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Analysis of Histopathology and Apoptosis Characteristics of Thymusb in Rats with High Altitude Polycythemia

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Abstract: Background:Hypoxia is the most important characteristic at high altitude area, which can cause a series of changes in human physiological function.This study aims to observe the effect of high altitude polycythemia (HAPC) on the pathological characteristics of thymus in rats, and to provide histomorphological basis for the pathogenesis of HAPC. Methods: SD rats were randomly divided into two groups: normal control group and HAPC model group. The rats in the control group were fed with normal oxygen, and the rats in the model group were placed in a cabin simulating the altitude of 5000 m.The rats in the model group with 40 days of hypoxia, the HAPC model was successfully established. The RBC, HGB, HCT in the peripheral blood of all the rats were detected,the pathological morphology, ultrastructure and apoptosis were observed. Results: The levels of RBC, HGB and HCT in HAPC model group were significantly higher than those in control group ($P < 0.01$); the thymus in HAPC model group had obvious pathological damage, and the apoptosis rate was also significantly increased ($P < 0.05$). Conclusions: The thymus injury of rats with high altitude polycythemia mainly showed characteristic pathological changes such as lymphocyte atrophy,decreased number,cell necrosis and apoptosis.

1. Introduction

Hypoxia is one of the most important pathophysiological segments in the development of many diseases. It inhibits the respiratory center and destroys vascular endothelial cells, leading to a series of diseases, such as high altitude pulmonary edema, hypoxic encephalopathy and so on [1]. HAPC is one of the diseases caused by hypoxia. It is a common disease in high altitude areas, which seriously affects the health of plateau residents. The embolization of important organs caused by HAPC is a serious threat to the lives of patients. At present, a considerable number of people in the world live in plateau areas. At the same time, plateau tourism is more and more popular among the

public. Therefore, plateau diseases are paid more and more attention by the medical community.

According to the differentiation time and function, immune organs can be divided into central and peripheral immune organs. The former is the place for immune cells to occur, differentiate and mature; the latter is not only the site for T and B lymphocytes to settle and proliferate, but also an important place for the immune response [2]. Thymus belongs to the central immune organ. It is the earliest immune organ and the site of T cell to differentiate and mature. Some immune organs, such as lymph, thymus, spleen and so on, form a series of regulated gene expression in order to maintain the homeostasis in the high altitude hypoxia environment, suppress the immune system [3]. As fatal diseases at high altitude usual occur in the early stage of hypoxic exposure [4], in order to intervene in specific aspects of diseases at high altitude, it is of great significance to study the changes of immune system at the early stage of hypoxic exposure.

2. Materials and Methods

2.1 Materials

2.1.1 Laboratory Animals

24 SPF SD rats,half male and half female,weighing 240-270g(Chengdu Dashuo Experimental Animal Co.Ltd.Laboratory production license No.:scxk (Chuan) 2013-24,use license No.:syxk (Chuan) 2014-189).

2.1.2 Reagents and Instruments

Polyoxymethylene (Batch number: 20150813, produced by Tianjin Jinfeng Chemical Co.Ltd.); PBS phosphate buffer (0.01M Ph7.2-7.4,Batch number: ZLI-9062,produced by Beijing Zhongshan Jinqiao Biotechnology Co.Ltd.); Xylene(Batch number:20150810,produced by Chengdu Kelong Chemical Reagent Factory); Neutral balsam (Batch number:20150710, produced by Shanghai Yiyang Instrument Co.Ltd.); Potassium dichromate (Batch number:20150421, produced by Chengdu Kelong Chemical Reagent Factory).

Bp221s electronic balance (Germany sartorius company); Rotary slicer (Germany sartorius company); TSJ-Ⅱ automatic closed tissue dehydrator (Germany sartorius company); BMJ-Ⅲ embedding machine (Germany Leica company); Phy-Ⅲpathological tissue bleaching and drying instrument (Germany Leica company); Digital three eye camera microscope (Olympus company); Image production and analysis software Motic images advanced, etc.

2.2 Methods

2.2.1 Experimental Grouping and Model Replication

After a week of adaptive feeding, SD rats were randomly divided into normal control group and model group with 12 rats in each group. The rats in the control group were fed with normal oxygen in the IVC air-conditioned animal room. The ambient temperature was 20 ℃~ 24 ℃, the relative humidity was 40% ~ 70%, and the light and dark were alternately illuminated for 12h / 12h. The rats in the model group were fed in the plateau environment simulation cabin (simulated altitude 5000 m) [5] for 40 days.

