Original Article

Hyperbaric oxygen treatment reduced the lung injury of type II decompression sickness

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Abstract: Objective: To detect the ultrastructural changes in rabbits with type II decompression sickness (DCS), and study the therapeutic effects of hyperbaric oxygen (HBO). Methods: Twenty-seven male New Zealand rabbits were randomly divided equally into the DCS group, HBO treatment group and control group. Experimental models of each group were prepared. Lung apex tissues were harvested to prepare paraffin- and EPON812-embedded tissues. Results: In the DCS group, macroscopic and histological examination revealed severe and rapid damage to lung tissue. Ultrastructural examination revealed exudation of red blood cells in the alveolar space. Type I alveolar epithelial cells exhibited retracted cell processes and swollen mitochondria, and type II cells showed highly swollen mitochondria and decrease in cytoplasmic lamellar bodies. Dilatation and congestion of capillary vessels were accompanied by swelling of endothelial cells and incomplete basement membrane. In the HBO treatment group, the findings were somewhat similar to those in the DCS group, but the extent of damage was lesser. Only a small amount of tiny bubbles could be seen in the blood vessels. Type I alveolar epithelia cells and endothelial cells of the capillaries illustrated slight shortening of cells, swollen cytoplasm and decreased cell processes. Type II alveolar epithelial cells showed slight swelling of the mitochondria, decreased vacuolar degeneration of lamellar bodies, and increase in the number of free ribosomes. Conclusions: Our microscopic and ultrastructural findings confirm that the lung is an important organ affected by DCS. We also confirmed that HBO can alleviate DCS-induced pulmonary damage.

Keywords: Alveolar epithelial cells, decompression sickness, hyperbaric oxygen, lung, ultrastructure

Introduction

Decompression sickness (DCS) is caused by intravascular or extravascular bubbles during or after a reduction in environmental pressure (decompression). DCS starts with the formation and increase in size of bubbles when a person gradually moves from a high-pressure environment to a normal-pressure environment. Inert gases, especially nitrogen, are more soluble in tissues and body fluid in a high-pressure environment, and the dissolved load of nitrogen is directly associated with the level and duration of pressure [1]. When a person gradually moves from a high-pressure environment to a normal-pressure environment, the excess nitrogen in the body is released from tissues into the blood stream. During such a transition, gas present in blood shifts from the unsaturated or saturated state to the supersaturated state; therefore, inert gases in the dissolved state form gas bubbles. Gas bubbles are transported to alveolar capillaries, where they diffuse into the lungs and are expired through respiration. Therefore, the lung is one of organs involved in stress response to air pressure changes [2, 3]. The amount of bubbles exceeds the capacity of the pulmonary capillary, inducing lung damage. Meanwhile, the continuous integration of microbubbles results in the formation of large bubbles, inducing more severe lung injury [4, 5]. Bubbles can also cause angiospasms, which lead to tissue ischaemia, oedema and haemorrhage [6, 7]. Thus, bubble formation is the primary factor in the pathogenesis of DCS [8]. The respiratory system is the main part affected by DCS [2, 9, 10], and damage to the lungs is closely related to sudden death.

It has been confirmed that hyperbaric oxygen (HBO) is useful for the prevention and treatment of DSC, as it reduces and eliminates the bubbles present in tissue and blood [9-11]; it is also the main treatment method. HBO treat-
ment promotes the formation of a gas concentration gradient between air bubbles and blood, which leads to gradual discharge of nitrogen and decrease in bubbles. In addition, HBO treatment can reduce the extent of oedema by reducing vascular permeability, eliminating activated leucocytes, and reducing damage to vascular endothelial cells [12, 13]. Further, HBO effectively improves oxygen delivery to body tissue, and promotes the absorption of inert gas and reduces the volume of bubbles that dissociate from tissue and blood [13, 14].

