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Serum mRNA Levels of Interleukin-1α and Interleukin-1β Increase upon Exposure to Intermittent Hypoxia

[1]Hyun-Soo Kim and [2]Youn Wha Kim
[1]Aerospace Medicine Research Center, Aerospace Medicine Training and Research Wing, Republic of Korea Air Force, 635 Danjae-ro, Namil-myeon, Cheongwon-gun, Chungcheongbuk-do 363-849, Republic of Korea
[2]Department of Pathology, School of Medicine, Kyung Hee University, 26 Kyunghee-daero, Dongdaemun-gu, Seoul 130-701, Republic of Korea

Abstract: Intermittent Hypoxia (IH) is the most common pattern of hypoxic exposure. The pro-inflammatory cytokine Interleukin (IL)-1 is present in the tissues or systemic circulation in many inflammatory conditions. Although, some earlier studies have shown that hypoxia enhanced the production of pro-inflammatory cytokines, the effects of IH on the expression of *IL-1* gene have not been investigated yet. The aim of this study was to examine whether IH affects the mRNA expression levels of two IL-1 molecules, IL-1α and IL-1β in rat serum. To obtain IH, 11 male Sprague-Dawley rats were placed in a hypobaric chamber (282 mm Hg, 30 min day^{-1}, 14 days). The mRNA expression levels of IL-1α and IL-1β in serum were determined using quantitative real-time RT-PCR. All of the rats exposed to IH exhibited significantly higher levels of IL-1 mRNA expression than the normoxic control rats. Exposure to IH resulted in 6.0 and 6.3 fold increases in the amount of IL-1α and IL-1β mRNA when compared to that of the control group ($p<0.001$, both). Researchers demonstrated that the serum levels of IL-1α and IL-1β mRNA were significantly increased in rats following 14 days of exposure to IH. The result suggests that IH has a positive effect on transcription of the *IL-1α* and *IL-1β* genes.

Key words: Intermittent hypoxia, serum, interleukin-1α, interleukin-1β, rat

INTRODUCTION

Hypoxia is one of the most frequent stress factors encountered in both health and disease and it is often implied as the common cause of tissue and cell injury. However, recent studies have indicated that the effects of hypoxia on the body can vary depending on the duration, severity and frequency of the hypoxic exposure (Neubauer, 2001). Individuals may be exposed to acute, chronic or intermittent hypoxia during their lives. The effects of acute and chronic hypoxia have been assessed for many decades. Although, Intermittent Hypoxia (IH) is the most common pattern of hypoxic exposure, studies on this issue have just begun in recent years. IH is defined as repeated episodes of hypoxia interspersed with normoxic breathing (Neubauer, 2001). IH may be observed in physiological and pathophysiological conditions such as during severe exercise, air travels, exposure to altitude, obstructive sleep apnea and various respiratory diseases.

The Interleukin (IL)-1 family is a group of 11 cytokines which induce a complex network of pro-inflammatory cytokines and regulates and initiates inflammatory processes via an activation of endothelial cells and leukocytes (Dinarello, 2011). IL-1α and IL-1β are the most studied members of the IL-1 family because of their role in mediating inflammatory responses such as the induction of acute phase proteins, release of other pro-inflammatory cytokines, increased expression of adhesion molecules or synthesis of nitric oxide (Dinarello, 2009). At normal physiological conditions, IL-1α and IL-1β are undetectable or of low concentrations in the blood (Bendtzen, 1991). Although, some earlier studies have shown that exposure to hypoxia enhanced the production and release of pro-inflammatory cytokines including IL-6 and Tumor Necrosis Factor (TNF)-α (Ertel *et al*., 1995; Yan *et al*., 1995; Klausen *et al*., 1997; Guner *et al*., 2013), the effect of hypoxia on the expression of IL-1 remains unclear. Furthermore, the relationship between exposure to IH and the transcription of the *IL-1* gene has not yet been investigated (Lam *et al*., 2008, 2012). The aim of this study was to determine the serum

Corresponding Author: Youn Wha Kim, Department of Pathology, School of Medicine, Kyung Hee University, 26 Kyunghee-daero, Dongdaemun-gu, Seoul 130-701, Republic of Korea

mRNA levels of pro-inflammatory cytokines IL-1α and IL-1β in rats exposed to 14 days of IH in comparison with normoxic rats.

