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Research Article

The effect of the endothelial nitric oxide synthase on hypoxia-induced factor-1 alpha level in a state of endothelial dysfunction after hyperbaric oxygen therapy

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ABSTRACT

Aim: This research aims to know the effect of eNOS on hypoxia-induced factor-1 alpha (HIF-1α) levels in endothelial dysfunction after hyperbaric oxygen therapy (HBOT). **Materials and Methods:** The design of this study was experimental research; the study sample consisted of 30 Sprague Dawley (*Rattus norvegicus*) white rats and divided into three groups p1, p2, and p3; p1 rats with a standard diet, p2 rats with a high-cholesterol diet, and p3 rats with a high-cholesterol diet continued HBOT at 2.4 ATA with 98% O_2 for three sessions with a duration of 30 min/session, and air brakes for 5 min between each session for 10 consecutive days. eNOS and HIF-1α were examined using the enzyme-linked immunosorbent assay method. **Results:** From the results of testing the significance of the regression model, the results showed that there was a relation of HIF-1α on eNOS levels ($P = 0.009$) after treatment with HBO 2,4 ATA with 98% oxygen for three sessions with the duration of 30 min/session, and air brake for 5 min between each session for 10 days consecutively. **Conclusion:** The effect of eNOS on HIF-1α levels in HBOT shows that HBO can significantly affect the levels of HIF-1α through endothelial nitric oxide synthase (eNOS) level.

KEY WORDS: Endothelial dysfunction, Endothelial nitric oxide synthase, Hyperbaric oxygen, Hypoxia-inducible factor-1α, Nitric oxide

INTRODUCTION

Nitric oxide (NO) is able to stabilize hypoxia-induced factor-1 alpha (HIF-1α) in normobaric conditions through the prolyl hydroxylase domain (PHD) protein activity.[1] If there is a decrease in oxygen levels in the blood, the body will respond like an increase in respiration and blood flow and if hypoxia occurs the body will also immediately respond through cellular signals starting from the PHD class enzyme, the enzyme from this class contains oxygen-sensing hydroxylases will hydroxylate proline-specific residues on subunit a of the HIF transcription factor-1α.[2]

Endothelial dysfunction is an imbalance between vasodilator release and an endothelium delivered vasoconstrictor factor that affects the reduction of NO 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 endothelial NO synthase (eNOS) and inactivation of NO by oxidative stress.[3]

Hypoxic conditions caused by changes from microvascular conditions such as endothelial dysfunction will result in the secretion of HIF-1α, which will stimulate the production of VEGF and NO. The level of HIF-1α has an essential role in increasing the level of NO, which turns out to be vasodilating in blood vessels.[4]

Endothelial dysfunction is associated with an increase in the degradation of extracellular NO secretions, which will be seen with the appearance of ROS formed by peroxynitrite.[5] The oxidation

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of NADPH that occurs in tunica adventitia will produce superoxide in very high amounts so that it will affect endothelial function.[6] The process of NO synthesis by eNOS is highly dependent on eNOS expression and functional activity of eNOS enzymes. eNOS expression decreases due to important factors in the pathogenesis of endothelial dysfunction.[7]

The endothelial dysfunction leads to a disturbed production of different hemoglobin NO derivatives, which not only affects NO release at various sites of the arterial bed but also hemoglobin–oxygen affinity and optimal blood oxygenation and deoxygenation in capillaries. These data support the notion that endothelial dysfunction may alter hemoglobin-oxygen relationship and tissue oxygen supply.[8]

Hyperbaric oxygen therapy (HBOT) 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.[9] HBOT is based on the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) molecules. ROS and 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.[9]

HBO is very beneficial for ischemic conditions.[9] HBO therapy has an advantage in the treatment of endothelial dysfunction, which is the initial lesion of atherosclerosis through eNOS because eNOS has been reported that eNOS conferred a protective effect on the endothelial dysfunction.[10]

In normobaric condition, NO is able to stabilize HIF-1α, but in hypoxic conditions, it is the opposite, whether in hyperbaric states, it is able to restore this ability through eNOS level. This research aims to know the effect of eNOS on HIF-1α levels in endothelial dysfunction after HBOT.

MATERIALS AND METHODS

Experimental Animal

This study was an experimental study with used randomized post-test only control group design that proved the effect of HBO on endothelial dysfunction by the enhancement of eNOS and HIF-1α, using 36 white rats (*Rattus norvegicus*) Sprague Dawley strains. The fulfillment of the investigation was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga of Surabaya, using the number 776-KE. The sample animals are rats of Sprague Dawley strain, weighing about 150 g weight with the healthy physical condition.

The followings are the division of groups:

1. Group 1 (p1): Twelve male Sprague-Dawley rat strains which are given a standard diet
2. Group 2 (p2): Twelve male Sprague-Dawley rat strains which are given a high-cholesterol diet
3. Group 3 (p3): Twelve male Sprague-Dawley rat strains which are given a high-cholesterol diet, then given HBO 2,4 ATA for 10 days consecutively.

This study was conducted for 3 months relatively.

Endothelial Dysfunction

Endothelial dysfunction is made by giving a high-cholesterol diet with a unique formula, administration of atherogenic diet by providing a mixture composition of Comfeed PAR-S 50%, 25% flour, 2% cholesterol, 2.5% pork oil, 20.5% starch, and water. High-cholesterol diet was administered for 2 months.

