Dose-Response:
An International Journal
October-December 2020:1-9

DOI: 10.1177/1559325820970821
journals.sagepub.com/home/dos
SAGE

Expression of Aquaporin-1 and Aquaporin-5 in a Rat Model of High-Altitude Pulmonary Edema and the Effect of Hyperbaric Oxygen Exposure

Jiewen Tan, MD[1], Chunjin Gao[2], Cong Wang, PhD[2], Linlin Ma, PhD[2], Xiaomin Hou, PhD[2], Xuehua Liu, PhD[2], and Zhuo Li, PhD[1]

Abstract

Objective: To investigate the therapeutic roles of hyperbaric oxygen exposure on high-altitude pulmonary edema and to determine whether aquaporin-1 and aquaporin-5 were involved in the pathogenesis of HAPE in rats.

Methods: Rats were divided into 5 groups: The control group, the HAPE group (HAPE model), the HBO group (hyperbaric oxygen exposure), the NBO group (normobaric oxygen exposure), and the NA group (normal air exposure). Western blot and real-time PCR were used to analyze the pulmonary expressions of AQP1 and AQP5. The wet-to-dry (W/D) weight ratio and the morphology of the lung were also examined.

Results: The lung W/D weight ratio in the HAPE group was increased compared with the control group. The injury score in the HBO group was noticeably lower than that in the control group. The mRNA and proteins expressions of AQP1 and AQP5 were significantly downregulated in the HAPE group.

Conclusions: Oxygen exposure alleviated high-altitude hypobaric hypoxia-induced lung injury in rats. Additionally, HBO therapy had significant advantage on interstitial HAPE.

Keywords

high-altitude pulmonary edema, hyperbaric oxygen therapy, aquaporin-1, aquaporin-5, lung wet-to-dry weight ratio, lung morphology

Introduction

High-altitude pulmonary edema (HAPE) is a life-threatening disorder among climbers and tourists who ascend to altitudes >2500 m. It was first described in South American high altitude dwellers who returned from a sojourn at low altitude[1] and subsequently in acclimatized lowlanders.[2]

HAPE usually develops within the first 2-5 days after the arrival at high altitude and presents with cough, dyspnea, tachycardia, and even with orthopnoea and pink sputum at advanced stage.[3-5] The incidence of HAPE among trekkers in the Himalayas and climbers in the Alps ascending at a rate of >600 m/day is around 4%.[6,7] In the alpine setting, when an altitude of 4559 m was reached within 22 h, the incidence increased to 7% in mountaineers without a history of radio-graphically documented HAPE and to 62% in mountaineers with such a history.[8]

The pathogenesis of HAPE has not been fully elucidated. Recent evidence suggests that aquaporins (AQPs) may play an important part in fluid clearance and edema formation in the lung after acute lung injury (ALI).[9] AQPs are a family of water-selective channels that increase plasma membrane water permeability and provide a route for rapid fluid movement.[10] AQPs are expressed in many tissues, such as the kidney, eye, and

[1] Department of Rehabilitation Medicine, XinHua College, Sun Yat-Sen University, Guangzhou, China
[2] Department of Hyperbaric Oxygen, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China

Received 16 July 2020; received revised 24 September 2020; accepted 8 October 2020

Corresponding Author:
Zhuo Li, Department of Hyperbaric Oxygen, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100020, China.
Email: zhuoli_172@126.com

lung, where rapid water transportation is necessary.[11] The main AQPs in peripheral lung tissues are AQP1 and AQP5. The former is expressed in microvascular endothelia throughout the lung and airways, and the latter is expressed in type-I alveolar epithelial cells and submucosal gland acinar cells.[12] Studies on AQP1 knockout mice and AQP5 knockout mice demonstrated that airspace-to-capillary osmotic water permeability was reduced 10-fold by the depletion of AQP1 or AQP5, and was further reduced by 2-3-fold in AQP1/AQP5 double-knockout mice. AQP1 facilitates hydrostatically-driven lung edema, but is not required for active near-isosmolar absorption of alveolar fluid.[13,14] In a model of adenovirus-induced lung inflammation, reduced pulmonary levels of AQP1 and AQP5 may be associated with abnormal fluid fluxes during pulmonary inflammation.[15] In a rat model of lipopolysaccharide (LPS)-induced ALI, the pulmonary expressions of AQP1 and AQP5 were decreased at 4 to 48-h post-LPS treatment.[16] It has been proposed that AQPtype water channels may be important in physiological and pathophysiological processes in the lung.

