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Original article

Hypoxia-induced brain cell damage in male albino wistar rat

Xiaoli Niu, Siyuan Li, Simin Zheng, Hongfei Xiong, Junlin Lv, Huijuan Zhang, Hongtao Liu *

Department of Anesthesiology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi Province 710004, China

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ABSTRACT

The biochemical markers of rat under low oxygen concentration, including brain water level, lactic acid, necrosis and Na+-K+-ATPase, was detected to analyze the hypoxia-induced brain damage, and to analyze the mechanism of brain injury. Histopathological alteration in brain tissue induced by hypoxia were investigated through hematoxylin and eosin stain (HE). Hypoxia induced factor-1a (HIF-1a) expression level in the brain was carried out using immunohistochemistry. Lactic acid level was positively correlated with the level of hypoxia, while concentration-dependent decrease in total Na+-K+-ATPase activity was noted. Hypoxia induced rathad a significant difference on brain water content compared to controls. The level of necrosis and lactic acid level was increased, and the decrease of Na+-K+-ATPase activity was observed. Histopathological examination of brain confirmed that there was neuronal cell death in hippocampal region. HIF-1a expression increased the hypoxia adaptation capability of the rat through the expressions of genes. Lactic acid, Na+-K+-ATPase and HIF-1a plays an important role in brain injury.

1. Introduction

Hypoxia is considered as a vital pathological process in several diseases, and alters body functions (Kanstrup et al., 1999; Chen, 2001). Under the condition of airtight atmosphere, due to metabolic and impairment of gas exchange between the organization and environment, quality of the air in the cabin gets worse gradually, and concentration of oxygen decrease rapidly and concentration of carbon dioxide increase rapidly. Hypoxia environment occurs immediately after long time. Hypoxia may lead to the functional impairment, disturbance of consciousness, reaction dullness, retardation and damage of learning-memory.

Severe hypoxia may cause pathological damage. There are several studies on hypoxia concentrated on hypoxic-ischemic encephalopathy (HIE) (Xia, 2005; Christiane and Brahimi, 2007), plateau hypoxia (Aramjit and Manoj, 2007; Fau et al., 2007), learning-memory (Liu et al., 2009), and various diseases induced by hypoxia and mechanisms (Funk et al., 2008; Peter, 2008). The topic hypoxia-induced brain damage is a hot research area of brain research field. Energy exhaustion, overexpression of excitatory amino acids, oxygen free radical damage, apoptosis and inflammation may lead to the brain damage. The brain is easily susceptible to oxidative stress due to the high level of polyunsaturated fatty acids, increased oxygen consumption, high concentrations of iron, and low antioxidant capacity. These factors may contribute the premature infant, apoplexy patients to brain damage.

There are few mechanisms of hypoxia-induced brain damage have been elucidated partially. Analyses of changes in energy metabolites and brain damage during hypoxia, and brain hypoxic preconditioning may lead to the finding of a way to protect the brain from hypoxia injury. The present study was aimed to investigate the biochemical effects of hypoxia on brain damage of male albino rats in the airtight cabin. Brain water level, necrosis, lactic acid level and Na+-K+-ATPase activity were measured. HIF-1a (hypoxia induced factor-1a) expression was carried out using immunohistochemistry. Histopathological alteration in the rat brain was investigated with use of hematoxylin and eosin stain (HE).

2. Materials and methods

2.1. Animals

Male wistar rats weighing 160–180 g were purchased from Institute of Laboratory Animal Science, Chinese Academy of

* Corresponding author at: Department of Anesthesiology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi Province 710004, China.
E-mail address: hongtaoliu4@hotmail.com (H. Liu).
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Medical Science. Rats were allowed to acclimatize for at least 15 days prior to experiment. Rats were housed at a room temperature of 22 ± 2 °C and a relative humidity of 50 ± 10% with controlled light. Food and water were provided ad libitum. All rats received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, Beijing.

2.2. Hypoxia equipment

Rats were placed in a custom-made 16-liter plastic hypoxia chamber. Fresh soda lime was placed on the bottom of chamber. O_2 and N_2 cylinders were connected with the chamber. The level of O_2 was controlled by infusing N_2 at flow rate of 8.0 L/min. The level of O_2 and CO_2 were observed continuously respectively (Rice et al., 1981). 21%, 18%, 15%, 12%, 10%, 8% O_2 were designed, and used in the experiment respectively. Control group exposed to normoxia (24% O_2) without food and water.

