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ORIGINAL ARTICLE

Significance of Oxidative Stress Level Changing in Rat Cerebral Tissue following Hypobaric Hypoxia Exposure to Extreme/High Altitude and Different Exposure of Times

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ABSTRACT

Aim: To elucidate brain damage under hypobaric hypoxia and changes in oxidative stress with ac. exposure to hypoxia in rats.
Study Design: Experimental study.
Place and duration of study: Department of Basic Medicine, Medical College of Qinghai University, Qinghai Xining from 1st October 2020 to 15th March 2021.
Methodology: Twenty four Sprague Dawley rats were randomly divided into 4 groups; control, 24hr, 48hr, and 72hr. Hypoxia was simulated at the altitude of 7000m in an automated hypobaric hypoxia simulator. In the control and hypoxia treatment groups, the blood sample and brain tissue samples were collected. For the biochemical estimation, activities of CAT, GPx, SOD and content of MDA were evaluated.
Results: A significant reduces in the activities of catalase, glutathione peroxidase and superoxide dismutase was observed when it is compared to that of the control group. Furthermore, the hypoxia treatment group had a higher content of MDA compared to that of the control group.
Conclusion: Acute hypobaric hypoxia promotes oxidative stress which can cause serious damage to the brain tissues.
Keywords: Hypobaric hypoxia, Oxidative stress, Brain injury, Peroxidation, Rats

INTRODUCTION

Population living at high altitudes may experience hypobaric hypoxia conditions which lead to the damage of the brain, heart, and multiple other organs.[1-4] Number of low landers were increasing day by day who travelled to high altitude zones for tourism and recreational purposes become a trend throughout the year. Unexpectedly, itinerants travelling from low altitudes to the high-altitude areas are more vulnerable to the development of mountain sickness encountered hypobaric hypoxia environment at high altitude may develop deleterious effects including high altitude cerebral oedema (HACE), acute mountain sickness (AMS), and high altitude pulmonary oedema (HAPE)[5-7]. These conditions become fatal at an extreme altitude of 7000m or above in mountaineers during acclimatization[8].

Following the above-mentioned conditions, it was considered that an exposure to such extreme altitude can trigger the increase of oxidative stress at cellular level with sequential damage to lipids, and proteins[9-11]. Oxidative stress-induced under hypobaric hypoxia is sensitive to the brain because of rich in poly-unsaturated fatty acids (PUFA) and a weak antioxidant defence system[12]. Thus, to determine the oxidative insults certain biological markers have been investigated which are considered as the key oxidative parameters including SOD, GPx, CAT and MDA[12,13]. Therefore, the present study model was established at an altitude of 7000m in the hypobaric cabinet to evaluate the oxidative damage to the brain at different exposure times 24, 48 and 72 hrs respectively.

The purpose of this study is to deliver the basic foundation in the development of therapeutic drugs and preventions under acute hypoxic brain damage.

MATERIALS AND METHODS

Adult clean grade Sprague Dawley male rats weighing between 200-220g were provided from the Beijing Vital River Laboratory Animal Technology Co. Ltd. Pathogen-free animal were placed in highly facilitated animal care center for 12 days prior to the experiment at the Medical College of Qinghai University, China under strict laboratory conditions.

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All SD rats were randomly divided into 4 groups with 6 rats in each group: 24-hour, 48-hour, and 72-hour hypobaric hypoxia group simulated at 7000m above sea level in a hypobaric chamber and a control group at Xining. The experiment was repeated twice. Animals were housed under proper life conditions with complete access to the diet apart from different exposure of time at the same altitude. The rats were placed in hypobaric cabinet at 7000m as mentioned in the previous studies[14].

Animal care guidelines were applied and timely experimental procedures had been performed following to Laboratory Animals guide[15] and the ethically approval was obtained from Animal Ethics Committee of Qinghai University (Xining, China).

An automatic hypobaric cabinet (model no: HCP-III) was obtained from Fukang Air Purification Equipment Engineering Co. Ltd. Shaanxi with following dimensions diameter of 1.8m, length of 1.4m), which was simulated at an altitude of 7000m (atmospheric barometric pressure of 310mmHg, PO_2 of 65-67mmHg). Hematological analysis was carried out using BC-5000vet model blood analyzer (Mindray, Shenzhen, China), automated microtome (Leica RM 2255) for paraffin sectioning was purchased from (Leica Microsystems, Wetzlar, Germany). BCA Assay Kit (Cat no: 23225) obtained from Thermo Fisher Scientific to evaluate the concentration of protein contents. Assay kits for Antioxidants (SOD, GPx, CAT) and lipid peroxidation (MDA) were bought from Nanjing Jiancheng Bioengineering Co. Ltd. Nanjing, China.

Following to the designed experiment for different duration of exposure, 5~7ml of blood were collected using EDTAK2 from rats. An automatic hematology vet analyzer was used to determine the hemoglobin (HGB) levels and hematocrit (HCT) count. Rats were anesthetize through 3% trichloroacetaldehyde and coronal matrix was used to performed the dissection. Herein, middle section was collected to perform the histopathology for tissues. Residual sections of brain were properly homogenized to evaluate the certain extents of SOD, CAT, GPx and MDA via ELISA reader.

The statistical data was analyzed by SPSS-20.0. Comparisons multiple groups were done through one-way ANOVA, comparison 2 groups were performed using the LSD test. P value <0.05 was considered to be significant.