Hyperbaric oxygen therapy (HBOT) is defined as breathing 100% oxygen under increased atmospheric pressure.[17] HBOT is highly recommended during the acute phase of HAPE. In particular, HBOT may be life-saving when descent is impossible.[18] Patients with hypobaric hypoxia-caused HAPE resulting may benefit from HBOT with hyperbaric hyperoxia. Some clinical studies reported HBOT in HAPE,[19,20] but few experimental studies have been carried out. As a result, the application of HBOT in respiratory disorders remains controversial.[21,22] More fundamental research is needed to investigate the efficacy of HBOT on HAPE. Our prior research on hyperbaric oxygen preconditioning has revealed that high altitude pulmonary edema may be prevented by hyperbaric oxygen preconditioning in rats.[23]

In this study, we aimed to (i) characterize a model of HAPE in rats; and (ii) evaluate the therapeutic role of HBOT and normobaric oxygen therapy (NBOT) on lung edema using wet weight/dry (W/D) weight ratios and histological studies of lung tissue. We also observed changes in mRNA and protein expressions of AQP1 and AQP5 in the lung tissues, and further investigated the role of AQP1 and AQP5 in HAPE.

Materials and Methods

Ethical Approval of Study Protocol

This study was approved by the Ethical Committee of the Animal Experimentation of Capital Medical University (Beijing, China) and performed in accordance with Guidelines for Ethical Review of Experimental Animal Welfare (GB/T 35892).

Study Design

Rats were divided into the 5 groups: control group, HAPE group, HBO group, normobaric oxygen (NBO) group, and normobaric air (NA) group. All rats were exposed to a simulated altitude of 6000 m for 24 h in a hypobaric chamber to produce a rat model of HAPE except for the control group. Immediately after being taken out to the ambient atmosphere, HAPE rats were anesthetized by administration of 10% chloral hydrate (5 mL/kg, i.p.); HBO rats were treated with HBO for 1 session; NBO rats were treated with normobaric oxygen for 2 h; and rats in the NA group were exposed to the ambient atmosphere without any treatment for 2 h.

At the different time-points mentioned above, rats were euthanized and thoroughly exsanguinated before their lungs were excised *en bloc*. The left lung lobe was used for determination of the W/D ratio (which was used as an index of water content in the lung). The right lower lobe was harvested for histopathology evaluation, and the right upper lobe was collected for molecular analyses.

Animals

Adult male Wistar rats (220-250 g) were obtained from the Military Medical Science Academy of the People's Liberation Army (Beijing, China). Rats were maintained at 25 $\pm$ 1°C under a 12-h:12-h light-dark cycle. They had *ad libitum* access to food and water. Forty-weight rats were randomly assigned into 5 groups (n = 8 in the control group, n = 10 in other groups).

Animal Model of HAPE

Rats were exposed to a simulated altitude of 6000 m (19,685 feet) in a hypobaric chamber (Institute of Aviation Medicine, Beijing, China) for 24 h. The rate of ascent to altitude was maintained at 300 m/min and it took 20 min to reach the desired altitude. After exposure for 24 h, the altitude descended to sea level at the same rate as the ascent to altitude. The temperature of the hypobaric chamber was maintained at 25 $\pm$ 1°C and the humidity was at 40-50%. The air flow rate was 4 L/h and the barometric pressure 355 mmHg. Rats were provided with adequate quantities of food and water during exposure to hypoxia.

HBO Exposure

Rats were placed into a custom-made pressure chamber of transparent acrylic plastic (701 Space Research Institute, Beijing, China) and received 1 h of HBO at 2.0 ATA in 100% oxygen (O_2) for 1 session. Compression was carried out at a rate of 1 kg/cm^2/min to 2.0 ATA/100% oxygen, and maintained for 60 min. The chamber was flushed with 100% oxygen at a rate of 5 L/min to avoid accumulation of carbon dioxide. Decompression was done at 0.2 kg/cm^2/min. During HBO exposure, the content of oxygen and carbon dioxide were continuously monitored and maintained at $\geq$ 98% and at $\leq$ 0.03%, respectively. The chamber temperature was maintained between 22°C and 25°C.

NBOT

Rats were placed into a custom-made chamber of transparent acrylic plastic (701 Space Research Institute, Beijing, China)

Table 1. Sequences of Primers.

Gene	Primer	Sequence
AQP1	Primer (upstream)	5'-CTTGTCTGTGGCTCTTGG-3'
	Primer (downstream)	5'-ACCTTCATGCGGTCTGTA-3'
AQP5	Primer (upstream)	5'-ATCTTCGTCTTCTTTGGCCT-3'
	Primer (downstream)	5'-CAGCGAGATCTGGTTTCCTA-3'
Actin	Primer (upstream)	5'-CCC ATC TAT GAG GGT TAC GC-3'
	Primer (downstream)	5'-TTT AAT GTC ACG CAC GAT TTC-3'

and received 2 h of normobaric oxygen therapy. The chamber was flushed with 100% oxygen at a rate of 2 L/min to avoid accumulation of carbon dioxide and to maintain a stable oxygen concentration. The content of oxygen and carbon dioxide was continuously monitored and maintained at 35% and at $\leq$ 0.03%, respectively. The temperature of the chamber was maintained between 22°C and 25°C and humidity at 40-50%.

W/D Weight Ratios of Lung Tissue

Rats were euthanized and thoroughly exsanguinated before the lung tissues were excised *en bloc*. Lung tissue samples were blot-dried and placed in pre-weighed trays made of aluminum foil. The wet weight of lung tissue was registered immediately on an electronic balance to an accuracy of 0.1 mg. The tray containing lung tissue was then baked in an oven at 70°C for 48 h until a constant weight was achieved. The dry weight of the tissue was then recorded. The water content of the tissue was calculated as wet weight minus dry weight, and expressed as milligrams of water per milligrams of dry tissue.