2.3. Water content of rat brain

After exposed to hypoxia for 3 h, rats were anesthetized with diethyl ether, and then killed by cervical dislocation. The brain tissue was removed surgically and weighted. Water content of brain tissue determined by lyophilization was calculated as a measure of hypoxia-induced brain damage, i.e.% water content = 100 × ((wet brain weight − dry brain weight)/wet brain weight)%.

2.4. Determination of necrosis

Following exposure to hypoxia for 3 h, rats were anesthetized with diethyl ether, and then killed by cervical dislocation. The brain tissue was removed surgically and sectioned into five slices (2 mm thick), and then those slices were placed in 3% 2,3,5-triphenyltetrazolium chloride at 37 °C for 30 min. Slices were dried on filter paper, and then weighted respectively. Total damage area (grey section) was removed, and weighted. The relative damage percentage was measured by calculating the brain damage area percentage by total slice (100 × total damage section/total slice).

2.5. Measurement of lactic acid and Na+-K+-ATPase

Lactic acid level and Na+-K+-ATPase activity were measured with kits according to the manufacturer's instruction. Following exposure to hypoxia for 3 h, rats were anesthetized with diethyl ether, killed by cervical dislocation. The brain tissue was removed for biochemical analyses. Removed brain tissue was collected in 0.1 M phosphate buffer (pH 7.4) and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min. The supernatant was used for analysis of lactic acid and Na+-K+-ATPase activity respectively. Lactic acid and Na+-K+-ATPase activity were measured according to the description of the kits. These values were evaluated by measuring at 530 nm, 636 nm with a UV spectrophotometer respectively.

2.6. Histopathological examination

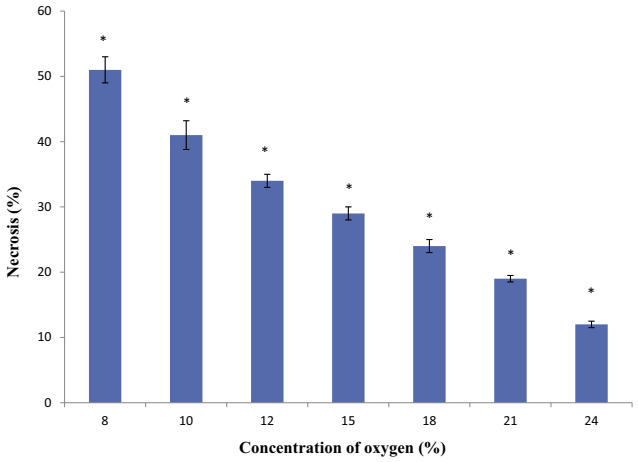
Rats were exposed to 6% O_2 for 3 h respectively, and sacrificed by decapitation. Brains were taken out and transferred to 4% paraformaldehyde. Hippocampus section was prepared (5 μm thick), and stained by hematoxylin and eosin. Stained sections were analyzed qualitatively by light microscope.

2.7. Immuno histochemistry

Following exposure to 8% O_2 for 3 h, the rats were anesthetized with diethyl ether, and perfused through the ascending aorta with 200 ml of 1% NaCl solution, followed with 200 ml of 4% paraformaldehyde. The brain tissue was removed, and kept in the 4% paraformaldehyde solution until slicing. The brains sections were dehydrated in 10% sucrose for 1 day, and then 30% sucrose solution for 2 days, till the brain sections sank to the bottom of the sample bottle. Hippocampus section were cut at 30 μm thickness on a freezing microtome, and processed for immunohistochemistry analyses. The sections were rinsed in PBS-T for three times. Then 3 ml of 1% $H_2O_2$was added at room temperature for 30 min. The slices were rinsed, and then added with 2 ml of 5% BSA solution for 20 min. 1:300 dilution of rabbit anti-HIF-1a antibody was added, and weaved in the refrigerator for 24 h. Biotin labeled monoclone mice anti-rabbit antibody was added. The slices were soaked in the SABC solution for 30 min. Then DAB was applied to stain for 20 min. The brain sections were then dehydrated in ascending alcohol concentrations and covered in xylene.

