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Expression of Hypoxia-Inducible Factor-1α and Myoglobin in Rat Heart as Adaptive Response to Intermittent Hypobaric Hypoxia Exposure

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ABSTRACT

Objective: The aim of this study was to investigate the influence of intermittent hypobaric hypoxia (IHH) on the expression of hypoxia adaptation proteins, namely hypoxia-inducible factor-1α (HIF-1α) and myoglobin (Mb).
Methods: Twenty-five male Sprague–Dawley rats were exposed to IHH in a hypobaric chamber in Indonesian Air Force Institute of Aviation Medicine, for 49.5 min at various low-pressure levels and at 1-week interval for four times (days 1, 8, 15, and 22). HIF-1α and Mb proteins were measured with enzyme-linked immunosorbent assay. Mb messenger RNA expression ratio was measured with one-step real-time reverse transcription polymerase chain reaction.
Results: The HIF-1α protein level increased after the induction of HH and continues to decrease after the induction of IHH (analysis of variance, $p = 0.0437$). Mb messenger RNA expression ratio and Mb protein level increased after the induction of HH and continues to decrease after induction of IHH (analysis of variance, $p = 0.0283$ and 0.0170, respectively), and both are strongly correlated (Pearson, $r = 0.6307$).
Conclusions: The heart of the rats adapted to intermittent hypoxia conditions by upregulating the expression of HIF-1α and Mb and then both returned to normal level.

Introduction

Hypoxia is a condition in which oxygen supply cannot fulfill the need of an organism, its organ, or even a cell. In this situation, the subject (organism, organ, or cell) has to activate various mechanisms to maintain the energy level. The hypoxia condition induces adaptation responses to defend homeostasis in the body. Physiologic responses such as increased heart, pulse, and respiratory rates increase oxygen supply. Transcription factor hypoxia-inducible factor (HIF)-1α will be stabilized with HIF-1β. This heterodimer regulates many of the genes in hypoxic condition. Altered cell metabolism, from aerobic to anaerobic, increases lactic acid production that makes acidosis. High blood pressure was recorded during hypoxia, to increase oxygen distribution through red blood cells (Loscalzo J, 2010).

In intermittent hypoxia, the subject is exposed to a certain low oxygen pressure frequently, and in an experiment, subject is exposed to it at a certain interval between each treatment. It can be presumed that any adaptation process would be repeated. Intermittent hypobaric hypoxia (IHH) is linked with oxidative stress, impairing cardiac function. However, early IHH also activates cardioprotective mechanisms (Herrera EA *et al.*, 2015).

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Clinical observation on preconditioning ischemia (Muray CE *et al.*, 1986) and previous experimental studies on the effect of intermittent hypoxia on the brain (Mulyawan W, 2012) and heart (Hidayat A, 2011) suggest that this condition might be beneficial for the subject.

The HH is relatively rare in the usual condition. Most people live at the sea level to 500 m altitude, where the influence of atmospheric pressure is relatively nil. The hypoxic conditions at high altitudes present a challenge for survival, causing pressure for adaptation. Interestingly, many people living at high-altitude regions (particularly in the Andes) are maladapted, with a condition known as chronic mountain sickness (Zhou D *et al.*, 2013). Studies of Tibetan highlanders showed that polymorphisms in candidate genes show signatures of natural selection as well as well-replicated association signals for variation in hemoglobin levels. Studies of Ethiopian highlanders found that variants associated with the hemoglobin variation among Tibetans or other variants at the same loci do not influence the trait in Ethiopians (Aranburu GA *et al.*, 2012; Peng Y, *et al.*; 2011).

When the body undergoes hypoxic conditions, adaptively, body will provide systemic and cellular responses to meet the oxygen requirements. Semenza has mentioned that the systemic hypoxic conditions increased the expression of erythropoietin (EPO), which led to an increase in the number of red blood cells in the oxygen-carrying molecule (Semenza, 2004). In these studies, it is known that in the condition of hypoxia, HIF-1α is a transcription factor that regulates the increased expression of EPO.

Circulation of blood throughout the body is regulated through blood pressure, which is dependent on the heart pumping and blood flow. Exposure of mice to hypoxia leads to a significant rise in blood pressure (Edckardt *et al.*, 2005). This rise is associated with microvascular endothelial changes, subtle tubulointerstitial injury, inflammation, and interstitial cell proliferation (Johnson RJ *et al.*, 2002). Therefore, the heart selected as samples in this study.

