, pasta) that characterize distinct lesions.^{3,4} The narrowing of the lumen from the arteries is due to the accumulation

of fatty substances, cell debris, and calcium called plaque.

Endothelial dysfunction is an imbalance between vasodilator release and an endothelium delivered vasoconstrictor factor that affect the reduction of NO (nitric oxide) bioavailability from rapid inactivation by endothelial production from ROS. Endothelial dysfunction plays an important role in the pathogenesis of atherosclerosis due to deregulation of enzymatic activity of eNOS (endothelial nitric oxide synthase) and inactivation of NO by oxidative stress.⁵

In the vascular system, Silent Mating Type Information Regulation 2 Homolog 1 (SIRT1) is the NAD + dependent class III histone deacetylase (HDAC) which is the metabolic sensor key in various metabolic networks providing vasodilating effects via eNOS and ROS scavenger pathways. In atherosclerosis, SIRT1 has an essential role in endothelial cells as an anti-inflammatory. In macrophages, it will prevent the formation of foam cells and is anti-thrombotic. Therefore, SIRT1 has a very

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Received: 2018-04-24

Accepted: 2018-5-21

Published: 2018-5-22

promising role in the prevention and approach of atherosclerosis therapy.⁶

Atherosclerosis leads to CHD. This causes a lack of blood and oxygen to the heart, which can cause myocardial infarction. Coronary heart disease is related to arterial narrowing, which causes insufficiency of blood supply containing a lot of oxygen, resulting in lack of oxygen supply.⁷ In the study of hyperbaric oxygen (HBO), it is mentioned that hyperbaric oxygen is very beneficial for ischemic conditions.⁸ In addition to these conditions HBO also has a beneficial effect of improving liver function tests.⁹ There are many other studies that can be done by utilizing HBO therapy.

Hyperbaric oxygen therapy is a type of treatment in which the patient breathes with 100% oxygen through the mask and is at a pressure of more than 1 ATA (2.4 ATA) over a period. Hyperbaric oxygen therapy is based on the role of ROS and RNS molecules. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) act as signaling molecules in transduction cascade for various transcription factors, growth factors, cytokines, and hormones. These reactive species molecules can have a positive or negative effect depending on the concentration of the molecule and the intracellular location.⁸

In this study, Hyperbaric Oxygen therapy has the advantage in the treatment of endothelial dysfunction which is the initial lesion of atherosclerosis via SIRT1 because SIRT1 has been reported that SIRT1 conferred a protective effect on the endothelial dysfunction and the heart through upregulation of antioxidants.

MATERIALS AND METHODS

Experimental Animal

This study was an experimental study with *randomized post-test only control group* design that proved the effect of hyperbaric oxygen (HBO) on endothelial dysfunction by the enhancement of SIRT1, using thirty white rats (*Rattus norvegicus*) Sprague dawley strains. The fulfillment of investigation was approved by the ethics committee of Faculty of Veterinary Medicine, Universitas Airlangga of Surabaya. The sample animals are rats of Sprague dawley strain, weighing about 150-gram weight with the healthy physical condition.

The followings are the division of groups:

1. Group 1 (p1): Male *Rattus norvegicus* group of Sprague dawley strains which are given a normal diet
2. Group 2 (p2): Male *Rattus norvegicus* group of Sprague dawley strains which are given an high-cholesterol diet

3. Group 3 (p3): Male *Rattus norvegicus* group of Sprague dawley strains which are given an high-cholesterol diet and given hyperbaric oxygen 2,4 ATA for ten days consecutively.

This study was conducted for 73 days with the following timing: adaptation period for 14 days, high cholesterol diet for 49 days and administration of hyperbaric oxygen (HBO) for ten days.

The hyperbaric oxygen was administered at a dose of 2.4 ATA for ten days consecutively. All of the intervention group consisted of ten mice.