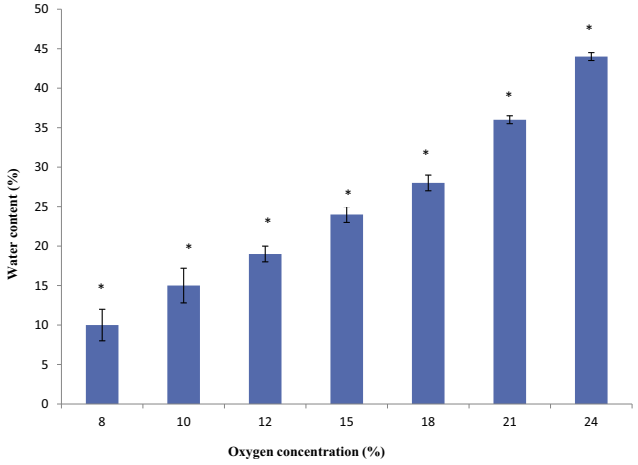
2.8. Statistical analysis

All the values were expressed as mean ± SEM. Statistical analysis of data was performed by "Student t" test. The p values less than 0.05 were considered as statistically significant difference.



*p<0.05

Fig. 1. Brain necrosis at different concentrations of oxygen. *p < 0.05.



*p<0.05

Fig. 2. Brain water content at different concentrations of oxygen. *p < 0.05.

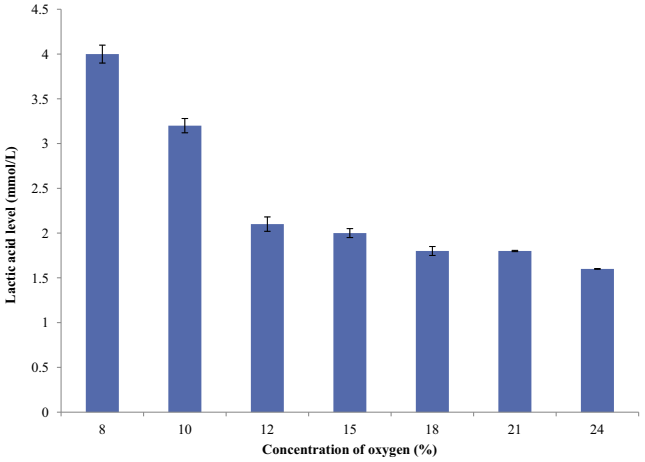


Fig. 3. Lactic acid levels in male albino rats at different concentrations of oxygen.

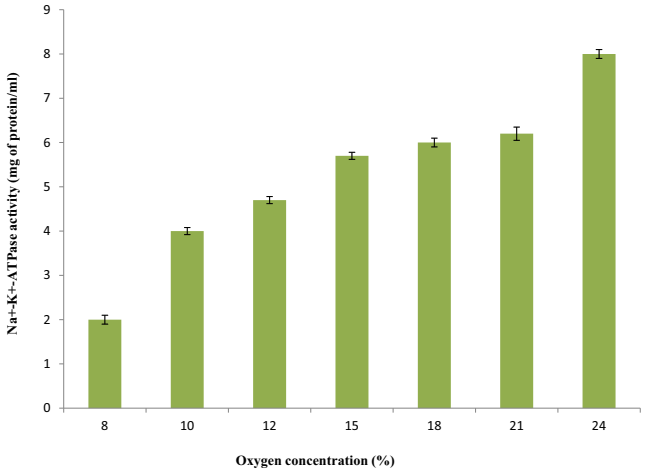


Fig. 4. Na+-K+-ATPase activity in male albino rats at different concentrations of oxygen.

3. Results

3.1. Necrosis

The reduction reaction of TTC begins under the effect of chondriosome succinate dehydrogenase in competent cell, and then red stabile, and indiffusible substance could be formed, whereas reduction reaction of TTC did not start in infarction section and the color of the section could be grey. This method might be used to investigate necrosis. Our experimental results showed that brain infarction ratio increased under serious hypoxia condition (8% O_2). There was a significant difference between group 24% O_2 and group 8% O_2 (Fig. 1, $p < 0.05$).

