RESEARCH ARTICLE

CHANGES ON OXIDATIVE STRESS-RELATED BIOMARKERS IN PLASMA AND CARDIAC TISSUE DUE TO PROLONGED EXPOSURE TO NORMOBARIC HYPEROXIA

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ABSTRACT

Background: Hyperoxia is a state of oversupply of oxygen in tissues and organs that can increase reactive oxygen species (ROS). When antioxidants cannot balance ROS levels, oxidative stress occurs. Catalase and reduced glutathione (GSH) are two of the antioxidants that can be very useful to counteract ROS. Increased production of ROS subsequently results in lipids damage and generates malondialdehyde (MDA). ROS interaction with cardiac cells causes remodeling thus leads to heart failure.

Objectives: The purpose of this study was to find out the changes on oxidative stress-related biomarkers in plasma and cardiac tissue.

Methods: Sprague Dawley rats were divided into 5 groups (n=6/group). Control group was exposed to normoxia (21% O_2), while each treatment group was exposed to hyperoxia (75% O_2) for 1, 3, 7, and 14 days. Blood and heart samples were used for blood gas analysis and hematology test, also for catalase specific activity measurement, GSH level, and MDA level measurement.

Results: Blood gas analysis of pO_2, pCO_2, and HCO_3 were increased, while the O_2 saturation and all hematological parameters were decreased. Plasma and cardiac tissue's catalase specific activity increased in day 1 to day 7 but declined in day 14. Cardiac tissue's GSH has the same result. Plasma GSH level increased in day 1 but decreased afterward. MDA level in plasma and cardiac tissue increased significantly since day 1.

Conclusion: Hyperoxia causes oxidative stress, marked by the increase of oxidative stress-related markers, and partially compensated respiratory acidosis.

INTRODUCTION

Oxygen is one of the substances needed by all living things, especially for breathing, doing activity, and more specifically for carrying out metabolic processes.[1] High levels of oxygen exposure are often therapeutic choice in several diseases.[2-4] However, oxygen therapy can trigger hyperoxia, a condition when there is excess oxygen in tissues and organs.[5] Hyperoxia is associated with multiple effects in different organ systems. It can directly damage tissues via the production of reactive oxygen species (ROS) that leads to increased cell death by apoptosis and increased release of endogenous damage-associated molecular pattern molecules (DAMPs) that stimulate an inflammatory response. In animal model, oxygen therapy greatly influences the progression and clinical manifestation of multiple system organ dysfunction.[6-7]

Hyperoxia induces an increase in reactive oxygen species (ROS), which are highly reactive molecules that mainly originate from the mitochondrial electron transport chain that can cause damage.[8-12] When hyperoxia occurs, the first body defence is antioxidants such as catalase and glutathione (GSH).[5,11] When antioxidants cannot balance ROS levels, oxidative stress occurs. ROS can then cause damage to lipids, proteins, DNA, and carbohydrates.[12] ROS damages lipids (Polyunsaturated Fatty Acids (PUFAs)) by carrying out lipid peroxidation and producing malondialdehyde (MDA).[8]

This research examined the levels of oxidative stress in heart because it is an obligate aerobic organ and consumes large amounts of oxygen. But oxygen is the center of ROS formation, which can induce irreversible damage and cell death.[13-15] This can then result in remodeling of the heart and leading to the heart failure.[8-10] In addition, blood levels of oxidative stress markers were also examined. Increased ROS due to hyperoxia can also result in changes in blood gas analysis and hematology test parameters.[16-18]

MATERIAL AND METHODS

This research was an in vivo experimental study, using male Sprague Dawley rats as experimental animals, and took place at the Laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University. Blood gas analysis and hematology test were carried out at the Clinical Pathology Laboratory of Dharmais Hospital. This research has obtained ethical approval from the Research Ethics Committee of the Faculty of Medicine, Trisakti University with number 81/KER/FK/X/2015.

The inclusion criteria for the experimental animals were male Sprague Dawley rats aged 8-12 weeks with body weight of 200-250 mg in healthy conditions, with normal activity and behaviour. Any Sprague Dawley rats that did not meet the criteria were excluded from the trial.

The number of samples used was determined by Federer's formula[19] and also followed WHO's[20] recommendations regarding the minimum number of animals in research, which is 6 rats in each group. Therefore, the number of rats used for this study was 30 rats.

The experimental animals were divided randomly into 5 groups: 1 control or no treatment group (P1) (21% O_2) and 4 treatment groups (P2 - P5) (75% O_2, normobaric). The treatment groups were exposed for 1 day, 3 days, 7 days, and 14

days respectively in a hyperoxia chamber. Examination of catalase specific activity was using Mates method[21], GSH examination with Ellman method, and MDA examination with Wills method[22]. The results were statistically analyzed with GraphPad Prism v. 7.0 with a Mann-Whitney test. The variables were considered significant if $p < 0.05$.

