The effect of intermittent hypobaric hypoxia exposure on reduced-glutathione level in rat lung and renal tissues

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Abstract
Hypobaric hypoxia is basically a hypoxia condition experienced in high altitude commonly during flight, that increase reactive oxygen species (ROS). When hypoxia hypobaric does not undergo continuation or in other word, intermittent, it will cause adaptation response in a form of protection mode into ROS. Moreover, ROS could be eliminated by reduced-glutathione (GSH) as an endogenous non enzymatic antioxidant. Therefore, the aim of this study was to analyze the effects of intermittent hypobaric hypoxia exposure on GSH level in rat lung and renal tissue. Lung and renal samples were collected from 6–8 weeks old male Sprague-Dawley rats weighing 150–200 g, previously exposed 1–4 times to intermittent hypobaric hypoxia in 35,000 ft (1 minute), 25,000 ft (5 minute) and 18,000 ft altitude (25 minute). Afterwards, GSH level was calculated from lung and renal extracts using the Ellman’s method. In lung tissues, GSH level was decreased in hypoxia 1×, 2×, 3×, 4× treatment, and were significant between the control–hypoxia 3×, control–hypoxia 4×, hypoxia 1×–hypoxia 3× and hypoxia 1×–hypoxia 4×. On the contrary, GSH level was increased in renal tissues on hypoxia 1× and hypoxia 2× treatment compared to control. Nevertheless, GSH level was decreased after 3× treatment and found almost stabilized at 4× treatment of hypoxia in renal tissues. Intermittent hypobaric hypoxia exposure affects GSH in rat lung and renal tissues with varying level as an adaptive response system.

Keywords: GSH, intermittent hypobaric hypoxia, lung, renal

1. INTRODUCTION

Hypoxia is a condition where there is a reduce amount of oxygen in the cellular level.\textsuperscript{1} Hypobaric hypoxia is a condition where the body craves for an adequate amount of oxygen from the air in order for the body to do work. This condition usually happens when a person reaches to a higher altitude.\textsuperscript{1,2}

When hypoxia hypobaric does not undergo continuation or in other word, intermittent, it will cause adaptation response in a form of protection mode into reactive oxygen species (ROS). An increased ROS can cause oxidative stress lead to macromolecule and cell membrane damages through DNA damage, protein oxidation, lipid peroxidation, and impaired ATP production.\textsuperscript{3}

Lungs plays an essential role in organism, it works to keep the balance level of oxygen in human physiological system. In hypobaric hypoxia condition, a lung is the first organ that affected between oxygen in the environment and gas exchange process.

Hypoxia decreases the systemic vascular resistance and yet the pulmonary vascular resistance increases. Hypoxia condition disturbs all the cells in the lung, and this disturbance can possibly leads to a non-contractile cells as a reaction on distorted construction and excretion of metabolites or distorted of cell membrane receptor expression.\textsuperscript{4} Moreover, kidney is an organ that is mostly exposed into hypoxia due to its anatomical structure shows significant difference in blood supply and oxygenation. The tension of oxygen found in kidney is comparatively low, particularly in renal medulla, despite its high rated blood flow and oxygen delivery. This circumstance is caused by parallel arrangement of arterial and venous pre glomerular and post glomerular vessels, since this allows oxygen to diffuse from arterioles into postcapillary venous system via shunt diffusion. Due to the restriction in renal oxygen supply, kidney is vulnerable to hypoxia and it contributes as mainly factor in pathogenesis of acute renal injury.\textsuperscript{5}

The body has endogenous antioxidant which protect...
it from oxidative damage. One of the important antioxidants is glutathione, or GSH. Glutathione (GSH) is a tripeptide protein that consists of 3 main amino acid (glutamic acid, cysteine, glycine). The presence of GSH commonly found in cytosol part of the body. In lungs specifically, GSH can be found in epithelial lining fluid, and held the function as the first line basis of the defense under oxidative stress condition. Previous study has reported that during the induction of hypoxia hypobaric intermittent condition, the levels of Manganese Superoxide Dismutase (MnSOD) as an endogenous antioxidant was decreased. Moreover, previous study also found that intermittent hypobaric hypoxic could induce the enhancement of lipid damage (malondialdehyde/MDA) and protein damage (carbonyl) in kidney rat. There were a different variation tend among the MDA and carbonyl concentration between the kidney of rats that generally increase upon the first exposure of hypoxia and will decrease at the next exposure. Regarding that, we would like to analyze whether the other antioxidant such as GSH also contribute in that process. Therefore, the aim of this study was to analyze the effects of intermittent hypobaric hypoxia exposure on GSH level in rat lung and renal tissue.

2. METHODS

This research was conducted in Department of Biochemistry at Faculty of Medicine Universitas Indonesia. Lung and renal samples were collected from 6-8 weeks old male Sprague-Dawley rats weighing 150-200g, previously exposed 1 - 4 times to intermittent hypobaric hypoxia in 35,000 ft (1 minute), 25,000 ft (5 minute) and 18,000 ft altitude (25 minute). Afterwards, GSH level was calculated from lung and renal extracts.

**GSH Measurement**

GSH level was measured by *Ellman’s method*. Glutathione added with Ellman’s reagent produced a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The color was observed in 412 nm wavelength. Glutathione level in lungs and renal tissue of each group was calculated on glutathione level per total protein. Protein was measured by *Christian Warburg’s method* at 280 nm wave length.