**Materials and methods**

**Experimental animals**

Twenty-seven healthy male New Zealand white rabbits (weight, 2.0-2.5 kg) were purchased from the Animal Center of Shandong Lukang Corporation. Before the experiment, the rabbits were reared for more than 1 week at the experimental centre so that they could adapt to the environment; the living and feeding conditions were normal (temperature, 23-25°C; humidity, 50.0-56.0%; light from 6:00 to 18:00). They were fed standard rabbit chow twice daily with free access to water. The animals were then randomly divided into the DCS group, HBO treatment group, and control group (n = 9 for each group). This study received the approval of the ethics committee of the General Hospital of Jinan Military Command. The animals were treated humanely in compliance with the Guide for the Care and Use of Laboratory Animals.

**Experimental models**

**DCS group:** The rabbits were placed in a compression chamber (YC3800/0.3-36 VII, Yantai Moon Hyperbaric Oxygen Chamber Co., Ltd.) with soda lime at the bottom. The pressure was increased to 800 KPa (absolute pressure) within 5 min and maintained for 60 min, then it was decreased rapidly to normal pressure within 5 min, during which anesthesia was not conducted.

**HBO treatment group:** The rabbits were treated as described above. After decompression, they were immediately placed in a new compression chamber for HBO treatment with more than 95% atmospheric oxygen concentration under 280 KPa pressure. The 280 KPa pressure was applied within 10 min and maintained for 60 min, after which the gas pressure was gradually reduced to normal for 20 min.

**Control group:** The animals were placed in the compression chamber and exposed to normal atmospheric pressure. They were removed from the cabin after 60 min.

Animal behaviours in each group were observed after the rabbits left the cabin. Three rabbits in the DCS group died as a direct result of the intervention. The others were humanely anesthetized within 30 to 60 min after the experiment induced with 10 mg/kg ketamine hydrochloride and droperidol solution by intramuscular injection. Specimens of the lung apex tissue were obtained. Then 10 times dose of anesthetic drugs was used for euthanasia. Finally, after confirming animal deaths, all rabbits were collected and were harmless burned.

**Microscopic examination**

Specimens of the lung apex tissue were fixed in 10% neutral formalin, and then embedded with paraffin and sliced using the conventional method. The specimens were then stained with hematoxylin-eosin (HE) and observed under an Olympus BX53F light microscope (Olympus, Tokyo, Japan). The specimens for electron microscopy were pre-fixed with 2.5% glutaraldehyde quickly after they were cut to a size of 0.2 cm × 0.2 cm × 0.2 cm. They were then fixed in 1% osmium acid and embedded in EPON812. Sections of thickness 60-70 nm were prepared with an ultrathin microtome. Uranyl acetate and lead citrate double-electron staining was used to visualize the sections under a JEOL-1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

**Results**

**Behavioural changes**

Rabbits in the DCS group exhibited varying degrees of DCS symptoms, including itching, increase in breathing frequency, restlessness, convulsions, and unilateral or bilateral lower limb dyskinesia. Three rabbits showed severe reaction and died within 30 min. In the HBO treatment group, three rabbits exhibited increased breathing frequency, restlessness, and mild lower limb dyskinesia, while the remaining six rabbits did not show obvious symptoms of DCS. The rabbits in the control group did not exhibit any abnormal symptoms.
Macroscopic findings

In the DCS group, a large amount of subcutaneous bubbles were observed in the dissected rabbits. Crepitus was noted on touching the subcutaneous bubbles. A large number of bubbles were also found in the blood vessels. The surface of lungs revealed hyperaemia, and a large quantity of pink frothy liquid flowed out when the lungs were pressed lightly. The three rabbits that died within 30 min had a large amount of blood containing bubbles in their chests.

In the HBO treatment group, a large amount of small subcutaneous bubbles were observed, and crepitus was noted on touching the bubbles. However, only a small amount of tiny bubbles could be seen in the blood vessels, and the lung was pale pink in colour with a soft texture. A small amount of pink frothy liquid was observed in a section of the lungs.