However, besides people living in high-plateau regions such as Tibet (Peng Y *et al.*, 2011), Andes (Zhou D *et al.*, 2013), or Ethiopia (Aranburu GA *et al.*, 2012), pilots are prone to unpleasant or even the deleterious effect of hypoxia, resulting from sudden low atmospheric pressure. Sudden hypoxia will reduce the psychomotor activities (Gradwell DP, 2006), which is very dangerous for the pilot. Fortunately, the danger can be reduced by a special training in which the pilot is exposed to a hypoxic condition by placing him in a low-pressure hypoxic chamber. The air pressure is made similar to various altitudes which occur in aviation.

It is well known that the heart and brain are the most aerobic organs in the body, and therefore, both consume a great portion of respiratory oxygen. However, heart is an obligate aerobic organ that consumes more oxygen than any other organ. Heart muscle cannot produce enough energy to sustain cardiac function and viability under hypoxic conditions. In the absence of sufficient oxygen, electron transport chain ceases, and cardiac energy demands are not met (Giordano FJ, 2005).

Therefore, we wondered if the intermittent hypoxia would have beneficial effects on the heart, they would be indicated by hearts' HIF-1α and myoglobin (Mb) levels. We realized the observation on the rats placed in various conditions of low atmospheric pressure in the hypobaric chamber.

Material and Methods

Animal model and sample preparation

All the following experimental designs and procedures have been reviewed and approved by a Scientific Ethic Committee. This experimental animal study was conducted in the Indonesian Air Force Dr Saryanto Institute of Aviation Medicine (Lakespra Dr Saryanto: Lembaga Kedokteran Penerbangan dan Ruang Angkasa Dr Saryanto) and the Department of Biochemistry and Molecular Biology, Faculty of Medicine University of Indonesia from 2015 to 2016. Twenty-five male Sprague–Dawley rats (200–250 g) were obtained from the Institute of Health Research and Development, Jakarta, divided into five groups. The rats of the control group were placed in environment atmosphere without exposure to HH. The other groups were placed in human hypobaric training chamber according to the number of experiments carried out (for one, two, three, and four times). Hypoxia group was exposed to HH for one time and hypoxia preconditioning groups were exposed to HH for two (IHH 1×), three (IHH 2×), and four times (IHH 3×).

After placing the rats in the chamber, the pressure is lowered quickly (within 7 min) to a value that is equal to the air pressure at 35,000-feet altitude. After 1 min in this condition, the pressure was increased progressively to a pressure equal to the one at 30,000-feet altitude (during 3 min), to a pressure equal to that at 25,000-feet altitude (during 5 min), and then to a pressure equal to the one at 18,000-feet altitude (during 30 min). At the end of the last altitude, the conditions were made to a normal high pressure. The procedure is shown in Figure 1. The experiment was repeated for three times after the first exposure, with a 1-week interval between each experiment. At the end of each experiment, animals of the correspondent group were killed under the ether anesthesia. The heart is taken and stored immediately in a deep refrigerator (−80°C).

Enzyme-linked immunosorbent assay for HIF-1α and Mb

For quantification of HIF-1α and Mb proteins, enzyme-linked immunosorbent assay (ELISA) was used. Negative control was used in the ELISA protocol. Samples were prepared for ELISA by sandwich technique using Rat HIF-1α ELISA Kit-96T (Elabscience) for HIF-1α protein and Rat MYO ELISA Kit-96T (Elabscience) for Mb protein. The method was performed according to the manufacturer's instruction. First, mince the tissue to small pieces and rinse them in ice cold PBS (0.01 M, pH 7.4) to remove excess blood thoroughly. Then, one hundred milligram of rat heart tissues were homogenate in 1-mL PBS with a glass homogenizer on ice. To further break the cells, subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×*g* to get the supernatant. Tissue extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals (Elabscience; 2014). An ELISA reader was used to measure the optical density at 450 nm in standards, blanks, and samples. Standard curve obtained with plotting the mean optical density value for each standard on the y-axis against the concentration on the x-axis and the equation based on the principle $y = ax + b$ was applied to determine concentration of HIF-1α and Mb proteins. Standard curve for HIF-1α protein is shown in Figure 2 and for Mb protein is shown in Figure 3.

RNA isolation and quantitative real-time reverse transcription polymerase chain reaction analysis for Mb

Total RNA was prepared from the rat heart tissue samples using Total RNA Mini Kit (tissue; Geneaid). The purity of the preparation was measured by the ratio between the absorbance at 260 and 280 nm using a spectrophotometer Varioskan Flash (Thermo Scientific). Primers of Mb and 18S as housekeeping genes were

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