



THE EFFECT OF INTERMITTENT HYPOBARIC HYPOXIA ON OXIDATIVE STRESS STATUS AND ANTIOXIDANT ENZYMES ACTIVITY IN RAT BRAIN

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ABSTRACT

Background: High altitude can cause hypobaric hypoxia (HH), resulted from the lower barometric pressure and hence partial pressure of oxygen. Hypoxia can lead to a lot of deleterious molecular and cellular changes, such as generation of free radicals or reactive oxygen species (ROS). Increasing of ROS can cause oxidative stress if the antioxidant enzyme does not increase simultaneously. Oxidative damage in brain has toxic effect on cognitive functions.

Objective: In this study, we investigate effect of acute intermittent HH on oxidative stress and antioxidant enzyme activity in rat brain.

Method: Wistar rats divided into 5 groups, consisting control group and four experimental groups which treated to HH. Rats were exposed to simulated HH equivalent to 35.000 feet in hypobaric chamber for 1 minute, repeated once a week.

Results: Level of malondialdehyde and carbonyl in rat brain under acute HH increased at HH exposure (group I) compare to control group. These levels decreased afterward at intermittent HH exposure (group II-IV). Specific activity of superoxide dismutase (SOD) shows increasing level at intermittent HH exposure, especially group IV was increasing of SOD level significantly. The increasing pattern of specific activity of catalase was inversely from SOD pattern, but it still has higher activity in intermittent HH compare to control group.

Conclusion: Brain tissue seems to be able to perform an adequate adaptive response to hypobaric hypoxia after the training, shown by its significantly decreased MDA and carbonyl level and also increased specific activity of SOD and catalase.

Keywords : Intermittent hypobaric hypoxia, Malondialdehyde, Carbonyl, Superoxide dismutase, Catalase

Received Dec 2, 2018 ; Revised 26 Dec, 2018 ; Accepted 26 Dec, 2018

INTRODUCTION

Hypoxia is a decreased oxygen concentration in tissues and cells, normally caused by reduction in the partial pressure of atmospheric oxygen like in high altitude (hypobaric hypoxia). Due to hypobaric hypoxia, reactive oxygen species (ROS), like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are increasingly generated and therefore increased risk of oxidative damage.[1,2] Normal metabolism produced ROS from electron transport chain in mitochondria. Increasing ROS production under hypoxic condition because lack of oxygen can inhibit transfer electron in mitochondria, thereby increasing superoxide radicals at complex III.[3,4]

There is a low oxygen partial pressure (hypobaric) in high altitude, so in this condition also cause lack of oxygen (hypoxia). Air force army and pilots have to routinely undergo training in hypobaric hypoxia conditions. Exposure to hypobaric hypoxia will also cause oxidative stress and affect their health. A study reported that high altitude exposure will increase the reactive oxygen species and decrease the activity of the antioxidant enzyme such as SOD and catalase.[5] Therefore, oxidative stress would be increased because of ROS generation in high altitude.

Oxidative stress is defined as increased free oxygen radicals, ROS and lack of antioxidant substances which are able to neutralize them. ROS generated by a number of processes in vivo are highly reactive and toxic. Some studies reported that ROS exert physiological conditions and play role in signaling pathways regulating cell growth and status redox cell.[6,7] However, when produced in excess, ROS exert unfavorable effects and

are able to attack cell macromolecules such as lipid, protein and DNA.[8]

Characteristic of ROS is very reactive to macromolecules in the cells. It can react with lipid and cause lipid peroxidation, results malondialdehyde. ROS also attack protein and DNA resulting carbonyl and 8-OHdG as end product.[8] We can detect these products to evaluate oxidative damage that occurs because of ROS elevation.

Biological systems have accomplished with enzymatic antioxidant as defense mechanisms against the deleterious effects of ROS. Superoxide dismutase (SOD) is the ultimate antioxidant enzymes that protect cell from oxidative damage by ROS. This enzyme converts O_2^- that very reactive to H_2O_2 that less reactive. Further hydrogen peroxide is detoxified become water by catalase enzyme and glutathione peroxidase.[9]

In this study, we want to explore the effect of acute intermittent hypobaric hypoxia exposure on oxidative status in rat brain. This condition is commonly experienced by the air force army through the simulation inside the hypobaric chamber. We want to know whether repeated hypobaric hypoxia exposure will be increasing or decreasing oxidative stress. The parameters of oxidative stress that will be measured are MDA and carbonyl. The antioxidant enzymes that will be measured are specific activity of SOD and catalase.

