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# Experimental Brain Injury Induced by Acute Hypobaric Hypoxia Stimulates Changes in mRNA Expression and Stress Marker Status

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## ABSTRACT

The present study was proposed to investigate the brain injury under acute hypobaric hypoxia following alteration in mRNA expression and stress markers in a time-dependent manner. SD clean graded male rats were randomly divided into four groups for this experimental brain injury, the control group at Xining (altitude, 2270m) and hypoxia treatment groups with different time exposure day1, day2, and day3 at (altitude, 7000m) in a hypobaric chamber. After day3 exposure, the brain tissues were collected. The level of mRNA expression of VEGF and HIF1-α was assessed using qRT-PCR. The oxidative stress level of superoxide dismutase (SOD) and malondialdehyde (MDA) were determined with commercial kits. AHH with time duration significantly increased the MDA level and decreased in the activity of SOD was seen in all hypoxia treated groups as compared to the control ($P< 0.001$). The mRNA expression level of HIF1-α and VEGF in day1, day2, and day3 AHH groups was markedly raised when it is compared to control ($P< 0.05$). Ultimately, in conclusion, such results indicate that AHH stimulates oxidative stress induces brain damage in rats.

***Keywords***: Acute hypobaric hypoxia, Brain injury, HIF-1α, Oxidative stress

## INTRODUCTION

Hypobaric hypoxia (HH) is the prime attribute of the high-altitude environment. Whereas the barometric pressure decreases with an ascent along with the partial pressure of oxygen (PO2) and 21% atmospheric oxygen is available at every altitude [1]. Multiple fatal effects of acute hypoxia at high-altitude were observed [2]. At 1800m, it was considered to affect the molecular physiology of humans, and such effects exacerbate after ascending to an altitude of 2400m which leads to certain deleterious disorders, which can be easily seen in sojourners. Whereas, the tolerance to the human body at an altitude above 5000m is completely unbearable for certain individuals to a certain extent of duration [3]. More than 140 million inhabitants live above 2500m, wherein 40 million of the population project to high-altitude regions as a traveler or employee all around the year [4].

Previous data reported that high altitude hypobaric hypoxia has several harmful stress-related effects on cellular functions where resulted in the extensive generation of ROS damage low-landers and mountaineers often experience multiple organ damage following different exposure

of time at high-altitude. In the last few years, several studies proposed alteration in the organ damage at the molecular level induced by acute hypobaric hypoxia (AHH) [5-7]. Previous studies also revealed that AHH can stimulate a range of pathological response, including biological, cellular, and genomic variations, the majority of which has centered on a definite tissue, such as the brain, heart, or lung. HIF-1α is one of the majority critical signaling molecules which arbitrate the reactions of mammalian cells to acute hypoxia by stimulating the expression of adaptive gene products, such as VEGF. Several studies were carried to know the extent of hypobaric hypoxia and its role in the regulation of angiogenic factors like VEGF and production of hypoxia-inducible factors. A recent study, wherein healthy male mice were exposed to an elevation of 7000m in a hypobaric chamber for one week divulged that acute exposure to hypoxia leads to an increase in master signaling molecule to hypoxia adaptation, HIF-1α, which in response prolonged the expression level of VEGF in the late stage of acute mountain sickness. That ultimately induced blood-brain barrier dysfunction, raised vascular permeability, and finally head to HACE and HAPE [8]. Oxidative stress-induced by acute hypobaric hypoxia leads to an imbalance between the production of ROS and the ability of an endogenous antioxidant system to protect from these reactive oxygen species.