Microscopic findings for the lungs

In the DCS specimens, a large number of irregular red blood cells were observed in the alveolar space of various sizes under low-magnification view. Occlusion of the alveolar space was seen around large vessels, while the alveolar spaces around these occlusive ones showed expansion and fusion (Figure 1A). Moreover, congestion was observed in the veins and capillaries with focal rupture. Under the high-magnification view, the alveolar wall covering the swollen alveolar epithelial cells showed focal rupture. Gas embolism and thrombosis were seen in small arteries, capillaries and veins; they were illustrated by transparent vacuoles of 5-15 μm in diameter present within the plasma or next

Figure 1. Microscopic features of lungs. A. DCS group: alveolar spaces revealed expansion and fusion, and veins and capillaries were congested (HE, ×100). B. DCS group: alveolar wall lining with swollen alveolar epithelial cells can be observed; gas embolism was found in a small vessel (black arrow) (HE, ×200). C. HBO treatment group: alveoli lined with a small amount of swollen alveolar epithelial cells can be seen, with some alveoli showing expansion and fusion; exudation of red blood cells was observed (HE, ×100). D. HBO treatment group: transparent bubbles of 5-15 μm in diameter were observed in small vessels (black arrow) (HE, ×200).
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Figure 2. Ultrastructure of the lungs in the DCS rabbits. A. Distorted red blood cells appeared in the alveolar spaces. Capillaries were dilated and congested with incomplete base membrane. B. Type I alveolar epithelial cells showed retraction of cell processes and swollen intracytoplasmic mitochondria. C. Type II alveolar epithelial cells showed swollen mitochondria with vacuolar degeneration. D. Vacuoles were gathered in the cytoplasm, leading to the manifest of extrusion nuclei, while the amount of lamellar bodies was reduced. E. Type II alveolar epithelial cells were swollen and vacuoles were found gathered in the cytoplasm. Microvilli appeared disarranged in the cell membrane, and some of them had dropped off. F. Type II alveolar epithelial cells revealed shrinkage of the cytoplasm, condensation of nuclear chromatin, and vacuolar degeneration in the lamellar bodies.

to the white blood cells (Figure 1B). In addition, pulmonary diffuse haemorrhage and atelectasis were also observed in the three rabbits that died. Furthermore, exudation and necrosis in the bronchi were observed to a large extent with considerable inflammatory response.

In the HBO treatment specimens, the alveoli were lined with slightly swollen alveolar epithelial cells, and some of the alveoli showed expansion and fusion. Exudation of red blood cells was also observed in the alveolar space. Expansion and congestion were observed in the veins and capillaries, in which gas embolism and thrombosis could also be found (Figure 1C). There were many transparent bubbles of 5-15 μm in diameter in the vessels (Figure 1D).

Ultrastructural features of the lungs

In the DCS specimens, the alveolar spaces were of various sizes and contained distorted red blood cells, and severe expansion or occlusion were seen in some of the alveolar spaces (Figure 2A). Type I alveolar epithelial cells were round and swollen with retracted cell processes and swollen intracytoplasmic mitochondria, which probably induced the loose connection between cells and basement membranes (Figure 2B). Type II alveolar epithelial cells showed obvious swelling (Figures 2C). A large number of vacuoles were found in the cytoplasm, leading to the manifest of extrusion nuclei, while the number of lamellar bodies was reduced (Figure 2D). Microvilli were found disarranged in the cell membrane, while some of them had dropped off (Figure 2E). In addition, some type II alveolar epithelial cells exhibited shrinkage of the cytoplasm, condensation of nuclear chromatin, and disappearance of lamellar bodies (Figure 2F). Capillaries were dilated and congested with incomplete base membrane. The changes in endothelial cells of
the capillary were similar to those of type I alveolar epithelia, with decreased cell processes and a swollen cytoplasm.

In the HBO treatment group specimens, ectatic alveolar spaces were observed. Type I alveolar epithelia cells and endothelial cells of the capillaries had similar features, including slight shortening of cells, swollen cytoplasm and decreased cell processes (Figure 3A). Type II alveolar epithelial cells showed slight swelling of the mitochondria, decrease and vacuolar degeneration of lamellar bodies, and increase in the number of free ribosomes (Figure 3B).