Lung Morphology

Lung tissue was fixed in 10% buffered formalin for 24 h. It was then embedded in paraffin and cut into 3 μm-thick sections. Sections were stained with hematoxylin and eosin, and images taken with an Olympus BX51 microscope (Olympus, Tokyo, Japan) with a 40× objective lens.

In addition to W/D weight ratios of lung tissue, a published scoring system was applied to evaluate the lung injury visible under a light microscopy. The degree of injury was scored based on alveolar and interstitial edema, neutrophil infiltration, and hemorrhage. Injury severity was graded for each variable: no injury = 0; injury to 25% of the field = 1; injury to 50% of the field = 2; injury to 75% of the field = 3; and diffuse injury = 4.[24] Samples were analyzed based on a scaled grading system by a pathologist who was blinded to the experimental protocol and the region of sampling. Three slides from each lung sample were randomly screened, and the mean taken as the representative value of the sample. The general injury score = edema score + neutrophil infiltration score + hemorrhage score. For presentation, we chose typical examples which were observed in all preparations for the same treatment.

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from frozen lung tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and a RNA kit (BioMed, Beijing, China). RNA was then reverse-transcribed to synthesize first-strand cDNA (Bioer, Hangzhou, China). Quantitative RT-PCR was done using a Line-Gene Sequence Detector (Bioer, Hangzhou, China). The primers of AQP1, AQP5, and beta-actin are listed in Table 1. PCR was performed using a BioEasy SYBR Green I Real-Time PCR Kit (BioMed). For the analysis of AQP1 and AQP5, the reaction mixture contained 25 μL of 2XSYBR Mix, 1 μL of 10 μM of forward primer, 1 μL of reverse primer, 0.3 μL of Taq DNA Polymerase, 2 μL of cDNA and 20.7 μL of ddH_2O. The thermal cycling conditions were: 95°C for 2 min for 1 cycle followed by 45 cycles of 95°C for 20 s, 60°C for 25 s, and 72°C for 30 s. Data was analyzed by the software attached to the detector (Bioer, Hangzhou, China). The size of RT-PCR products was confirmed by electrophoresis on 1% agarose gels. PCR products were sequenced, the sizes of amplicons for AQP1, AQP5 and beta-actin were 213 bp, 186 bp, and 150 bp, respectively. The relative quantification of mRNA expression was calculated by the $2^{-\Delta Ct}$ method.

Protein Preparation

Peripheral lung tissues were frozen in liquid nitrogen, and stored at -80°C until analysis. Tissues were homogenized in ice-cold isolation solution containing 250 mM sucrose, 10 mM triethanolamine, 1 μg/mL leupeptin, and 0.1 mg/mL phenylmethyl sulphonylfluoride. Homogenates were centrifuged at 12000 rpm for 10 min at 4°C. Supernatants were obtained, and protein concentrations measured using a protein assay kit (Sunbio, Beijng, China). An N-glycosidase F deglycosylation kit (Roche, Mannheim, Germany) was used for deglycosylation of proteins.

Western Blot

Total proteins (50 μg/sample) were diluted in 5× loading buffer (Tris HCl 0.25 M, pH = 6.8; sodium dodecyl sulfate (SDS) 10%; bromophenol blue 0.5%; glycerol 50%; dithiothreitol 0.5 M), then boiled for 5 min. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 12% gradient gels. Proteins were transferred electrophoretically to polyvinylidene difluoride (PVDF) (Bioer, Hangzhou, China). membranes treated with methanol. Proteins were blocked for 1 h at room temperature in TBS-T (Tris-buffered saline containing 0.1% Tween 20) containing 5% non-fat dry milk and incubated overnight at 4°C with anti-AQP1 antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-AQP5 antibody (1:200; Santa Cruz Biotechnology) in TBS-T containing 5% non-fat dry milk.

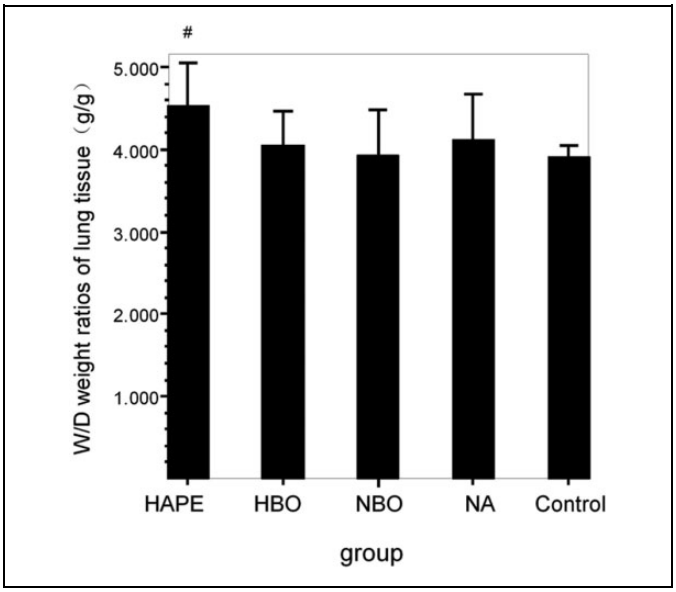

Figure 1. W/D weight ratio W/D weight ratio in the Control group (n = 7), the HAPE group, the HBO group, the NBO group and the NA group (N = 8). #P < 0.05 for HAPE group vs Control group. Values are presented as mean ± S.D.