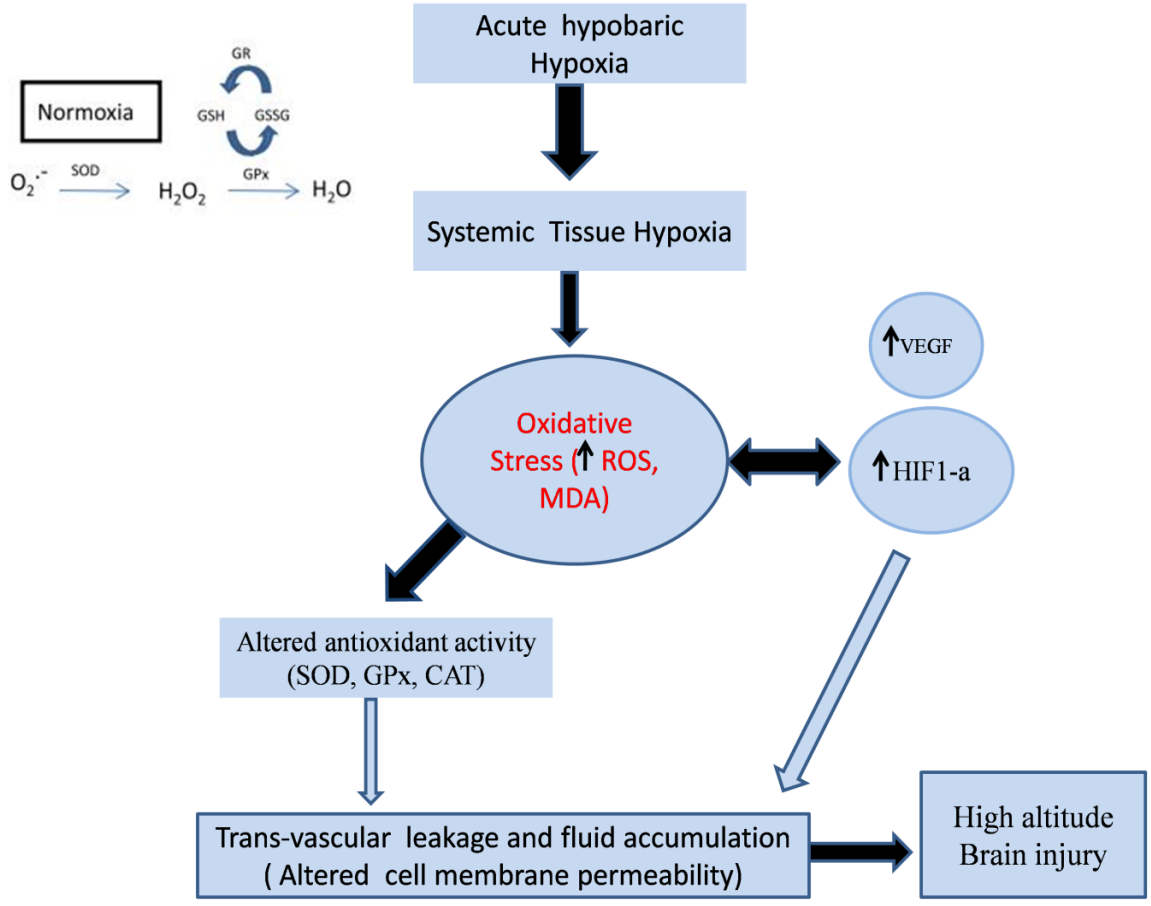


**Fig 1. Schematic targeted mechanism of action involved in acute hypobaric hypoxia induced brain injury. SOD, superoxide dismutase; MDA, malondialdehyde; ROS, reactive oxygen species; GPx, glutathione peroxidase; CAT, catalase; VEGF, vascular endothelial growth factor; HIF1-α, hypoxia-inducible factor 1 alpha; GR, glutathione reductase; GSH, glutathione; GSSG, glutathione disulphide.**

Following the concept mentioned above, the point of this study is to generally evaluate the oxidative stress (SOD, MDA), HIF-1α, and VEGF of rat exposure to the

AHH with different exposure of time at 7000m. Such a study should present a motivation for the investigation of the molecular mechanisms and targeted treatments for certain acute mountain illnesses.