Sample analysis is using blood from the heart of a mouse. Previously, rats were fasted for 10–12 h and were only given distilled water. Blood serum is taken and then checks the eNOS level.

eNOS and HIF-1α Level Check

The level of eNOS and HIF-1α in all groups of rats is examined by enzyme-linked immunosorbent assay (ELISA) from the serum in all groups. Measurement of this protein is done using ELISA to obtain quantitative data.

Statistical Analysis

The data obtained were analyzed statistically to get a description of descriptive values, with descriptive statistics. Normally distributed tested using Kolmogorov–Smirnov and further using the linear regression test, $P < 0.05$.

RESULTS

The overall study sample was 36 rats which were divided into three groups, and then, the blood samples were taken for the examination of eNOS and HIF-1 levels using ELISA.

The results of mean value descriptive statistics tests of eNOS were 0.407 ng/ml and HIF-1α were 0.292 [Table 1].

Normal distribution test results for the variables in eNOS and HIF-1α using the Kolmogorov–Smirnov normality test were normally distributed ($P \geq 0.05$) [Table 2].

Table 3 shows further testing of the regression coefficients with results: Significance value 0.009. In this study, because alpha is used 5%, the significance value is 0.009 smaller than alpha, so it can be concluded that the constants in this regression equation are meaningful and can be obtained regression equation based on HIF-1α level = 0.562–0.531 (eNOS level). It shows the influence of eNOS levels on HIF-1α level according to the regression equation above.

DISCUSSION

eNOS, hydrolyzing L-arginine to NO and L-citrulline, after release of NO produced by eNOS will diffuse rapidly in cell membranes and cause relaxation of vascular smooth muscle; the results of eNOS examination in this study showed a significant increase in the treatment group supported by the previous studies, namely, Buras study[11] which also showed an increase in eNOS after the administration of HBOT.

From the results of testing the significance of the regression model, the results showed that there was an effect of HIF-1α level through eNOS levels (P = 0.009). The previous research also showed an increase in eNOS mRNA and protein expression after exposure to HBOT.[11] *In vivo* and *in vitro* studies of fetal lung endothelial cells have shown an increase in eNOS mRNA and protein expression and eNOS activity with an increase in oxygen concentration at 1 ATA.[12]

Other studies also suggest that increased oxidative pressure from HBOT and the formation of ROS and RNS have essential roles as signaling molecules in the transduction cascade.[13-14] The increase in oxidative stress experienced by pulmonary endothelial cells during HBO treatment can be a catalyst for improving eNOS and mRNA activity and subsequent proteins in pulmonary tissue.[15]

HBO treatment is also associated with increased oxidative stress that could result in NO inactivation and the formation of the cytotoxic peroxynitrite.[16] Combined increases in extracellular superoxide dismutase and iNOS activity should protect NO bioactivity and avoid the formation of peroxynitrite anion during inflammation.[17] Clearly, maintaining the balance between increasing free oxygen radicals and the antioxidant defenses versus increasing NO production is critical in the impact of HBOT on endothelial dysfunction. The resulting RNS has a role in the protective effect on tissues that have suffered an injury in the study. The beneficial effects of HBO treatment depending on NOS can rely on an increase in the initial eNOS activity followed by upregulation of late-stage eNOS proteins from endothelial cells in tissues. The study also showed that the mechanism that might be carried out by HBO hypoxia and oxidative stress gave a paradoxical benefit to traumatized tissue.[15]

The direct effect of HBOT includes improvement in hypoxic conditions such as attenuated levels of HIF-1α but the presence of NO will have a different impact in hypoxia condition, by decreasing the level of HIF-1α. This is actually an advantage in HBOT because HBOT always returns a balanced state in accordance with the disease it is carrying out so it does

Table 1: The result of descriptive statistics of eNOS and HIF-1α level

Groups	Minimum	Maximum	Mean	Std. deviation
Hypoxia-induced factor	0.188	0.574	0.407	0.11464
Endothelial nitric oxide synthase	0.132	0.450	0.292	0.09296
Valid N (listwise)	36			

eNOS: Endothelial NO synthase, HIF-1α: Hypoxia-induced factor-1 alpha

Table 2: The result of linear regression test of eNOS and HIF-1α level

Model	Sum of squares	df	Mean square	F	Sig.
Regression	0.085	1	0.085	7.754	0.009
1 Residual	0.375	34	0.011		
Total	0.460	35			

Dependent variable: Hypoxia-induced factor, Predictors: (Constant), endothelial nitric oxide synthase. eNOS: Endothelial NO synthase

Table 3: The result of coefficient regression

Coefficients[a]					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
(Constant) 1	0.562	0.58		9.624	0.000
Endothelial nitric oxide synthase	−0.531	0.191	−0.431	−2.785	0.009

Dependent variable: Hypoxia-induced factor

not still reduce HIF-1α but always stabilizes HIF-1α toward optimal conditions.

This can be explained because in the administration of HBOT using the principle of redox hypotheses, hormesis, and hypoxia precondition which states that the HBOT response to disease can change according to its microenvironment.[18]

CONCLUSION

Giving HBO can significantly affect the levels of HIF-1α through eNOS level. The HBO should be administered at a pressure of 2.4 ATA with 98% O_2 for three sessions with the duration of 30 min/session, and air brake for 5 min between each session for 10 days consecutively.

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