After washing in TBS-T, membranes were incubated with horseradish peroxidase (HRP)-labeled anti-rabbit antibody (1:3000; Santa Cruz, Biotechnology) for 2-3 h at room temperature. Blots were developed with enhanced chemiluminescence agents (ECL Plus; Sunbio) before exposure to X-ray film. To confirm equivalent loading of samples, the same membranes were incubated with anti-β-actin antibody (1:1000; Santa Cruz Biotechnology) and visualized via enhanced chemiluminescence as described above. For quantification, films of western blots were scanned using a Minolta scanner and Adobe Photoshop software. The densitometry analysis was performed using LabWorks software (UVP, Upland, CA, USA). The relative density of AQP1 and AQP5 bands was normalized to the density of actin to represent the amount of AQP1 and AQP5 protein. The value of the control group was regarded to be 100%. The results of the HBO-PC and HAPE groups were expressed as a percentage of the value of the control group.

Statistical Analyses

Statistical analyses were carried out using SPSS15.0 software (SPSS, Chicago, IL, USA). All quantitative data were expressed as mean ± SD. One-way analysis of variance (ANOVA) or independent sample *t*-test were used to determine the differences among groups. $P < 0.05$ was considered significant.

Results

W/D Weight Ratio

The W/D ratio in the HAPE group was higher than that of the control group ($p < 0.05$), indicating increased lung water content in the HAPE group. However, no significant difference was observed between the HBO group and NBO group, or between the HBO group and NA group, suggesting no significant advantage of HBO over NBO and NA with respect to lung water content (Figure 1).

Lung Morphology

When the altitude was raised to 6000 m, rats demonstrated symptoms of lethargy, tachypnea and respiratory distress in the hypobaric chamber. However, after exposure for 24 h, with altitude descent, all above-mentioned symptoms were alleviated. When the chest was opened, the lungs of HAPE rats exhibited congestion, swelling, and hemorrhage. Histological examination of the lungs revealed marked interstitial edema in all sections in the HAPE group, suggesting that a rat model of HAPE was established. In this model, interstitial edema was described as a thickened interalveolar septum, expandable capillaries, and pink exudation in the alveolar space. The injury scores in the HBO group and NBO group were significantly decreased compared with the HAPE group (both $p < 0.01$). The injury score in the HBO group was significantly lower than that in the NA group ($p < 0.01$) and NBO group ($p < 0.05$). These results implied that oxygen exposure attenuated lung injury induced by hypobaric hypoxia at high altitude. Additionally, HBOT showed significant advantage over NBOT with respect to lung injury scores (Figure 2).

The Protein Levels of AQP

Western blot revealed that the expressions of AQP1 and AQP5 in the HAPE group were significantly reduced compared with the control group (both $p < 0.01$), implying that impaired movement of water resulting from the downregulation of AQP1 and AQP5 was correlated with the formation of HAPE. Significant differences were observed in the expressions of AQP1 and AQP5 between HBO and NBO ($p < 0.01$, $p < 0.05$), and between HBO and NA groups (both $p < 0.01$). AQP1 in the HBO and NBO groups were much higher than that in the HAPE group ($p < 0.01$, $p < 0.05$). AQP5 in the HBO, NBO, and NA group were much higher than that in the HAPE group (all $p < 0.01$). These results indicated that the downregulation of AQP1 and AQP5 in the lungs of HAPE rats could be upregulated by oxygen exposure. HBO had more significant effect on the expressions of AQP1 and AQP5 than NBO and NA (Figure 3).

The mRNA Levels of AQP1 and AQP5

The mRNA transcription level of AQP1 in the HAPE group was significantly lower than that in the control group ($p < 0.01$). Significant differences were also found between the HBO and NBO, and the HBO and NA groups ($p < 0.01$). The mRNA level of AQP1 in the HBO and NBO group (but not in the NA group) was markedly increased compared with that in the HAPE ($p < 0.01$). With respect to AQP5 mRNA expression,