2.2.2 Observation Indexes

(1) After successful modeling, anesthetized with 6% Pentobarbital Sodium, about 5ml arterial

blood was extracted from abdominal aorta of rats and put into EDTA-K2 purple head blood collection vessel and gently shaken.Then, RBC, HGB and HCT of the two groups were measured with blood routine instrument.

(2) Pathomorphology examination of immune organs: the thymus tissue was cut and fixed with 10% paraformaldehyde for 24 hours, then paraffin embedded sections were made, and the pathological changes of thymus tissue were observed by HE staining. The ultrastructure of thymus was observed by transmission electron microscope.

(3) Tissue apoptosis detection: part of thymus tissue was cut, fixed with 4% paraformaldehyde for 15 min, washed with PBS, then stained with TUNEL at room temperature for 10 min, washed with PBS again, cell morphology was observed under light microscope, and apoptotic cells were counted (per 100 cells).

2.2.3 Sstatistical Analysis

statistical analyses were performed with SPSS17.0. Independent sample t-test was used, the data were expressed as mean ± standard deviation(SD).P values of < 0.05 were considered as significant and of < 0.01 as highly significant.

3. Results

3.1 Determination of Rbc, Hgb and Hct in Peripheral Blood of Rats in Two Groups

It can be seen from table 1 that RBC, HGB and HCT of model group were significantly higher than those of control group ($P < 0.01$), indicating that the model has been successfully replicated.

Table 1 Comparison of Rbc, Hgb and Hct between the Two Groups

Groups	n	RBC ($\times 10^{12} \cdot L^{-1}$)	HGB ($g \cdot L^{-1}$)	HCT ($L \cdot L^{-1}$)
Control group	12	5.78±0.134	145.60±4.90	0.29±.0067
Model group	12	7.71±0.42**	244.85±14.74**	0.46±.023**

* $P < 0.05$, * * $P < 0.01$;significantly different from the control group.

3.2 Pathomorphology Examination of Thymus

3.2.1 General Pathology Observation of Thymus

It can be seen from Figure 1 that in the control group, the fibrous connective tissue of thymus capsule was intact, no hyperplasia, inflammatory exudation and other changes were observed, no reduction or atrophy of lymphocytes in cortex was observed, and no special abnormality was observed in medulla. In the model group, the number of lymphocytes in the cortex decreased significantly, and lymphocyte atrophy was observed.

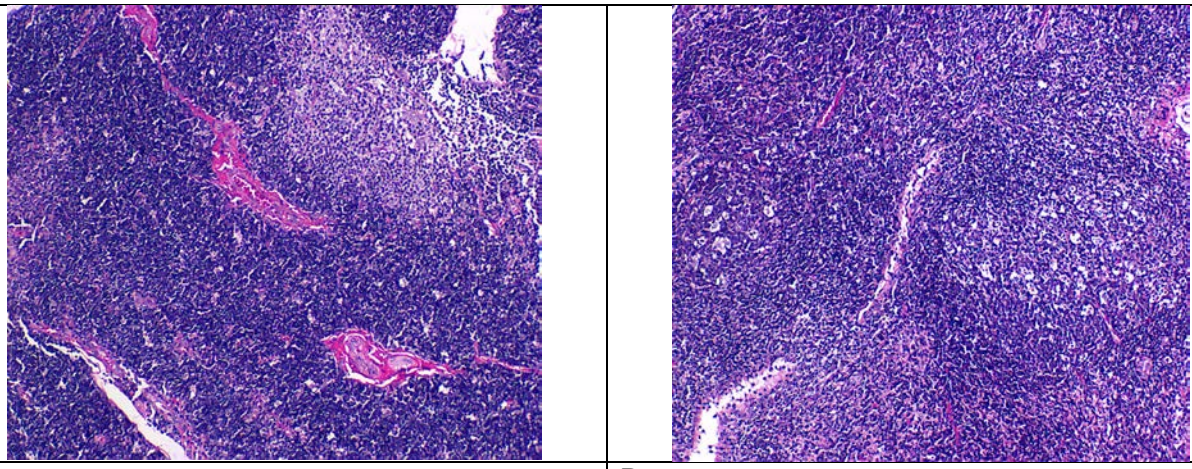


Fig.1 The General Pathological Morphology of Thymus Tissue

A: Thymus general micrograph of control group (× 100)
B: Thymus general micrograph of model group (× 100)