RESULTS

Changes in blood gas analysis and hematology test parameters can be seen in Table 1. Significant increase in pO_2 can be seen since 1-day ($p=0.002$) to 14-days ($p=0.002$) hyperoxia treatment, pCO_2 ($p=0.045$) and HCO_3 ($p=0.041$) increased significantly since day 7. Significant decrease of pH parameters ($p=0.008$), hemoglobin ($p=0.005$), and RBC ($p=0.029$) began since day 1. While O_2 saturation ($p=0.037$) and hematocrit ($p = 0.030$) decreased significantly since the third day.

The lowest specific activity of catalase in plasma began to experience a significant increase in the 3-days hyperoxia treatment group ($p=0.030$). The highest result was obtained in the 7-days group ($p=0.004$), whereas in the 14-days group ($p=0.010$) the specific activity decreased. This also occurred to the specific activity of catalase measured in the cardiac tissue. The specific activity of catalase in plasma was lower than that of the cardiac tissue (Figure 1).

Plasma GSH level increased unsignificantly in 1-day group ($p=0.067$). It began to increase significantly in the 3-days group ($p=0.005$). But in the 7-days group to 14-days group, there was a decrease. GSH levels of both plasma and cardiac tissue in 1-day group increased. But they went in the opposite direction afterward, the plasma GSH level decreased

while the cardiac tissue's GSH level increased. In the 14-days group, the cardiac tissue's GSH levels dropped drastically. Plasma GSH levels were always lower than cardiac tissue's (Figure 2).
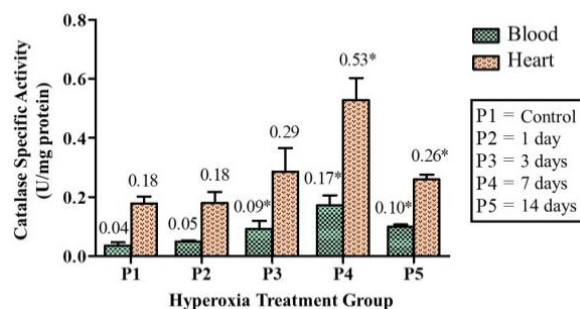

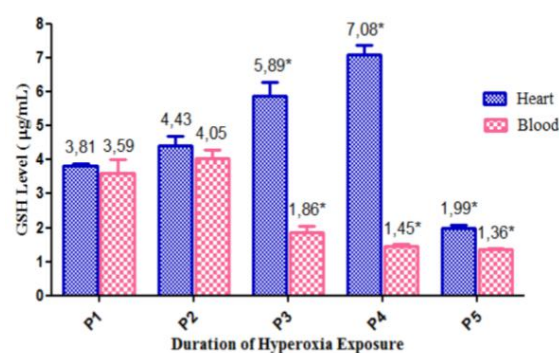**Figure 1.** Catalase Specific Activity
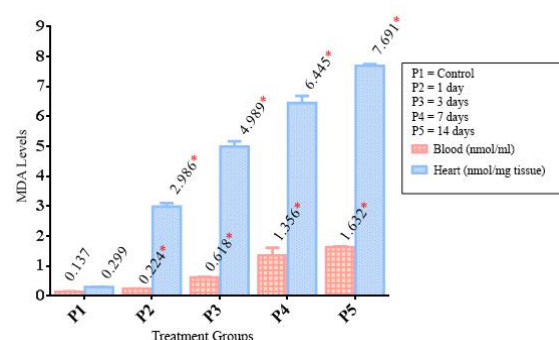

Figure 2. Glutathione Level

Figure 3. Malondialdehyde Level

MDA level of the plasma had a significant increase since 1-day $p=0.000$) until 14-days hyperoxia treatment

(p=0.000). MDA level of the cardiac tissue also increased significantly since 1-day treatment (p=0.000) until 14-days treatment (p=0.000). MDA levels of plasma were in line to MDA levels of the cardiac tissue, where both increase since day 1 to day 14 (Figure 3).