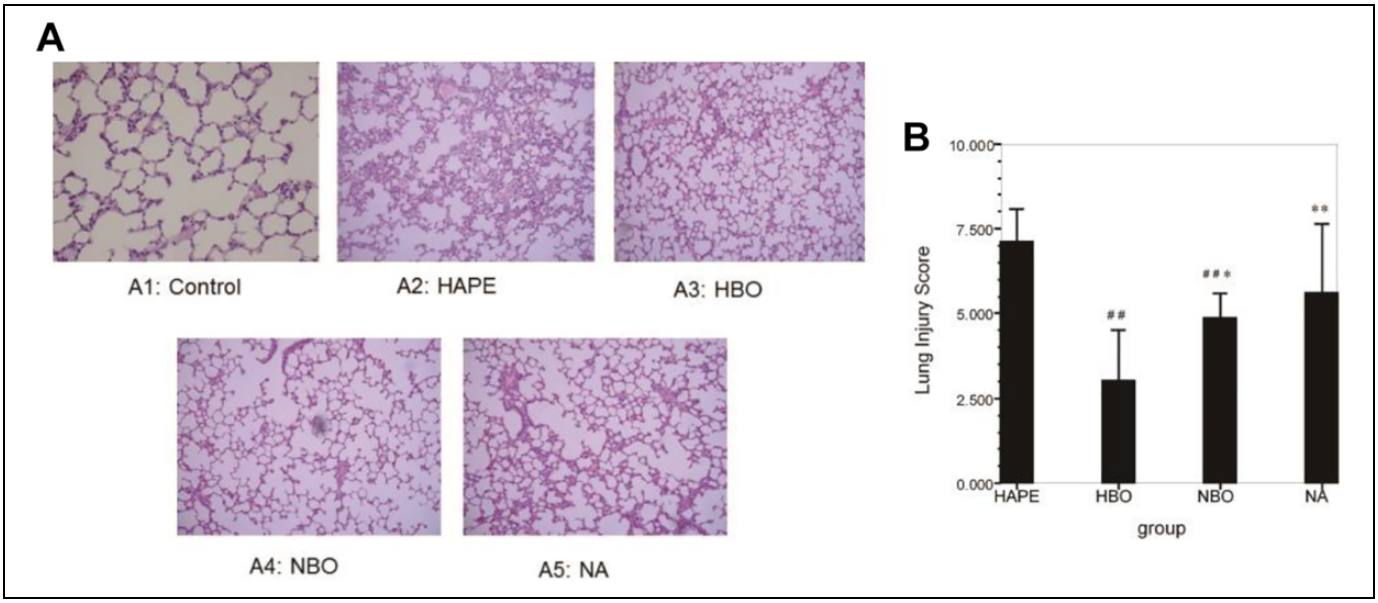

Figure 2. A) Histological changes of the lung (H&E stain. magnification 40×). A1: Control Group A2: HAPE Group A3: HBO Group A4: NBO Group A5: NA Group. B) Histology Injury Score. ##P < 0.01 for the HBO group and the NBO group vs the HAPE group; **P < 0.01 for the NA group vs the HBO group and *P < 0.05 for the NBO group vs the HBO group (all N = 8). Values are presented as mean ± S.D.

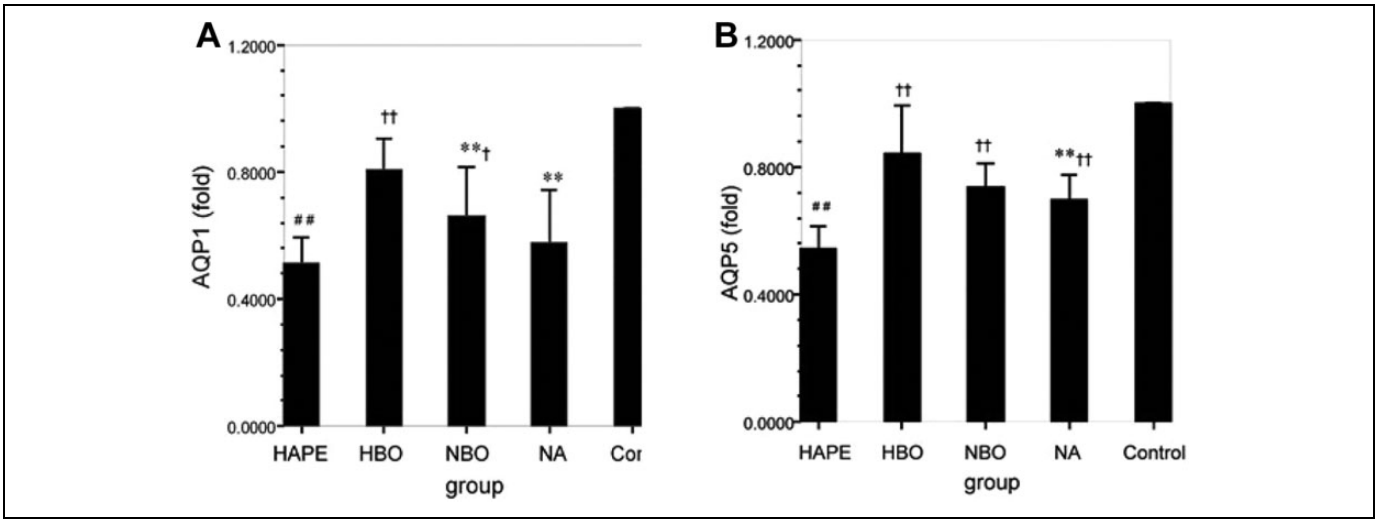

Figure 3. Western blot analyses of AQP1 (A) and AQP5 (B) in lungs. Western blot for AQP1, AQP5 and β-actin (C). Fold-change values represent a mean of 8 samples (n = 8) divided by the mean of the 7 controls (n = 7). ##P < 0.01 for HAPE group vs Control group; ††P < 0.01, †P < 0.05 for the HBO group, NBO group and the NA group vs the HAPE group. **P < 0.01, *P < 0.05 for the NA group and the NBO group vs the HBO group. Data are presented as mean ±S.D.