## MATERIAL AND METHODS

### Animals

Experimental clean grade SD male rats weighing 200-210g were obtained from the Beijing Vital River Co., Ltd., Beijing. Rats were housed in the experimental center of High Altitude Medical Application Research Center in a pathogen-free animal facility for 12 days prior to the experiment under strict laboratory conditions.

### Experimental Design

All 20 SD rats were randomly divided into 4 groups as follows: (1) control group at Xining (altitude, 2270m) and AHH groups (day1 group; day2 group and day3 group) of different time exposure simulated at 7000m above sea level. Rats were kept under living conditions and provided with complete access to ad libitum and water.

### Ethical Statement

Guidelines for animal care were followed and all the technical procedures had been carried out following the Health Guide for Laboratory Animals [9] and the experimental protocol was ethically approved and performed accordingly to the Animal administration and Animal Ethics Committee of Qinghai Medical College (Xining, China).

### Instruments and Reagents

The hypobaric chamber (model no: HCP-III) was bought from (Fukang Air Purification Equipment Engineering Co., Ltd., Shaanxi) (diameter of 1.8m, length of 1.4m), which was simulated at 7000m (atmospheric barometric pressure of 310 mmHg, PO2 of 65-67 mmHg). Total RNA was extracted from the brain tissues using the TaKaRa Universal RNA Extraction Kit according to the manufacturer's instructions. Specific primers were used for qRT-PCR: HIF-1α (F: 5′-CCAGATTCAAGATCAGCCAGCA-3′; R: 3′-GCTGTCCACATCAAAGCAGTACTCA-5′); VEGF (F: 5′-TCCTGCAGCATAGCAGATGTGA-3′; R: 3′-CCAGGATTTAAACCGGGATTTC-5′); and β-actin (F: 5′-CCTAAGGCCAAGTGAAAA-3′; R: 3′-CAGAGGCATACAGGGACAACAC-5′). Change in the expression level of mRNA for target marker was performed with housekeeping β-actin using a fold change by 2-ΔΔct method. Protein content was determined using BCA Assay Kit (Cat no: 23225) obtained from (Thermo Fisher Scientific). Antioxidants (SOD) and lipid peroxidation (MDA) biochemical assay kits were purchased from Nanjing Jiancheng Bioengineering CO.Ltd., (Nanjing, China).

## RESEARCH METHOD

Following the designed experiment 3% trichloroacetaldehyde hydrate was used to anesthetize the rats and the brain tissue was collected using coronal brain matrix following dissection; the mid coronal section was used to perform the morphology for tissue. The remaining brain tissue was homogenized and the content for MDA and level of SOD activity were measured using the ELISA technique. The change in the expression level of VEGF and HIF-1α was performed by qRT-PCR.

### Statistical Analysis

The statistical work was conducted using SPSS 20.0 software. Data were presented as the means ± SD or comparisons multiple groups was made by one-way ANOVA or comparison 2 groups were performed using the least significant difference (LSD) test was performed. A value of $P<0.05$ was considered to indicate a statistically significant difference.

## RESULTS

Expression of HIF1-α and VEGF was detected by qRT-PCR. As shown in

Table. 1, the expression of HIF1-α in day1, day2, and day3 hypoxia treatment group significantly raised compared to the control group (P< 0.05). AHH affect the level of VEGF in brain tissues, which was significantly increased by day2 and day3 hypoxia groups when compared with the control group (P< 0.05). While there is no significant changes in VEGF were observed by the day1 hypoxia treated group in comparison to the control group.

**Table 1. Estimation of the mRNA expression level of HIF1-α and VEGF**

| Group | HIF1-α | VEGF |
|---|---|---|
| Control | 1.20±0.90 | 1.00±0.24 |
| Day1 | 2.40±0.93* | 1.24±0.40 |
| Day2 | 2.22±0.60* | 1.83±1.00* |
| Day3 | 2.50±0.40* | 2.00±0.70* |
| F | 3.41 | 2.94 |
| P | 0.043 | 0.065 |