MATERIALS AND METHODS

Fourteen male Sprague-Dawley rats weighing between 200 and 230 g were used. The animals were randomly divided into IH and normoxic control groups. To obtain IH, 11 rats were placed in an altitude chamber at a barometric pressure of 282 mm Hg, corresponding to an altitude of 7,620 m. The temperature and moisture of the chamber were maintained at 20-24°C and 45%, respectively with 12 h light and dark cycles. The rats of the IH group were kept at this barometric pressure 30 min day^{-1} for 14 days. The remaining three rats of the control group stayed in the same environment as those of the IH group with access to food and water *ad libitum* with the exception of breathing normal room air. The chamber was opened daily to clean the cages and replenish food and water. At the end of exposure to IH both groups of animals were anesthetized by diethyl ether. The 10 mL blood sample was collected from each animal using the intracardiac puncture and immediately carried into the laboratory to minimize the degradation of RNA. For measurement of the mRNA level, 5 mL portions from the samples were immediately centrifuged at 3000 rpm for 15 min followed by careful aliquoting. RNase inhibitor was promptly added to the segregated serum. The serum samples were then stored at -70°C until the Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) was performed.

Quantitative real-time RT-PCR was performed on the Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) with the C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The total serum RNA was isolated using the NucleoSpin RNA II extraction kit (Macherey-Nagel GmbH and Co., KG, Dueren, Germany) according to the manufacturer's instructions. cDNA synthesis was performed with the ReverTra Ace-α-reverse transcriptase kit (Toyobo Co., Ltd. Osaka, Japan) also according to the manufacturer's instructions. The amount of standard cDNA was determined photometrically. The reverse-transcribed cDNA was used for the real-time RT-PCR reaction using SsoAdvanced SYBR Green Supermix (Bio-Rad Laboratories, Inc.). The primer sequence used for IL-1α was as follows: forward 5'-GTCTTCTTACAT CATTCAACTCAC-3'; reverse 5'-GGAACCCAGAGGAAA CAC-3'. The primer sequence used for IL-1β was as follows: forward 5'-GGATGATGACGACCTGCTA-3'; reverse 5'-ACTTGTTGGCTTATGTTCTGT-3'. The primer sequence used for β-actin was as follows: forward 5'-ATCTTCCGCCTTAATACTTCATT-3'; reverse 5'-ACC AAAGCCTTCATACATCAA-3'. The PCR reactions for IL-1α, IL-1β and α-actin were initiated with a denaturing step at 95°C for 3 min followed by 40 cycles at 95°C for 10 sec, 58°C for 10 sec and 72°C for 20 sec. A melting curve, ramping from 65-95°C was performed following each RT-PCR to test for the presence of primer dimers. When primer dimer formation was detected, the PCR was ran again using a separate aliquot of cDNA. Each measurement was repeated three times and the values were used to calculate the ratios of IL-1α/β-actin and IL-1β/β-actin with a value of 1.0 used as the control (calibrator).

Measurements are presented as mean±standard error. Statistical significance of the differences between IH and control groups was determined by the non-parametric Mann-Whitney U-test. Data analyses were performed using SPSS (Version 15.0, SPSS Inc., Chicago, USA) and significance was set at $p<0.05$ versus the control group.

RESULTS AND DISCUSSION

To study the effects of exposure to IH on the expression of the *IL-1* gene in the serum of rats, mRNA expression of IL-1α and IL-1β was analyzed by quantitative real-time RT-PCR. Exposure to IH induced an upregulation of gene transcription of IL-1α and IL-1β. There was a marked increase in the expression levels of IL-1α and IL-1β mRNA in the serum of rats exposed to IH which reached statistical significance. Exposure to IH resulted in 6.0 and 6.3 fold increases in the amount of IL-1α and IL-1β mRNA when compared to that of the control group ($p<0.001$, both) (Fig. 1).

Researchers observed that the serum mRNA levels of pro-inflammatory cytokines IL-1α and IL-1β were significantly elevated in rats exposed to 14 days of IH. It has been shown that hypoxic exposure is one of the factors that generate various mediators of inflammation. The result is consistent with the finding of a earlier study demonstrating that exposure to IH for 5 weeks significantly elevated the levels of pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α, in rat serum (Guner *et al*., 2013). Similarly, Lam *et al*. (2012) showed that IH induced a functional upregulation of the pro-inflammatory cytokines and their receptors both at mRNA and protein levels in the rat carotid body. Del Rio *et al*. (2010, 2011) also reported that IH increased the expression of IL-1β, IL-6 and TNF-α as well as endothelin-1, nitric oxide synthases and reactive oxygen species within the rat carotid body. Moreover, Leeper-Woodford and Detmer (1999) observed that