it was also significantly lower in the HAPE group than in the control group ($p < 0.01$). A significant difference was only found between the HBO and NA groups ($p < 0.01$), but not between the HBO and NBO groups. There were no significant differences between the HAPE and HBO, and between the HAPE and NBO groups. Furthermore, the mRNA level of AQP5 in the NA group was lower than that in the HAPE group ($p < 0.05$). Taken together, AQP1 and AQP5 play an important role in the pathogenesis of HAPE. Compared with NA, HBO had evident effect on the upregulation of AQP1 and AQP5 in lung tissues. Compared with NBO, HBO had more robust regulatory effect on the mRNA expression of AQP1 in the lung (Figure 4).

Discussion

Establishment of the HAPE Model

There are 2 types of pulmonary edema: interstitial pulmonary edema and alveolar pulmonary edema. If the fluid in

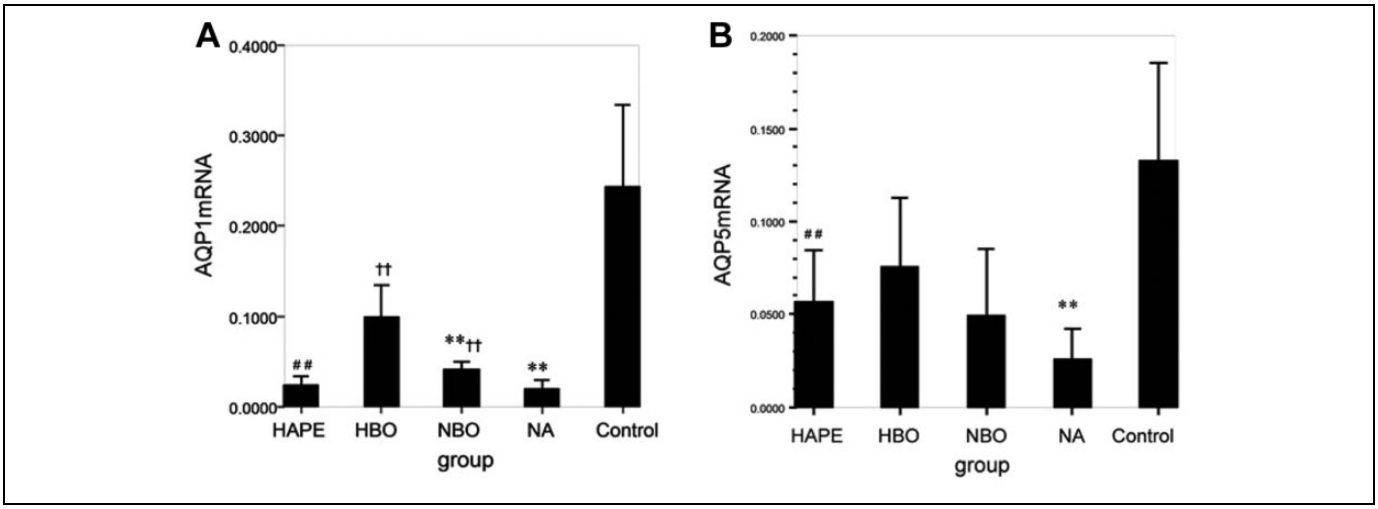

Figure 4. Real-time PCR analyses of AQP1mRNA (A) and AQP5mRNA (B) in lungs. $^{\#\#}P < 0.01$ for HAPE group (n = 8) vs Control group (n = 7); $^{\dagger\dagger}P < 0.01$, $^{\dagger}P < 0.05$ for the HBO group (n = 8), the NBO group (n = 8) and the NA group (n = 8) vs the HAPE group. $^{**}P < 0.01$, $^{*}P < 0.05$ for the NA group and the NBO group vs the HBO group. Data are presented as mean ± S.D.

pulmonary interstitial edema cannot be absorbed effectively, alveolar pulmonary edema will develop. Therefore, interstitial pulmonary edema is an important pathological phase of alveolar pulmonary edema. Observations in mountain climbers revealed that nearly 80% of fast-ascending climbers had subclinical interstitial pulmonary edema, whereas the incidence of full-blown HAPE is much lower,[25,26] suggesting that interstitial pulmonary edema is more common among mountaineers. In the current study, we investigated the effect of HBOT on the expressions of AQP1 and AQP5 in a rat model of interstitial pulmonary edema, in which rats were exposed to a simulated altitude of 6000 m for 24 h. Our results revealed that rats in the HAPE group exhibited typical interstitial pulmonary edema and demonstrated significantly increased lung water content, implying that the HAPE model in rats was successfully established.