MATERIAL AND METHODS

Experimental Design

This study was an experimental study carried out at Biochemistry and Molecular Biology Laboratory, Faculty of

Medicine, Universitas Indonesia and Lakespra Saryanto TNI AU. Samples are determined with Federer formula. Twenty five male Wistar rats (6-8 weeks old; body weight 150-250 gram at entry into protocol) were randomly divided into 5 groups (n = 5 per group), consisting control group and four experimental groups. Rats were subjected to acute hypobaric hypoxia by placing them into the hypobaric chamber for one, two, three and four times, respectively. All rats had free access to water and standard rat chow. Water and food consumption was assessed every 2 days. Protocol of hypobaric hypoxia is designed to altitude studies and training especially to train air marshal. Hypobaric chamber is simulated at 35,000 feet for 1 minute to get the effect of acute hypobaric hypoxia. Afterward, the altitude is lowered gradually to 18,000 feet and maintained this level to sacrifice rats and get the brain.

Tissue Preparation

One hundred milligram brain tissues are homogenized in 1 mL PBS. This homogenate is used to measure MDA and carbonyl level. Homogenate for analysis of specific activity of SOD and catalase was added with PMSF as a protease inhibitor.

Measurement of MDA Level

Measurement of MDA level using Wills method. This method is using thiobarbituric acid (TBA) that will react with MDA and form pink color. The homogenate was mixed with trichloroacetic acid (TCA) 20% to precipitate protein. Then centrifuge 6000 rpm for 5 minutes, keep the supernatant. Add thiobarbituric acid 0.67% and incubate at boiling water 95-100°C. MDA level was appropriate with pink color

formed that read by spectrophotometer at 530 nm.

Measurement of Carbonyl Level

Measurement of carbonyl level is using 2,4-dinitrophenylhydrazine (DNPH) that will react with carbonyl substances and form color.[10] The homogenate was added with 10 mM DNPH in 2.5 M HCl and incubated in dark at room temperature. Afterward, add trichloroacetic acid (TCA) 20%, incubate on ice for 5 minutes and centrifuge 10,000 g for 10 minutes, discard supernatant. Add TCA 10%, incubate on ice for 5 minutes and centrifuge 10,000 g for 10 minutes, discard supernatant. Resuspend the pellet by ethanol: ethyl acetate sol (1:1) Add Guanidine HCl and centrifuge 10,000 g for 10 minutes, keep supernatant and read the absorbance at wavelength 360-385 nm. and centrifuge 10,000 g for 10 minutes, discard supernatant.

Measurement of SOD Specific Activity

The specific activity of SOD enzyme was determined using RanSOD® kit (Randox). Superoxide dismutase (SOD) in samples will catalyze the dismutation of the superoxide anion ($O_2\bullet$) to hydrogen peroxide and oxygen. This method uses xanthine and xanthine oxidase to generate superoxide anion which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (I.N.T.) to form a red formazan dye that can be read at 505 nm. The SOD activity is then calculated by the inhibition degree of this reaction because SOD will inhibit superoxide anion generation. Sample was mixed with the reagent and read the absorbance using spectrophotometer at 505 nm after 30 seconds and 3 minutes later. The specific activity is calculated from activity (U/mL) divided by protein concentration (mg/mL).

Measurement of Catalase Specific Activity

Catalase activity is measured by using hydrogen peroxide (H₂O₂) mixed with homogenate.[11] Catalase in sample will convert rapidly hydrogen peroxide into oxygen and water. The decreasing of hydrogen peroxide can be read at 240 nm in 30 minutes (after mixing sample and hydrogen peroxide) and 3 minutes later. The specific activity is calculated from activity (U/mL) divided by protein concentration (mg/mL).

RESULTS

Measurement of MDA level in rat's brain has shown that there was increasing of MDA level at group I (once hypobaric exposure). Meanwhile at group II, III and IV, the level of MDA was decreasing significantly compare to control group ($p < 0.001$).

Measurement of carbonyl level has shown that there was increasing significantly carbonyl level at group I compare to control group ($p = 0.017$). Afterward, the carbonyl level was decreasing at group II-IV. The decreasing was statistic significantly at group III ($p = 0.045$) and IV ($p = 0.010$).

Specific activity of SOD in rat's brain under hypobaric hypoxia showed that decreased at group I, but not significant. Later, the activity was increasing group II-IV. However, the significant increasing found at group IV ($p < 0.001$).