**Discussion**

The lung was one of the main target organs of DCS [15]. In our study, three of the rabbits in the DCS group showed obvious respiratory failure and died within 30 min. Moreover, all the animals demonstrated increase in breathing frequency and restlessness, and haemorrhage and exudate were found with surrounding inflammatory cells. These findings indicated that lung tissue was impaired in the early stage of DCS. Obvious histological changes in lung tissue were observed in rabbits sacrificed within 1 h after the experiment in the DCS group. We think that the alveolar space occlusion observed around large vessels was caused by the constriction of adjacent lung tissue by endovascular and extravascular bubbles in the early stage of DCS. The peripheral alveoli swelled up as a compensation mechanism, in order to increase the area available for gas-blood exchange. With the aggravation of lesions, alveolar spaces expanded gradually and the alveolar walls were fractured, inducing the fusion of alveoli and formation of bullae of the lung tissue. Moreover, thrombi or bubbles present in veins and capillaries can lead to blood circulation disorders, further exacerbate the impairment in gas-blood exchange, and result in respiratory failure eventually. In agreement with this, it has been reported that gas micro-nuclei formed during rapid decompression can enhance intravascular coagulation and thrombosis via damage to the vascular endothelium and activation of platelets [16].

The ultrastructural findings for type I alveolar epithelial cells and capillary endothelial cells suggest that the blood-gas barrier was damaged. With regard to type II alveolar epithelial cells, the results were inconsistent, with some rabbits showing indication of mild injury and others showing indication of serious injury. Based on the ultrastructural findings, we speculated that the pathological changes in type I alveolar epithelial cells and capillary endothelial cells occurred as early as the time at which...
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Lung damage was induced by DCS, and that type II alveolar epithelial cells secreted more surfactants in order to compensate for and maintain the stability of the respiratory membrane. With the aggravation of lung injury and time, the amount of surfactants in type II alveolar epithelial cells was gradually decreased and used up, and lamellar bodies presented with vacuolar degeneration or disappeared. Simultaneously, owing to cell damage and energy loss, the mitochondria swelled and degenerated, and the cristae appeared fractured or disappeared. Pyknosis of the nuclei and symptoms of proapoptosis were found in type II alveolar epithelial cells. In the safe decompression group, damage to the lung tissue was milder than that in the DCS group. These findings indicate that changes in the lung tissue might be a regulatory stress response to environmental changes during compression.

Our study showed that rabbits treated with HBO had mild symptoms of DCS and exhibited normal activity. However, on gross examination, we found a lot of bubbles in the subcutaneous adipose tissues, probably because nitrogen solubility in fat tissues is far greater than that in blood. The mild pathological changes were confirmed by the histological and ultrastructural findings in the HBO treatment group. The alveolar epithelial cells were slightly swollen, and although bubbles were present, they were small in number and volume and were only partly present in blood vessels. Ultrastructural examination revealed that occlusion of alveolar spaces and obvious embolism of the capillaries was rarely observed, and that type II alveolar epithelial cells showed only mildly swollen mitochondria. The findings thus indicated that timely HBO treatment is helpful in reducing or preventing the occurrence of DCS. This treatment may therefore be useful for divers and high-altitude workers. However, since we did find some bubbles with our treatment, repetitive or comprehensive HBO treatment may be more effective. In agreement with our findings, Richard et al. also reported that HBO could reduce or eliminate the gas micronuclei within tissues [14, 19]. Furthermore, the recompression regime was also reported to cause a significant reduction in bubble size [20, 21]. In our study, ultrastructural examination revealed that the damage to alveolar epithelial cells in the HBO treatment group was lesser than that in the DCS group, which suggests that the HBO treatment and recompression regime can effectively reduce and avoid pulmonary damage induced by decompression. Moreover, Bosco and Landolfi found that HBO could reduce the risk of platelet activation during decompression, and prevented the formation of venous thrombi [22, 23]. This may explain the significant reduction in venous and capillary embolism observed in the HBO treatment group in our study.

In conclusion, our findings indicate that damage to the respiratory epithelium and destruction of the blood-gas barrier affected alveolar gas-exchange function in DCS. Further, the benefits of HBO treatment in DCS were confirmed.

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Disclosure of conflict of interest

None.

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