3.2. Water content

The increased water content found in brain tissue was induced by hypoxia. While exposed to 10%, 8% O_2, and water levels were the highest in the brain. Brain water content was 77.8% and 77.9%

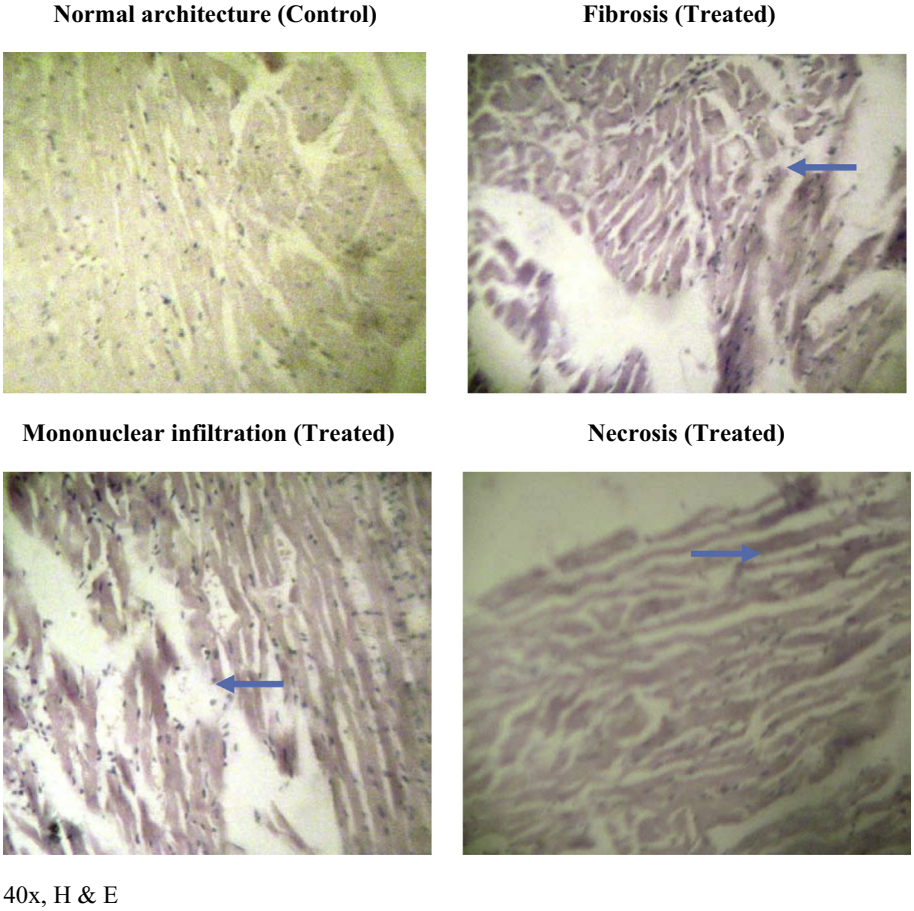


Fig. 5. Representative photographs of histopathological examination (HE) in the male albino rat hippocampus under hypoxia conditions.

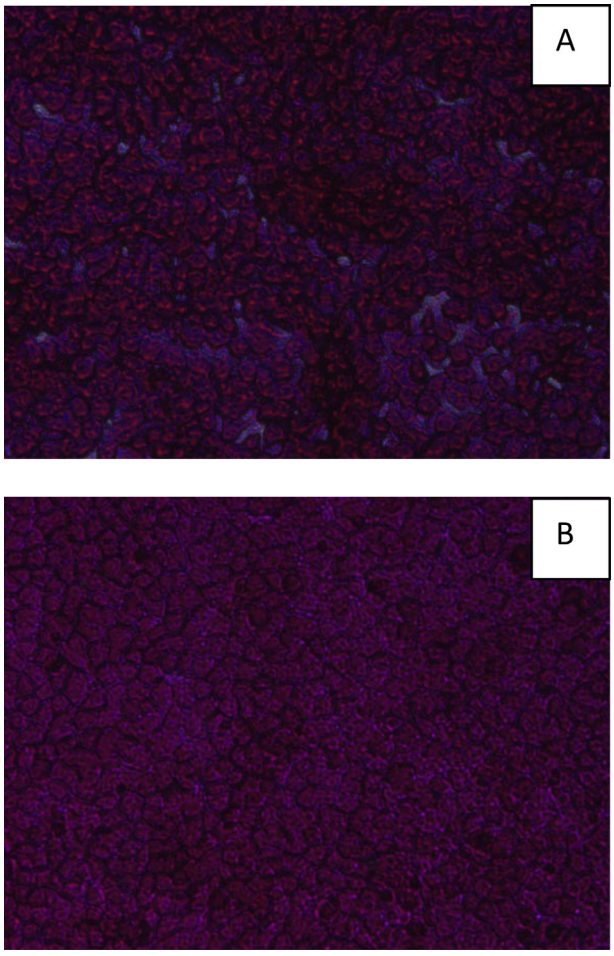


Fig. 6. HIF-1α immunohistochemistry photomicrographs of male albino rat hippocampus sections.

respectively. When exposed more than 12% O_2, the brain water contents were 76.8%. There was significant difference in the brain water content of 10%, 8% O2 group compared to 24% O_2 group (Fig. 2, $p < 0.05$).