HBOT and HAPE

The beneficial effects of HBOT on HAPE include increased tissue oxygenation, inhibition of ischemia–reperfusion injury, modification of neutrophil function, and impairment of bacterial replication, which are involved in the overall antioxidant and anti-edema effect.[27-29] HBO specifically suppresses tumor necrosis factor-alpha (TNF-α) and hypoxia-inducible facor-1 (HIF-1), which have been proven to be related to the pathogenesis of HAPE.[30] The exposure to HBO enhanced K-ATPase activity, increased active Na^+ transport, and accelerated lung edema clearance.[31] All of these features may significantly improve the outcomes in HAPE. Liu et al. compared the therapeutic effect of HBOT plus conventional therapy with conventional treatment alone in 90 patients with HAPE. They found that HBOT, if used as an adjuvant, could shorten the duration of pulmonary edema.[19] Duan et al. observed 32 HAPE patients from a plateau of 4636-5130 m and reported that, HBOT significantly reduced the elevated pulmonary arterial pressure in HAPE compared with NBOT. HBOT is highly recommended in treating HAPE among patients at extremely high altitude.[20]

In the present study, oxygen exposure had more beneficial effect on HAPE than normal air exposure. Except for W/D ratio and mRNA expression of AQP5, HBO had significant advantage over NBO and NA, which was consistent with the above-mentioned studies. During the procedure of establishing HAPE models, rats gradually developed lethargy, tachypnea, and respiratory distress with the simulated increase in altitude in the hypobaric chamber. Also, the intake of food and water was reduced and some rats did not eat and drink during the exposure. The findings might result in a reduction in water content. After being taken out of the hypobaric chamber and treated with normal air, all these symptoms were improved, which accordingly led to varied degree of increase in water content.

The main concern in the use of HBO for pulmonary disorders is the fear of oxygen-induced pulmonary toxicity.[32] A review showed that HBOT-induced lung injury was related to prolonged treatment at high therapeutic pressure, and appeared to be less of a concern at hyperbaric pressures < 2.0 atmospheres.[33] Our results indicated that the properly use of worked well on HAPE and had advantages over normobaric oxygen therapy.

AQPs and HAPE

The pathogenesis of HAPE is complicated. In general, the accepted mechanism is a sequential process of high-altitude hypoxia-induced pulmonary hypertension, increased capillary permeability, and compromise of the alveolar epithelial barrier resulting in pulmonary edema.[34] In this process, injury to the capillary endothelium and alveolar epithelium appear to play

an important role in HAPE development. More than 90% of the internal surface area of the lung is lined by alveolar epithelial Type-I cells, and AQP5 is mainly distributed in the apical membrane of Type-I alveolar epithelial cells. AQP1 in primarily located in microvascular endothelia.[35] Therefore, it is likely that the injury in the capillary endothelium and alveolar epithelium affect the function and expression of AQP1 and AQP5. Studies have indicated that AQP1 and AQP5 provide the principal route for osmotically-driven water transport across the microvascular endothelial barrier and alveolar epithelia, respectively.[13,14] Considering the important role of AQP1 and AQP5 in ALI and formation of pulmonary edema, we hypothesize that water transport mediated by AQP1 and AQP5 may be involved in the pathogenesis of HAPE.

The expressions of AQP1 and AQP5 in the lungs of rats in the HAPE group were significantly decreased compared with the control group. Except for the mRNA level of AQP5, the expressions of AQP1 and AQP5 in the HBO and NBO groups were increased with the relief of symptoms. These data suggested the important roles of AQP1 and AQP5 in the pathogenesis of HAPE. In the lungs of HAPE rats, the lower expression of AQP1 and AQP5 may affected the absorption of water in the airspace, interstitial, and capillary compartments, which could consequently result in disordered fluid transportation and hypobaric hypoxia edema. Recently evidence suggested that AQP-1 transports nitric oxide (NO) across cell membranes.[36]

The present study also demonstrated that the decrease in the level of AQP5 was less than that of AQP1. Because of the different distribution of AQP1 and AQP5 in the respiratory system, their functions on water transport in the lungs differ. AQP1 mainly affects interstitial edema and AQP5 affects alveolar edema. In the present study, most of the rats in the HAPE group had interstitial edema, which might explain why AQP1 expression decreased more than AQP5 expression.

Another interesting finding in the present study was that different from AQP1, the AQP5 protein, AQP1 mRNA, and AQP5 mRNA were decreased in the NBO and NA groups compared with the HAPE group, which may be due to the fact that the effect of hypobaric hypoxia at high altitude on the downregulation of AQP5 lasted for several hours. Jiao et al. obtained a similar result in a LPS-induced model of ALI in rats, in which AQP1 expression was partly resumed at 24 h after LPS treatment and steroid administration, whereas the expression of AQP5 was unchanged.[16] In the present study, only the mRNA expression of AQP5 in the HBO group was increased compared with the HAPE group, suggesting a significant effect of HBO on the upregulation of AQP5 mRNA.