Table 1. Blood Gas Analysis and Hematology Test

Parameter	Normoxia	Hyperoxia			
		1 Day	**3 day**	**7day**	**14 Day**
pO_2 mmHg	95.9 ± 2.1	134.7 ± 1.7*	173.8 ± 1.7*	248.1 ± 1.2*	412.3 ± 1.1*
pCO_2 mmHg	35.5 ± 1.2	36.2 ± 1.1	37.7 ± 1.5	39.6 ± 0.8*	42.1 ± 1.2*
pH	7.46 ± 0.006	7.43 ± 0.003*	7.40 ± 0.004*	7.35 ± 0.004*	7.00 ± 0.005*
HCO_3 Mmol/L	24.2 ± 0.4	24.7 ± 0.4	25.2 ± 0.4	25.8 ± 0.4*	26.8 ± 0.3*
Saturated O_2 %	94.7 ± 0.6	93.6 ± 0.7	92.2 ± 0.5*	91.0 ± 0.6*	88.6 ± 0.8*
Haemoglobin g/dL	12.0 ± 0.12	11.2 ± 0.08*	10.7 ± 0.04*	9.5 ± 0.07*	8.6 ± 0.10*
Haematocrit %	40.8 ± 0.4	40.2 ± 0.2	39.5 ± 0.2*	38.9 ± 0.3*	37.6 ± 0.2*
RBC µl/1000	6.2 ± 0.1	5.9 ± 0.07*	5.3 ± 0.2*	4.9 ± 0.2*	4.2 ± 0.2*

Mean result ± SEM. * meaningfully different compare with normoxia (p<0.05, Mann Whitney test)

DISCUSSION

As seen in Table 1, starting from the beginning of the hyperoxia treatment, the pO_2 began to increase and showed systemic hyperoxia. High pO_2 induces a strong physiological response, resulting in hypoventilation and CO_2 retention. This resulted in a significant increase in pCO_2 since the 7-days of treatment. High pCO_2 caused respiratory acidosis. This situation was also supported by a decrease in pH that has occurred since the beginning of treatment. Partial compensation for respiratory acidosis began on the seventh day and showed a significant increase in HCO_3. ROS caused lipid peroxidation in the membranes of red blood cells, resulting in hemolysis. Hemolysis caused all hematological parameters and O_2 saturation to decrease.

A significant increase of catalase activity in cardiac tissue in the 7-days hyperoxia treatment group can be seen as a heart defense response to ROS, in this case, H_2O_2. The 14-days group showed a significant decrease in the catalase activity in cardiac tissue indicating a failure of defense response to the increasing H_2O_2 resulting in oxidative stress. This is in accordance with research by Miguel.[23] The specific activity of catalase in the plasma was lower than the cardiac tissue. In similar research by Marina [24], it was found that an increase in specific activity of catalase in the plasma indicated that the cell membrane became unstable and leaked the catalase enzyme into the blood.

Increased levels of GSH is due to an increase in uptake of Glutamic acid that is a precursor in the formation of GSH.

Plasma GSH levels are always lower than the levels of tissue GSH because most of GSH residing within plasma came from cell leakage. This is consistent with research by Malmezat *et al*.[25]

Hyperoxia will increase ROS and inflammation due to increase of NF-κB. Radical oxygen species will cause decrease of nitric oxide and cause oxidative/nitrosative species. Radical oxygen species will cause uncoupling of mitochondrial respiration that will lead to decrease oxygen consumption and ATP synthesis. The decrease of nitric oxide will decrease microvascular perfusion and may cause cell death. The longer the duration of hyperoxia, the more ROS is formed, so that more lipids are damaged and more MDA is produced. MDA in the cardiac tissue can then go out into the plasma so that the plasma MDA levels also increased. In this study, MDA levels of plasma and cardiac tissue of all experimental groups increased significantly. This is in line with similar research by Loiseaux-Meunier *et al*.[26] MDA level of cardiac tissue in the 1-day group increased significantly when compared to the control group, similar to the research by Sifringer *et al*[27] and Bandali *et al*.[28]

CONCLUSION

There was an increase of catalase activity in the plasma and cardiac tissue in 1-day to 7-days treatment group, which then decreased on day 14. The same condition was found in cardiac tissue's GSH level. While plasma GSH levels increased just for one day, then it decreased. MDA levels of plasma and cardiac tissue increased significantly since the beginning until the end of the treatment. This showed that in the plasma and cardiac tissue of rats induced by prolonged normobaric hyperoxia, oxidative stress occurs.

REFERENCES

1. Sherwood L. Human physiology: from cells to systems. 6th Ed. Jakarta: EGC; 2011.

2. Kilgannon JH, Jones AE, Shapiro NI, Angelos MG, Milcarek B, Hunter K, et al. Association between arterial hyperoxia following resuscitation from cardiac arrest and in-hospital mortality. The Journal of the American Medical Association. 2010;303(21):2165-71.

3. Lau YY, Tay YY, Shah VA, Chang P, Loh KT. Maintaining optimal oxygen saturation in premature infants. The Permanente Journal. 2011;15(1):108-13.