High Cholesterol Diet

Endothelial dysfunction is made by giving a high cholesterol diet with a special formula, many other methods that can be done to make a high cholesterol diet. In this study high cholesterol diet was administered with the composition of modification formula containing sodium acids via sonde to each rat/day and high cholesterol diet with mixed compositions of comfeed PAR-S, flour, cholesterol, pork oil, starch, and water, which were made into pellet then dried, to group2 (p2) and group 3 (p3) only. The high cholesterol diet is a formulation of a preliminary study carried out by examining the lipid profile and examining the level of lipoprotein-associated phospholipase A2 (Lp-PLA2) as a marker of endothelial dysfunction. High cholesterol diet was administered for seven weeks (49 days), to make endothelial dysfunction subject.

SIRT1 level measurement

The activity of SIRT1 in all three groups of rats are examined by Enzyme-linked immunosorbent assay (ELISA) from the serum in all groups. Measurement of this protein is done by using ELISA to obtain quantitative data.

Statistic Method

From the experimental results, the data were analyzed using statistical package for social science version 20.0 software, with ANOVA. Normally distributed and homogeneous data were analyzed further using Post-Hoc Multiple Comparison items Tukey HSD, the value of $p < 0,05$.

RESULTS

The study used *Rattus novergicus* mice, which were divided into three groups equally, each of 10 mice per group, the mean weight of mice in this study was 150 grams under healthy conditions. During the 73-day intervention period, there was no drop out of the sample. Analysis of data normality and homogeneity can be seen in [table 1](#).

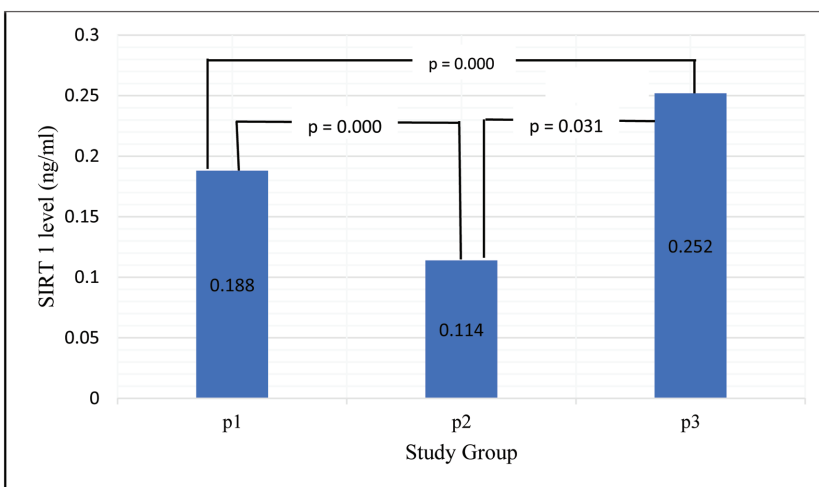
Table 1 Normality and homogeneity test of SIRT 1

Variable	Saphiro-Wilk Test	Levene's Test of Homogeneity
SIRT 1 p1 group (normal control)	0.275	
SIRT 1 p2 group (negative control)	0.187	0.464
SIRT 1 p3 group (treatment)	0.205	

Table 2 Comparison of SIRT1 level between groups

Variable/Group	n	Mean \pm SD	p-value
SIRT1 level			
p1 (control group)	10	0.188 \pm 0.017	
p2 (negative control)	10	0.114 \pm 0.029	0.000
p3 (treatment)	10	0.252 \pm 0.027	

*significant (p<0.05)

**Figure 1** Post hoc analysis of SIRT 1 level

Based on [table 1](#), there was a normal distribution of each SIRT 1 variable in all intervention groups ($p > 0.05$), and the data had the homogeneity variety ($p > 0.05$) so that ANOVA test was used to compare the difference of SIRT1 in the three intervention groups. Comparison of SIRT1 level in three intervention group can be seen in [table 2](#).

Based on [table 2](#), the mean value of SIRT 1 in group p1 was 0.188 ± 0.017 , mean SIRT 1 in group p2 was 0.114 ± 0.029 , and mean of SIRT 1 in group p3 was 0.252 ± 0.027 . Through ANOVA analysis obtained p-value = 0.000, so it can be concluded that there is difference of SIRT1 value between three intervention group. The results will then proceed through a post hoc analysis to determine the difference between each intervention group in pairs. Post hoc analysis can be seen in [figure 1](#).