3.2.2 Ultrastructural Pathology Observation of Thymus

It can be seen from Fig. 2 that the thymus tissue of the control group is rich in lymphocytes with round nuclei, rich in heterochromatin, slightly swollen mitochondria in the cytoplasm, and rich in ribosomes. In model group, thymus tissue was rich in lymphocytes, with round nuclei and abundant heterochromatin.Mitochondria in cytoplasm were severely swollen, and ribosomes were also abundant.

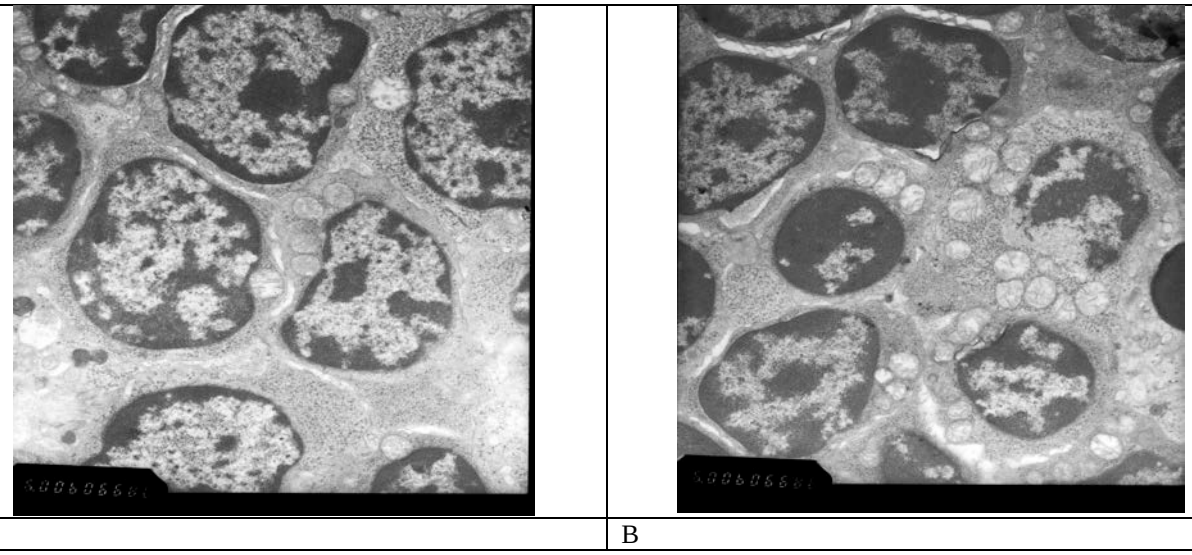
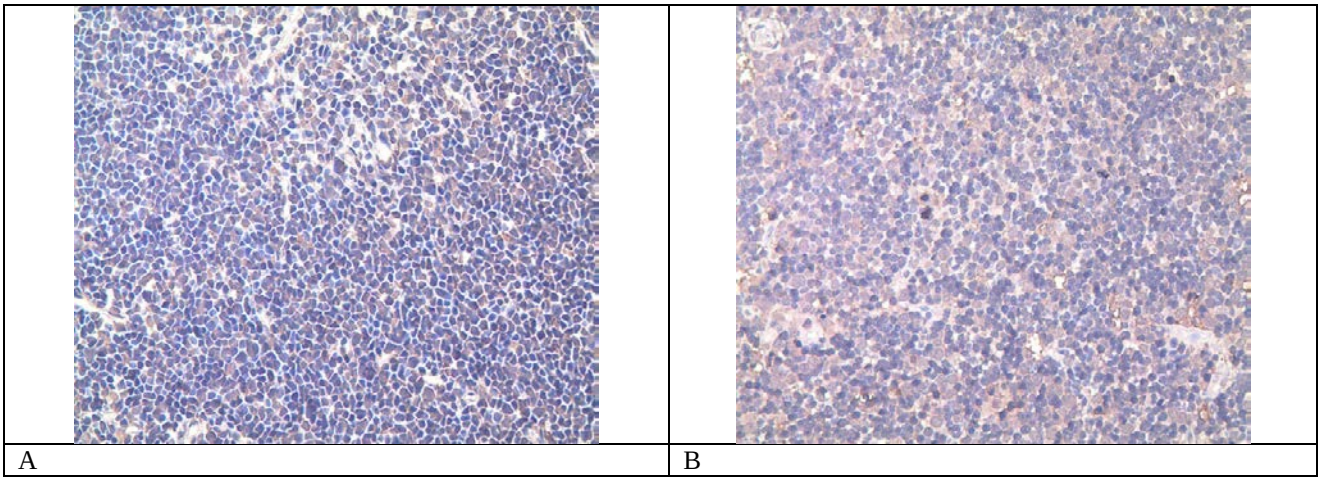