*Effect of mRNA expression like Hypoxia-inducible factor 1 alpha (HIF1-α) and vascular endothelial growth factor (VEGF) level in brain tissue of rat simulated to AHH 7000m for 1, 2 and 3 days. Data is presented as means ± SD (n=5). * P<0.05 vs control and hypoxia group 1, 2 and 3 days.*

Oxidative stress-induced under AHH plays an extensive role in the pathogenetic factor and its involvement in the mechanism of brain injury. Table 2. This shows that by day2 and day3 AHH group notably increased the MDA content in the brain compared to the control (P< 0.001). In addition, the day3 group strikingly increased the MDA content (P< 0.001) in the brain compared to the day1 AHH group. The activity of SOD was markedly decreased in day1, day2, and day3 AHH group compared with the control group (P< 0.001). While the SOD activity of the brain within all the AHH groups was also significantly downregulated (P< 0.001).

**Table 2. Estimation of oxidative stress activity in brain tissues**

| Group | MDA (nmol/mgprot) | SOD (u/mgprot) |
|---|---|---|
| Control | 12.42±3.01 | 419±22.42 |
| Day1 | 15.00±4.74 | 379±31.33* |
| Day2 | 21.20±2.90* | 287±14.04*# |
| Day3 | 23.40±0.91*# | 131±30.00*#a |
| F | 12.16 | 121.91 |
| P | <0.001 | <0.001 |

*Effect of lipid peroxidation marker like malondialdehyde (MDA) content and antioxidant enzyme like superoxide dismutase (SOD) activity in brain tissue of rat simulated to AHH 7000m for 1, 2 and 3 days. Data is presented as means ± SD. * P<0.001 vs control and hypoxia group 1, 2 and 3 days. # P<0.001 vs hypoxia group day1 and 2 & 3days group. a P<0.001 vs day 2 and day 3.*

## DISCUSSION

Brain counts for one of the most sensitive organs and it is highly susceptible to high-altitude brain injury. Multiple studies reported that AHH can cause serious damage to the brain which can ultimately lead to certain high-altitude illnesses including cerebral edema [10]. The cerebral structure is highly vulnerable following the hypoxic stress where this sensitive organ has less survival viability to oxidative damage. Herein, this stress leads to the production of ROS and providently weakens the antioxidant defense system. Hence, evidence reported that HACE under oxidative insult causes destruction of BBB structural formation and finally leads to cellular swelling [11]. HIF-1α is abundantly known as the master regulator in response to any hypoxic changes that stimulate cellular adaptation in the brain. It also plays an important role in regulating certain genes such as VEGF which involves cell survival under hypoxia conditions. The up-regulation of VEGF confirms that the increase in the BBB injury occurs with hypobaric hypoxia-induced by angiogenesis [8].

In the present study, we examined the VEGF and HIF-1α expression by qRT-PCR that induced by AHH in the brain, showed that the cerebral tissue opposed the AHH brain injury by up-regulating the hypoxia-inducible factor and angiogenesis by increasing the consumption of oxygen and certain nutrients in response to compensate the hypoxia mechanism. Herein, VEGF is a well-known mediator engaged in BBB permeability, vascular leakage, and angiogenesis induced by AHH [5, 11].

Brain damage in the experimental rat model of AHH can develop due to certain physiological stress which leads to the excessive generation of ROS production and lipid peroxidation. Such oxidative damage weakens the antioxidant defense system at the cellular level in the brain [12]. This study results reported that a gradual decrease in the SOD activity resulted in the incapability of cerebral tissue to scavenge

hydroxyl radical, superoxide anions, and other chain reaction lipid peroxide end products. AHH can notably reduce the antioxidant activity, thereby increasing ROS generation and lipid peroxidation, which ultimately leads to brain damage.

## CONCLUSION

In conclusion, the study revealed that hypoxia caused by AHH induces the cerebral vulnerability of oxidative damage and elevates the expression level of HIF-1α and VEGF which consequently leads to extensive brain tissue damage.

## Declaration of competing interest:

The authors proclaim no competing of interest.

## ACKNOWLEDGMENT

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