Based on [Figure 1](#), there was a significant difference between SIRT1 levels in the p1 vs. p2 ($p < 0.05$), p1 vs. p3 ($p < 0.05$), and p2 vs. p3 ($p < 0.05$) and the highest level of SIRT1 was found in p3 group

(0.252 ng/ml), while the lowest level of SIRT 1 was found in group p2 (0.114 ng/ml).

DISCUSSION

In accordance to the specific objective of this study which was to prove the increase of Sirtuin (SIRT1) levels after HBO administration, in this study, there was a significant increase of Sirtuin (SIRT1) levels in the treatment group compared to 2 other groups: normal control group and negative control group, with a significance value of 0.000 ($p < 0.005$). The average result of normal control group is 0.188 ± 0.017 , the average result of negative control group is 0.114 ± 0.029 , while in treatment group, the average result is 0.252 ± 0.027 .

Silent information regulator factor 2 related enzyme-1 (SIRT1) is a nicotinamide adenine dinucleotide-dependent deacetylase, which can deacetylate histone and non-histone proteins as well as transcription factors, and also engages in physiological cell regulation including aging, gene transcription, energy balance and oxidative stress.¹¹ In endothelial dysfunction, there is a decrease in sirtuin expression because of the imbalance of energy metabolism which decreases ATP levels which also means the decrease of nicotinamide adenine dinucleotide (SIRT1).¹²

Hyperbaric Oxygen could affect the SIRT1 level because HBO exposure can mainly produce H_2O_2 molecules as Reactive Oxygen Species (ROS) molecules which are expected to increase the activity of Sirtuin (SIRT-1), as mentioned that this protein can be activated at the time of the increase of H_2O_2 molecules inside the cells through activation of Mitogen-Activated Protein Kinase (MAPK). There is a cross-talk between the redox state within the cell and the signaling pathway inside the cell, in which the increase of ROS molecule will trigger cellular response such as cessation of the cell cycle, senescence mechanism, apoptosis or necrosis, depending on the level of damage due to the increase of oxidants.¹³

In the endothelial dysfunction occurs ischemia, during ischemia, decreased cerebral blood flow reduces the availability of oxygen, leading to inflammation, and the production of nitric oxide (NO), reactive oxygen species and toxic prostanoids.¹⁴

The mechanism of HBO therapy provoked endothelial dysfunction remains unclear. However, we have shown that HBO moderately increases the level of endogenous ROS, which stimulates the increased production of endogenous antioxidant enzymes including heme oxygenase-1 (HO-1) and catalase (CAT). Furthermore, oxygen free radicals scavenger or antioxidant enzyme inhibitor

attenuates endothelial protection of HBO, indicating that ROS and antioxidant enzymes play a pivotal role in ischemic tolerance elicited by HBO.¹⁵ Other than using the mechanism of HBO appears to increase the expression of antioxidant enzymes, alleviate oxidative stress, reduce the formation of hydroxyl radicals, enhance superoxide dismutase and catalase activity.¹⁶⁻¹⁸ It has been reported that SIRT1 conferred a protective effect on the heart through upregulation of antioxidants through activation of FoxO.¹⁹ Further work is needed to explore the signaling pathways of SIRT1 in endothelial dysfunction of HBO.

Similar findings from research conducted by Yan et al. (2013) that examined the role of SIRT1 in neuroprotection in ischemic model mice, where the increase of SIRT1 protein and mRNA expression can enhance the behavior of the experimental animals. Besides, HBO can decrease infarct volume if the HBO is given immediately after infarction occurs within 24 hours and HBO may improve the morphological features of ischemia when it is administered within seven days after the cerebral ischemia.²⁰

CONCLUSION

Giving hyperbaric oxygen can significantly affect the endothelial dysfunction due to an high-cholesterol diet, by increasing the levels of Sirtuin 1. The HBO should be administered at a pressure of 2.4 ATA with 98% O₂ for three sessions with the duration of 30 min/session, and air break for 5 minutes between each session for 10 days consecutively.

ACKNOWLEDGMENT

The authors wish to thank Hang Tuah University of Medical Science for its financial support and all my teacher who participated in the study.

CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest regarding all elements of the research.

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