4. Farquhar H, Weatherall M, Wijesinghe M, Perrin K, Ranchord A, Simmonds M, et al. Systematic review of studies of the effect of hyperoxia on coronary blood flow. Am Heart J. 2009;158(3): 371-7.

5. Dean JB, Mulkey DK, Henderson RA, Potter SJ, Putnam RW. Hyperoxia, reactive oxygen species, and hyperventilation: oxygen sensitivity of brain stem neurons. J Appl Physiol. 2004;96(2):784-91.

6. Vincent JL, Taccone FS, He X. Harmful effects of hyperoxia in postcardiac arrest, sepsis, traumatic brain injury, or stroke: The importance of individualized oxygen therapy in critically ill patients. Canadian Respiratory Journal. 2017;2017:1-7.

7. Gonzales RR, Barrasa MJL, Nuez RA,Pedrosa CAM, Saavedra MMT,Bello GMA, et al. Multiple

system organ response induced by hyperoxia in a clinically relevant animal model of sepsis. Shock. 2014;42(2):148-53.

8. Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014;2014:1-31.

9. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. The J of Clin Investigation. 2005;115(3):500-8.

10. Alfadda AA, Sallam RM. Reactive oxygen species in health and disease. Journal of Biomedicine and Biotechnology. 2012;2012:1-14.

11. Matés JM. Effect of antioxidant enzymes in the molecular control of rective oxygen species toxicology. Toxicology. 2000;153(1-3):83-104.

12. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. 5th ed. New York: Oxford University Press; 2015.

13. Taverne YJ, Bogers AJ, Duncker DJ, Merkus D. Reactive oxygen species and the cardiovascular system. Oxidative Medicine and Cellular Longevity. 2013;2013:1-15.

14. Moradkhan R, Sinoway LI. Revisiting the role of oxygen therapy in cardiac patients. J Am Coll Cardiol. 2010;56(13):1013-6.

15. Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, et al. Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. J Biol Chem. 2003;278(47):47129-35.

16. V Marina M, V Evgeniya K. Special features of deviations and correction pro and antioxidant blood plasma balance of rats under stress of different genesis. Advance Research in Scientific Areas. 2012;7(15):2158-60.

17. Rostami E, Rocksen D, Ekberg NR, Giony M, Ungerstedt U. Brain metabolism and oxygenation in healthy pigs receiving hypoventilation and hyperoxia. Respir Physiol Neurobiol. 2013;189(3):537-42.

18. Byrd RP. Respiratory acidosis. [updated 2015 Jul 31, cited 2015 Dec 3]. Available from: http://emedicine.medscape.com/article/301574-overview#a7

19. Federer WT. Experimental design: theory and application. New York: Oxford & IBH Publishing Company; 1955.

20. Siantur M. Penentuan besar sampel. Semarang: Universitas Diponegoro; 2007.

21. Matés J, Pérez-Gómez C, De Castro I. Antioxidant enzymes and human diseases. Clinical Biochemistry. 1999; 32(8):595-603.

22. Wills ED. Evaluation of lipid peroxidation in lipids and biological membranes. In: Snell K, Mullock B, editors. Biochemical toxicology: A practical approach. Oxford: IRL;1987:127-52.

23. Miguel F, Augusto AC, Gurgueira SA. Effect of acute vs chronic H2O2-induced oxidative stress on antioxidant enzyme activities. Free Radical Research. 2009;43(4):340-7.

24. Marina V, Evgeniya V. Special features of deviations and correction

pro and antioxidant blood plasma balance of rats under stress of different genesis. Advance Research in Scientific Areas. 2012;7(15):2158-60.

25. Malmezat T, Breuille D, Capitan P, Mirand PP, Obled C. Glutathione turnover is increased during the acute phase of sepsis in rats. The Journal of Nutrition. 2000; 130(5):1239-46.

26. Loiseaux-Meunier MN, Bedu M, Gentou C, Pepin D, Coudert J, Caillaud D. Oxygen toxicity: simultaneous measure of pentane and malondialdehyde in humans exposed to hyperoxia. Biomed Pharmacother. 2001;55(3):163-9.

27. Sifringer M, Haefen CV, Krain M, Paeschke N, Bendix I, Buhrer C, et al. Neuroprotective effect of dexmedetomidine on hyperoxia-induced toxicity in the neonatal rat brain. Oxidative Medicine and Cellular Longevity.2015;2015:1-10

28. Bandali KS, Belanger MP, Wittnich C. Hyperoxia causes oxygen free radical-mediated membrane injury and alters myocardial function and hemodynamics in the newborn. Am J Physiol Heart Circ Physiol. 2004;287(2):553-9.