RESULTS

The present study indicated that the hypoxia treated group showed a decrease in the SOD, GPx and CAT activity following to the exposure of hypoxia in a time-dependent manner, and the MDA content is significantly raised in 48hr and 72hr groups when compared to that of a control group. Following within the hypoxia treatment group the results for the 48hr and 72hr groups also exhibited significantly reduction in the activity of GPx and SOD when compared to that of the 24 hr group. MDA content is raised within the brain tissue of hypoxia treated 48hr and 72hr when compared to that of the 24hr group as shown in (Tables 1-2). The results for the hematological markers indicates that the HGB level is raised in the 48 hr and 72 hr group, while the HCT count is also raised in 48hr and 72hr group when it is compared to that of the control group. Whereas their level is significantly higher within the hypoxia treated 72hr group when it is compared to that of the 24hr group as shown in (Table 3).

Results for H&E sections of the CA1 region shows disorganization in the arrangement of neuron cells with extensive swelling and broaden in the pericellular spaces along with highly dark stained shrunken pyknotic nuclei is visible in all the hypoxia treated group on micro-level (Fig 1).

Table 1: Changes in MDA content and SOD level in brain tissue under hypobaric condition

Group	MDA (nmol/mgprot)	SOD (U/mgprot)
Control	11.80±2.01	429.71±20.71
24hr	12.49±3.99	398.73±10.36[a]
48hr	19.43±3.83[ab]	307.56±29.58[ab]
72hr	22.75±1.70[ab]	132.25±26.94[abc]
F value	18.30	200.62
P value	<0.001	<0.001

[a-c]Compare with the Control, 24hr, 48hr and 72hr group respectively. Means with certain superscripts are presented. *P*<0.001 compared with two groups

Table 2: Changes in GPx and CAT activity in brain tissue under hypobaric condition

Group	CAT(U/mgprot)	GPx (U/mgprot)
Control	9.63±2.63	10.36±1.52
24hr	5.43±0.58[a]	9.08±1.08[a]
48hr	5.19±3.16[a]	7.75±0.75[ab]
72hr	4.91±0.46[a]	6.55±0.82[ab]
F value	11.213	10.752
P value	0.011	0.001

[a-b]Compare with the Control, 24hr, 48hr and 72hr group respectively. Means with certain superscripts are presented. *P*<0.01 compared with two groups

Table 3: Changes in hematological parameters under hypobaric condition

Group	HGB(g/l)	HCT(%)
Control	111.2±12.49	39.2±4.47
24hr	121.0±21.67	43.2±7.60
48hr	132.7±18.32[a]	47.7±6.86[a]
72hr	151.2±9.49[ab]	53.2±2.91[ab]
F value	6.75	6.55
P value	0.003	0.003

[a-b]Compare with the Control, 48hr and 72hr group respectively. Means with certain superscripts are presented. *P*<0.01 compared with two groups.

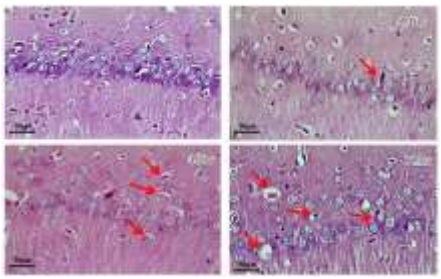

Fig 1: Histopathological changes of CA1 region of neuron cells is presented using H&E staining. Original magnification, 40X. C: control, 24hr: 24 hour hypoxia, 48hr: 48 hour hypoxia, 72hr: 72 hour hypoxia. Arrow head: presents the dark stained pyknotic nuclei with shrunken neurons, pericellular space enlargement and, extensive neuronal disarrangement.

DISCUSSION

The brain, a multifaceted and the most sensitive organ that can be highly affected by a number of stressful condition. Number of studies has reported that the high-altitude is most susceptible to brain damage above 2500m[16]. Here, the concentration of hemoglobin always takes into count whenever the human adaptive response to the hypoxia conditions is discussed at a high altitude[17]. Therefore, elevation at 4500m in Tibetan people can cause high-altitude polycythemia[18]. However, hypoxia under a hypobaric situation can lead to certain deleterious physiological stress which induces oxidative stress at high altitudes[13]. It is reported that ROS involved in an extensive role of hypoxia situations and damages to the mitochondrial DNA and proteins in the brain that can lead to cell death.[19] In nutshell, oxidative insult is developed when ROS production overcomes the antioxidant capacity[20, 21].

Herein, the results of hematological parameters showed that hypobaric hypoxia was successfully established at 7000 m in the hypobaric chamber.22 The histopathological change indicates that the brain tissues are more susceptible to hypoxia-related injury and the extent of brain damage depends on the exposure time[23,24]. A decrease in the antioxidant activities of SOD, GPx and CAT in the following study showed progressive inadequacy of brain tissues at molecular level to remove OH-group radicals, superoxide anions and several lipid peroxide final products[25]. Moreover, this model of hypoxic brain-injury would be useful for developing novel drug discovery for high-altitude illnesses.

CONCLUSION

Hypoxia at extreme altitude induces oxidative stress which suppressed the antioxidant defence system and also the degree of oxidative damage to the brain tissues is time-dependent.

Conflict of interest: Nil

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