Fig.2 The Ultrastructure of Thymus Tissue

A: Thymus ultrastructural micrograph of control group (× 100)
B: Thymus ultrastructural micrograph of model group (× 100)

3.3 Detection Results of Thymocyte Apoptosis

Compared with the control group (16.83 ± 4.67), the apoptosis rate of thymocytes in model group (25.33 ± 7.53) was significantly increased ($P < 0.05$). The thymocyte apoptosis of the two groups is shown in Figure 3.



A B

Fig.3 Thymocyte Apoptosis Picture

A: Thymocyte apoptosis in control group (× 100)
B: Thymocyte apoptosis in model group (× 100)

4. Discussion

Human body is in the environment of high altitude and hypoxia. Due to the stimulation of hypoxia, a large number of red blood cells will be generated in bone marrow, leading to high altitude polycythemia (HAPC). Excessive erythropoiesis can lead to increased blood viscosity, increased microcirculation resistance and hypoxia of tissue cells [6]. Previous studies have shown that hypoxia controls innate and adaptive immunity by regulating immune cell proliferation, development and effector function [7]. Thymus is an important central immune organ of human body. It is the site of T lymphocyte differentiation, development and maturation. It can export mature T lymphocytes to peripheral T cell bank. It can maintain immune homeostasis by domesticating thymocytes and establishing its own immune tolerance. In addition, thymus can coordinate B lymphocytes to play the role of humoral immunity [8].

In order to better understand the effect of high altitude hypoxia on the morphology of thymus tissue, we constructed an animal model of hypoxia, and observed the pathological characteristics of thymus in normoxia group and HAPC group. We found that the number of lymphocytes in the thymic cortex of HAPC group decreased significantly, the mitochondria swelled seriously, and the apoptosis rate increased significantly. Hypoxia leads to the above characteristic pathological damage of thymus tissue, which may affect the immune function to a certain extent. Studies have found that hypoxia can induce morphological changes of thymocytes, apoptosis, thymic tissue atrophy, and the telomere length,the expression of TERT mRNA and protein were significantly increased [9]. In addition, histological analysis of the effect of prenatal hypoxia on thymus development of offspring of rodents showed that the cortical area of thymus of offspring exposed to hypoxia was smaller, and the decrease of cortical T lymphocytes was corresponding to the increase of caspase 3 (CASP3) mRNA abundance and thedecrease of proliferation marker Ki67

(mki67)expression[10]. These findings are similar to the results of this study. Hypoxia can lead to pathological changes in thymus tissues of immune organs, which indicates that hypoxia can affect the immune function of the body. However, the specific regulatory mechanism of how the immune system regulates and tolerates hypoxia is not clear. At present, researches on pathophysiology of altitude sickness and hypoxia at home and abroad mainly focus on respiratory system, circulatory system and motor system [11], while researches on immune dysfunction caused by altitude sickness are less. The abnormal immune function caused by hypoxia will affect the health of migrants at high altitude. It is an urgent problem to clarify the pathophysiological mechanism of immune damage caused by hypoxia and to carry out intervention according to the pathogenic mechanism.

Revealing the pathogenesis of HAPC is one of the important ways to study the occurrence and development of HAPC, and the study of pathological characteristics of target organs through animal experiments can lay an important foundation for the study of molecular mechanism of HAPC. In this study, the effect of high altitude hypoxia on the immune system is not comprehensive enough, and long-term continuous in-depth observation and research are needed in the future.

5. Acknowledgment

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