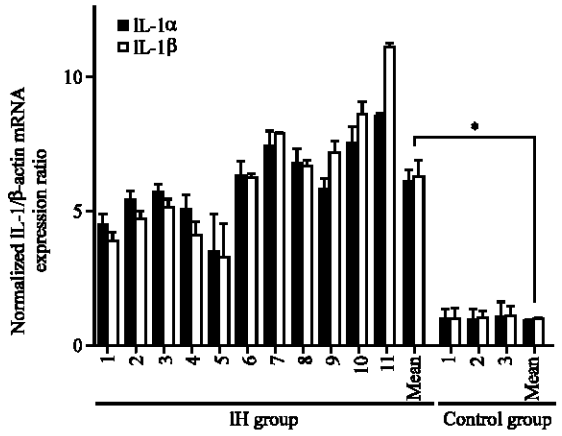

Fig. 1: Effect of exposure to IH on IL-1 mRNA expression in the serum of rats was examined using quantitative real-time RT-PCR analysis. Results are mean values of three independent experiments with each animal. All of the 11 rats exposed to IH showed significantly higher levels of IL-1 mRNA expression than the normoxic rats. Exposure to IH resulted in 6.0 and 6.3 fold increases in the amount of IL-1α and IL-1β mRNA when compared to that of the control group ($p<0.001$, both). *$p<0.001$ in comparison to the corresponding values of the control

pro-inflammatory cytokines were increased in rat lung macrophages exposed to hypoxia *in vitro*. Weinberger *et al.* (2001) found that immunohistochemical expression of the pro-inflammatory cytokines was increased in lung macrophages following 2 weeks of exposure to hypoxia. The result supports the earlier data showing the effects of hypoxia on the production of IL-1β. In addition, to the best of the knowledge, researchers first demonstrated a significant increase in the IL-1α mRNA level following IH in rat serum.

By contrast, other studies have failed to show a consistent effect of IH on the markers of inflammation. Tam *et al.* (2007) found a significant increase in IL-6 and TNF-α following a 90 min hypoxic exposure however, a subsequent exposure did not produce any further increase in IL-6 and TNF-α. Klausen *et al.* (1997) showed increased serum levels of IL-6 without changes in those of other cytokines in human subjects exposed to 4 days of hypoxia. Furthermore, in another study using the human model of IH, Querido *et al.* (2012) observed no significant change in any of the pro-inflammatory cytokines. The following reasons may underlie such a discrepancy: Differences in rat species and hypoxic chamber, differences in pattern of hypoxic exposure including the inspired oxygen level, duration and frequency, differences in the primers used and differences in the temperature protocol and PCR process with varying degrees of sensitivity.

Pro-inflammatory cytokines are produced by mononuclear phagocytes as well as neutrophils and endothelial cells. They possess metabolic, physiological and hematopoietic activities and play a role in the regulation of the immune responses (Bankers-Fulbright *et al.*, 1996). The findings that the serum levels of IL-1α and IL-1β are elevated following IH exposure is significant because these cytokines perpetuate the inflammatory response and are thought to mediate many of the disturbances which are characteristic of sepsis.

The limitations of the data presented herein should be acknowledged. First, the number of animals in this study was small, with 11 IH-exposed rats in the data set. Second, the upregulation of IL-1 should be confirmed on serum protein level. Third, researchers do not as yet understand which cellular pathways are responsible for the process of *IL-1* gene regulation of the serum in response to IH exposure. Investigation of the time course of altered expression may provide clues to the mechanisms that are responsible for the effects of IH on *IL-1* gene regulation. Additionally, to determine the primary sources for IL-1, subsequent studies need to focus on additional molecular methods such as enzymatic dissociation and density gradient centrifugation for separating leukocytes from other cell types.

CONCLUSION

Researchers demonstrated that exposure to IH for 14 days significantly elevated the serum mRNA levels of IL-1α and IL-1β when compared with those of normoxic rats. This result suggests that IH has a positive effect on the transcription of *IL-1* genes in the serum of rats. Further, investigations are necessary to explain the mechanism by which IH causes up-regulation of serum IL-1.

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Researchers declare that they have no competing interests. The views and opinions expressed in this study are those of the researchers and do not reflect the official policy or position of the Republic of Korea Air Force or Republic of Korea Ministry of National Defense.

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