



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% O₂), while each treatment group was exposed to hyperoxia (75% O₂) 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 pO₂, pCO₂, and HCO₃ were increased, while the O₂ 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.

Keywords : Cardiac tissue, Catalase, Glutathione, Hyperoxia, Malondialdehyde

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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 Dharmas 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% O₂) and 4 treatment groups (P2 - P5) (75% O₂, 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).

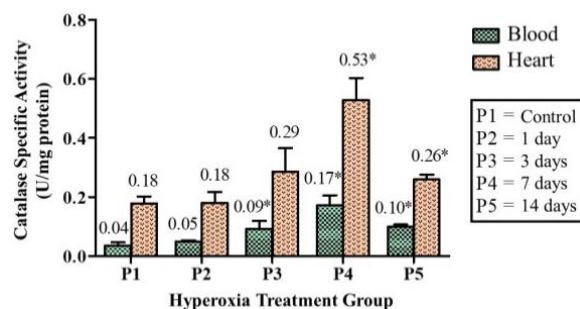


Figure 1. Catalase Specific Activity

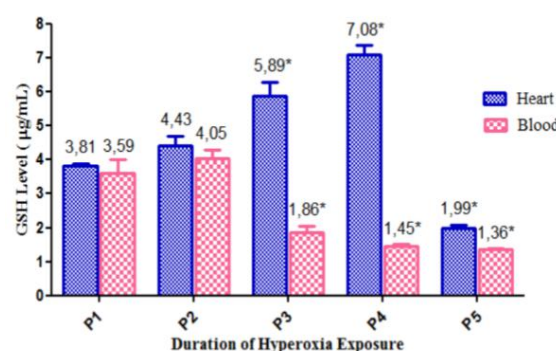


Figure 2. Glutathione Level

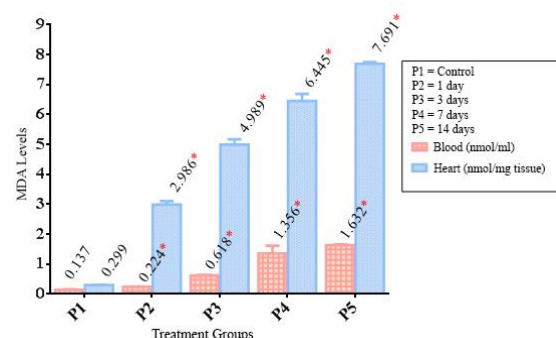


Figure 3. Malondialdehyde Level

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	95.9	134.7	173.8	248.1	412.3
mmHg	± 2.1	± 1.7*	± 1.7*	± 1.2*	± 1.1*
pCO ₂	35.5	36.2	37.7	39.6	42.1
mmHg	± 1.2	± 1.1	± 1.5	± 0.8*	± 1.2*
pH	7.46	7.43	7.40	7.35	7.00
	± 0.006	± 0.003*	± 0.004*	± 0.004*	± 0.005*
HCO ₃	24.2	24.7	25.2	25.8	26.8
Mmol/L	± 0.4	± 0.4	± 0.4	± 0.4*	± 0.3*
Saturated O ₂	94.7	93.6	92.2	91.0	88.6
%	± 0.6	± 0.7	± 0.5*	± 0.6*	± 0.8*
Haemoglobin	12.0	11.2	10.7	9.5	8.6
g/dL	± 0.12	± 0.08*	± 0.04*	± 0.07*	± 0.10*
Haematocrit	40.8	40.2	39.5	38.9	37.6
%	± 0.4	± 0.2	± 0.2*	± 0.3*	± 0.2*
RBC	6.2	5.9	5.3	4.9	4.2
μl/1000	± 0.1	± 0.07*	± 0.2*	± 0.2*	± 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 pO₂ began to increase and showed systemic hyperoxia. High pO₂ induces a strong physiological response, resulting in hypoventilation and CO₂ retention. This resulted in a significant increase in pCO₂ since the 7-days of treatment. High pCO₂ 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 HCO₃. ROS caused lipid peroxidation in the membranes of red blood cells, resulting in hemolysis. Hemolysis caused all hematological parameters and O₂ 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, H₂O₂. The 14-days group showed a significant decrease in the catalase activity in cardiac tissue indicating a failure of defense response to the increasing H₂O₂ 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- κ B. 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.

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