3.3. Lactic acid

When the rat was exposed more than 10% O_2, lactic acid level in 10% brain homogenate tissue changed from 1.23 mmol L^{-1} to 1.26 mmol L^{-1}. Whereas rat exposed to 6% O_2, lactic acid level was increased significantly (changed from 1.26 mmol L^{-1} to 4.2 mmol L^{-1}. Lactic acid level was increased under serious the degree of hypoxia. Lactic acid was accumulated in the brain at 8% O_2 (Fig. 3).

3.4. Na+-K+-ATPase

The hyperactivity of Na+-K+-ATPase was pretty sufficient to maintain the ion homeostasis in the range of 24–12% O_2. Na+-K+-ATPase activity was reduced significantly at serious hypoxia (10% O_2) which induced the cell function disorder due to atrophia and cell edema (Fig. 4).

3.5. Histopathological analysis

The brain tissue was removed surgically from rat after exposure to 8% O_2 for 3 h. Hippocampus section was prepared for histopathological investigation. Histopathological analysis of brain tissue confirmed that there was neuronal cell death in hippocampal gyrus of hypoxia group compared to their respective controls (Fig. 5A). When oxygen level was 8%, 10% separately, histopathological investigation of brain showed that there was no cell death in the male albino wistar strain rats brain (Fig. 5B and Fig. 5C).

3.6. Immunohistochemistry

Expression of HIF-1a in the male albino rat hippocampus section was obvious. Our experiment confirmed that dilution ratio was a key factor to complete the HIF-1a immunohistochemistry due to instability and low abundance of HIF-1a (Fig. 6).

4. Discussion

Our experimental results showed that the levels of lactic acid elevated significantly under the severe hypoxia condition (8–10% O2). Severe hypoxia could cause acidosis easily and may lead to tissue edema, and cell death (Bernhardt et al., 2007; Marta et al., 2008). It clearly indicates that hypoxia may lead to anaerobic metabolism, and metabolic acidosis. Pyruvic acid was converted to lactic acid under anaerobic glycolysis.

Reduction in Na+-K+-ATPase activity showed that leads to the loss of ion homeostasis occurred. Na+-K+-ATPase could transport Na+ ions and K+ ions against their concentration gradient. It is believed that loss of ion homeostasis may play a key role in the pathogenesis of brain cell damage. Severe hypoxia-induced perturbation of ion homeostasis may lead to the intracellular accumulation of sodium and calcium ions, and subsequent activation of proteases and phospholipases. In addition the formation of oxygen and nitrogen free radicals may occur (Kintner et al., 2007). This may leads to the alteration of functional and structural including cerebral edema, eventually lead to cell death. Energy exhaustion may induce inhibition of Na+- K+-ATPase activity and accumulation of lactic acid, and acidosis and cell apoptosis under hypoxia.

HIF-1 is composed of a HIF-1ß and O_2 regulated HIF-1a subunit. HIF-1 is a transcriptional activator that regulates the expression of multiple genes under continuous hypoxia (Semenza, 2004). HIF-1 may play a general role in coordinating adaptive physiologic responses to hypoxia at the level transcription. HIF-1a have been implicated in the coordinate transcriptional activation of genes en-coding glycolytic enzymes in hypoxia cells, which provide an alternative means of energy under conditions of limited oxygen availability (Charles et al., 1996; Semenza, 2001). Immunohistochemistry study confirmed that HIF-1a was induced by hypoxia at 8% O_2. HIF-1a expression enhanced the hypoxia adaptation capability of the rat through the regulation of expression of multiple genes.

5. Conclusion

Lactic acid levels are positively correlated with the level of hypoxia, whereas total Na+-K+-ATPase activity shows a concentration-dependent reduction. Hypoxia induced rat has a significant difference in brain water content under severe hypoxia condition compared to control. Brain necrosis and lactic acid levels were increased. Reduction of Na+-K+-ATPase activity, neuronal cell death and HIF-1 expression appear in hippocampal gyrus. Na+-K+-ATPase, lactic acid and HIF-1α may play a vital role in pathogenesis of brain injury.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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