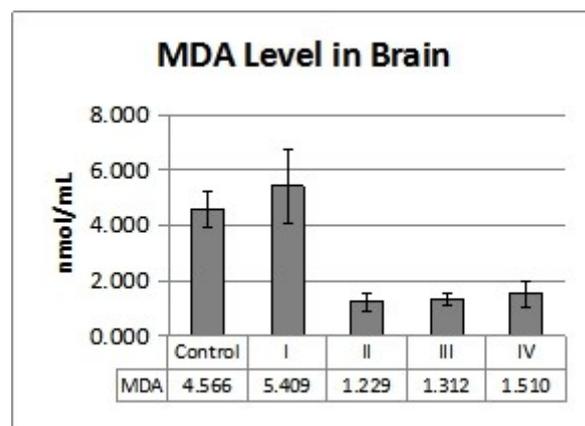


Figure 1. Graphic of MDA level in rat's brain under acute intermittent hypobaric hypoxia

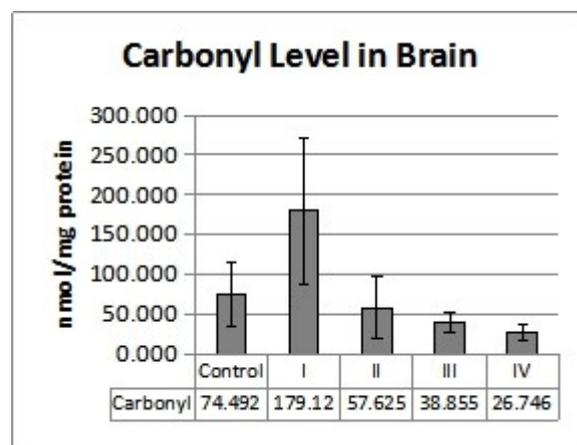


Figure 2. Graphic of carbonyl level in rat's brain under acute intermittent hypobaric hypoxia

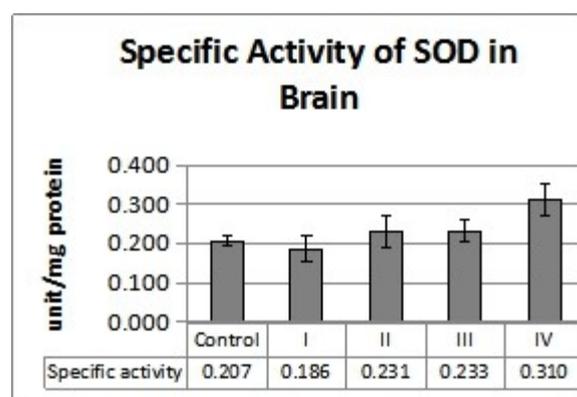


Figure 3. Graphic of specific activity of SOD in rat's brain under acute intermittent hypobaric hypoxia

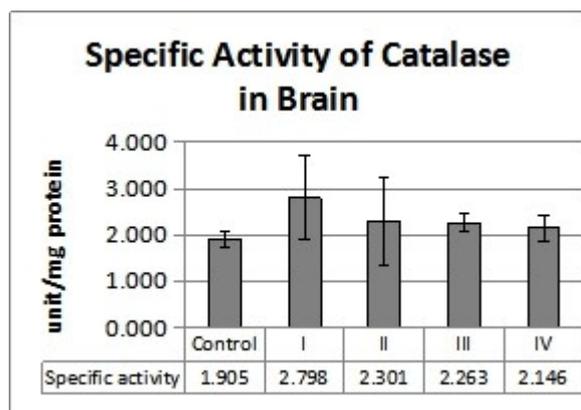


Figure 4. Graphic of specific activity of catalase in rat's brain under acute intermittent hypobaric hypoxia

Results of Specific activity of catalase in rat's brain under hypobaric hypoxia showed inversely with specific activity of SOD. There was increasing of specific activity of catalase at group I, and then the activity was decreased gradually at group II-IV, but the level still higher than control group.