**Statistical analysis**

The data were analyzed using ANOVA in SPSS version 21. Shapiro Wilk test was done before being statistically analyzed using to check normal distribution data. The result were estimated to be significant if the p value < 0.05.

3. RESULT

**GSH Content of the Lung Tissue Samples**

The overall GSH substance was calculated and with the principle of \[ X = \frac{Y}{a+b}\]. The normality of the data was calculated by Shapiro Wilk and revealed that there were groups which scored in p>0.05 indicating that the data had abnormal distribution. Moving forward, the data then be analyzed through Kruskal Willis test, and been found out that there was significant difference between the control – hypoxia 3, control – hypoxia 4, hypoxia 1 – hypoxia 3 and hypoxia 1 – hypoxia 4 as shown in figure 1.

**GSH Content of the Renal Tissue Samples**

The total content of GSH in renal samples was based on the linear regression function of \[ y = 0.0418x + 0.0045 \] that measured from the GSH standard curve. The total GSH content is in \( \mu g/mL \) was calculated using the formula of: \[ X = \frac{Y}{a+b} \] yet the value is represented in mean ± SD. GSH concentration was calculated by dividing GSH content with protein concentration (\( \mu g/mg \) protein). All of these data suggested that level of GSH in firstly increased in line after exposure until the second hypoxia, yet following a decreased trend in third hypoxia and almost showing stable level on the fourth hypoxia as shown on the figure (Figure 2) although statistically, the data were not significant according to ANOVA and Post Hoc LSD Analysis (\( p = 0.268 \)).

**DISCUSSION**

This research was held in order to measure the amount of lung and renal GSH level after being exposed to intermittent hypobaric hypoxia for several periods of time. Reduced glutathione (GSH) was picked as the parameter of this research since GSH plays important role in maintaining the intracellular redox equilibrium and protect tissues from oxidative stress. This suggested the main benefits of GSH system as one of the major
non-enzymatic antioxidant defenses in lung and renal.\textsuperscript{3} The lung and renal samples measured in this research was taken from 6-8 weeks old rats, this was based on their adolescent period within those range of time, so any oxidative-related disturbance will not be found interfering the data results. The GSH level was affected with hypobaric hypoxia condition and it was seen that GSH in lung tissue was significantly decreased in the 1x, 2x, 3x, and 4x treatment. Our result actually had the similar result with the previous research on Manganese superoxide dismutase (MnSOD) level on the same hypobaric hypoxia intermittent condition. MnSOD level in that condition were also decreased compared to the control variable.\textsuperscript{6} MnSOD is an endogenous antioxidant enzyme which catalyzes the conversion of superoxide radical into H$_2$O$_2$. While, the function of GSH is to catalyzes H$_2$O$_2$ into H$_2$O.\textsuperscript{3} Therefore, it might be possible when the activity of MnSOD decreased; GSH is being used to stabilize the condition so that the ROS and oxidative stress are under control. Moreover, catalase activity on lungs also decreased under intermittent hypobaric hypoxia condition. Specifically, under the intermittent hypobaric hypoxia on 1x induction, the catalase activity hits the lowest level. It might be caused by the adaptation of the lungs to suppress the oxidative stress.\textsuperscript{8} Perhaps, catalase and glutathione works together to protect cells from further damage due to hypoxia condition.

In renal tissues, GSH level increased on the first and second hypoxia exposure then decreased and slightly elevated again. It trend level has matched with carbonyl level trend according to previous study.\textsuperscript{7} Carbonyl is an oxidative stress marker which reflected protein damage.\textsuperscript{3} Therefore, GSH level trend as if followed the trend of oxidative stress so perhaps when ROS level is found to be high, it will also be high, and vice versa. The GSH level within hypoxia has increased until a certain amount of time. The peak was found on 2 times of hypoxia exposure testing (0.053±0.024) and this was perhaps due to the maximum GSH concentration which can produced by renal under hypoxia condition has reached to overcome ROS. Next, there was an almost stabilized amount of renal GSH level on 4 time exposure of hypoxia (0.028±0.007), which probably described that the GSH level has risen a little, again, after certain adjustment. The increasing of hypoxia exposure leads to elevated mitochondrial ROS production, since GSH become oxidized faster in order to scavenge excessive ROS to keep it within normal range. An increasing trend in renal GSH level upon increasing exposure to hypoxia was most likely due to a high overcome of GSH amount until it reached maximum point, whereas when it finally decreased, it was most likely due to to elevated mitochondrial ROS production; since the more hypoxic the tissue is, the higher the ROS production.\textsuperscript{9} Within this state, renal GSH played role as antioxidant yet oxidized faster in order to neutralize any excessive ROS so that it can be maintain at normal range.\textsuperscript{3} Although the results of this research began with an increasing trend of renal GSH upon exposure to hypoxia in a time-dependent manner, the level of renal GSH decreased on the 3 days post-exposure, perhaps due to maximum adaptation to the hypoxic condition. However, further studies are necessary to address this issue.

GSH was used to counter balance the increased formation of ROS during intermittent hypobaric hypoxia condition. It protects tissues from oxidative stress that might cause cells damage. In general, it can be stated that lung and renal, within an intermittent hypobaric hypoxia is equipped with adapting mechanism in response to the exposure. Antioxidants, such as glutathione, is very essential to support lung and renal function. Future studies such as pulmonary and renal function test are needed to prove that the lung and kidney still works fine and really protected by glutathione.

5. CONCLUSION

Intermittent hypobaric hypoxia exposure affects GSH in rat lung and renal tissues with varying level as an adaptive response system.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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