DISCUSSION

Graphics of MDA and carbonyl level have the same pattern. This result showed that at group got first hypobaric exposure lead to increasing MDA and carbonyl level compare to control group. Some previous studies revealed that high altitude exposure will increase oxidative stress conditions, such as lipid peroxidation, DNA damage and nitric oxide production.[12,13]

However, at the group that got intermittent hypobaric exposure, the level of MDA and carbonyl were decreasing. It is because at the group that got repeated hypobaric exposure, occurred adaptive mechanism to hypobaric hypoxia exposure. This can be seen at the parameter of oxidative stress, which decreasing after repeated hypobaric exposure. This result is supported by specific activity of SOD in rat's brain

under acute intermittent hypobaric hypoxia. The SOD activity found increasing at group that got intermittent hypobaric exposure. Although there is slightly decreasing SOD activity at group that got the first exposure. The increasing of SOD activity at repeated exposure was meant to combat free radicals (ROS) accumulation because of hypobaric hypoxia exposure.

The specific activity of catalase showed that increasing activity at group that got first exposure. It occurs to against increasing ROS generation at the first hypobaric exposure, before increasing of SOD activity found. Decreasing of catalase activity at group that got repeated exposure is because of adaptive mechanism bearing repetitive hypobaric exposure. The accumulation of ROS under intermittent hypobaric hypoxia was neutralized enough by increasing SOD activity.

Previous study demonstrated that exposure of short-term chronic hypobaric hypoxia will cause the upregulation of transcription factor NRF2 that mediated by oxidative stress. This transcription factor will control the expression of antioxidant enzymes.[14] Another study reported that intermittent hypobaric hypoxia can increase antioxidant capacity in rats brain and protect the neuron cells from oxidative damage.[15]

CONCLUSION

Intermittent hypobaric hypoxia exposure can induce adaptive mechanism against oxidative stress. It is demonstrated by decreasing of MDA and carbonyl level and increasing of specific activity of SOD and catalase in intermittent exposure groups.

REFERENCES

1. Solaini G, Baracca A, Lenaz G, Sgarbi G. Hypoxia and mitochondrial oxidative metabolism. *Biochim Biophys Acta - Bioenerg.* 2010;1797(6-7):1171-7.
2. Maiti P, Singh SB, Sharma AK, Muthuraju S, Banerjee PK, Ilavazhagan G. Hypobaric hypoxia induces oxidative stress in rat brain. *Neurochem Int.* 2006;49(8):709-16.
3. Bleier L, Dröse S. Superoxide generation by complex III: From mechanistic rationales to functional consequences. *Biochim Biophys Acta - Bioenerg.* 2013;1827(11-12):1320-31.
4. Wheaton WW, Chandel NS. Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol.* 2011;300(3):C385-93.
5. Wozniak A, Drewa G, Chesy G, Rakowski A, Rozwodowska M, Olszewska D. Effect of altitude training on the peroxidation and antioxidant enzymes in sportsmen. *Med Sci Sports Exerc.* 2001;33(7):1109-13.
6. Zhou D, Shao L, Spitz DR. Reactive oxygen species in normal and tumor stem cells. *Adv Cancer Res.* 2014;122:1-67.
7. Siomek A. NF- κ B signaling pathway and free radical impact. *Acta Biochim Pol.* 2012;59(3):323-31.
8. Dayem AA, Choi H-Y, Kim J-H, Cho S-G. Role of oxidative stress in stem, cancer, and cancer stem cells. *Cancers.* 2010;2(2):859-84.
9. Venditti P, Di Stefano L, Di Meo S. Mitochondrial metabolism of reactive oxygen species. *Mitochondrion.* 2013;13(2):71-82.
10. Levine RL, Williams JA, Stadtman EP, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. In: *Methods in Enzymology.* 1994:346-57.
11. George P. Reaction between catalase and hydrogen peroxide. *Nature.* 1947;160:41-3.
12. Joanny P, Steinberg J, Robach P, Richalet JP, Gortan C, Gardette B, et al. The effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. *Resuscitation.* 2001;49(3):307-14.
13. Serrano J, Encinas JM, Salas E, Fernández AP, Castro-Blanco S, Fernández-Vizarra P, et al. Hypobaric hypoxia modifies constitutive nitric oxide synthase activity and protein nitration in the rat cerebellum. *Brain Res.* 2003;976(1):109-19.
14. Sethy NK, Singh M, Kumar R, Ilavazhagan G, Bhargava K. Upregulation of transcription factor NRF2-mediated oxidative stress response pathway in rat brain under short-term chronic hypobaric hypoxia. *Funct Integr Genomics.* 2011;11(1):119-37.
15. Costa DC, Alva N, Trigueros L, Gamez A, Carbonell T, Rama R. Intermittent hypobaric hypoxia induces neuroprotection in kainate-induced oxidative stress in rats. *J Mol Neurosci.* 2013;50(3):402-10.