Studies on the regulation of AQPs showed that the expression of AQP is regulated by growth factors, TNF-α, inflammatory mediators, and osmotic stress (9). We wondered how HBO regulates the expression of AQP1 and AQP5 at the mRNA and protein levels. We hypothesized that HBO changes osmotic stress by improving the epithelial Na^+ channel (ENaC) and the enzymatic activity of Na^+-K^+-ATP kinase,[31] and HBO regulates HIF-1 and its downstream genes, including vascular endothelial growth factor (VEGF), TNF-α, and some inflammatory mediators.[37]

There are some limitations of this study. First, the sample size was relatively small and only male rats were analyzed. Second, the varied food and water intake among groups might affect the W/D ratio of the lung tissues. Third, the regulation of AQPs on other signaling pathways or ion channels needs to be explored.

Conclusions

The present study demonstrated that HBOT alleviated lung injury induced by high-altitude hypobaric hypoxia in rats. HBOT showed significant advantage over NBOT. The pulmonary edema in rats induced by high-altitude hypoxia were associated with the downregulation of AQP1 and AQP5 in the lung, and this downregulation could be attenuated by HBO therapy. This is the first study showing the effect of HBO exposure on the mRNA and protein expressions of AQP1 and AQP5 in a rat model of HAPE. Further investigations are needed to identify the therapeutic role and the mechanisms of HBOT on HAPE.

Author Note

The authors thank Professor Huaping Dai and Dr. Christian Heiden (Traunstein, Germany) for their helpful advice and suggestions. We also wish to thank Professor Huajun Xiao for his excellent technical assistance.

Acknowledgments

This study was supported by the International Science and Technology Cooperation Program of the Ministry of Science and Technology of China (2012DFA31240).

Author Contribution

The conception or design of the work: Jiewen Tan, Chunjin Gao, Zhuo Li. The acquisition, analysis, or interpretation of data: Chunjin Gao, Cong Wang, Linlin Ma, Xiaomin Hou, Xuehua Liu. The creation of new software used in the work: Linlin Ma. Drafted the work and substantively revised the work: Xuehua Liu, Zhuo Li.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The International Science and Technology Cooperation Program of the Ministry of Science and Technology of China (2012DFA31240).

ORCID iD

Zhuo Li, PhD https://orcid.org/0000-0002-8184-6678

References

1. Pasha MA, Newman JH. High-altitude disorders: pulmonary hypertension: pulmonary vascular disease: the global perspective. *Chest*. 2010;137(6 Suppl):13S-19S.
2. Liptzin DR, Abman SH, Giesenhagen A, Ivy DD. An approach to children with pulmonary edema at high altitude. *High Alt Med Biol*. 2018;19(1):91-98.
3. Eide RP 3rd, Asplund CA. Altitude illness: update on prevention and treatment. *Curr Sports Med Rep*. 2012;11(3):124-130. doi:10.1249/JSR.0b013e3182563e7a
4. Imray C, Wright A, Subudhi A, Roach R. Acute mountain sickness: pathophysiology, prevention, and treatment. *Prog Cardiovasc Dis*. 2010;52(6):467-484. doi:10.1016/j.pcad.2010.02.003
5. Guo L, Tan G, Liu P. Three plasma metabolite signatures for diagnosing high altitude pulmonary edema. *Sci Rep*. 2015;5:15126. Published October 13, 2015.
6. Hou YP, Wu JL, Tan C, Chen Y, Guo R, Luo YJ. Sex-based differences in the prevalence of acute mountain sickness: a meta-analysis. *Mil Med Res*. 2019;6(1):38.
7. Luks AM, Swenson ER, Bärtsch P. Acute high-altitude sickness. *Eur Respir Rev*. 2017;26(143):160096.
8. Bhagi S, Srivastava S, Singh SB. High-altitude pulmonary edema: review. *J Occup Health*. 2014;56(4):235-243. doi:10.1539/joh.13-0256-ra
9. Verkman AS. Role of aquaporins in lung liquid physiology. *Respir Physiol Neurobiol*. 2007;159(3):324-330. doi:10.1016/j.resp.2007.02.012
10. King LS, Agre P. Pathophysiology of the aquaporin water channels. *Annu Rev Physiol*. 1996;58:619-648. doi:10.1146/annurev.ph.58.030196.003155
11. Agre P, Bonhivers M, Borgnia MJ. The aquaporins, blueprints for cellular plumbing systems. *J Biol Chem*. 1998;273(24):14659-14662. doi:10.1074/jbc.273.24.14659
12. Zea B, Verkman AS. Invited review: role of aquaporin water channels in fluid transport in lung and airways. *J Appl Physiol*. 2002;93(6):2199-2206. doi:10.1152/japplphysiol.01171.2001
13. Bai C, Fukuda N, Song Y, Ma T, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J Clin Invest*. 1999;103(4):555-561. doi:10.1172/JCI4138
14. Ma T, Fukuda N, Song Y, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-5 knockout mice. *J Clin Invest*. 2000;105(1):93-100. doi:10.1172/JCI8258
15. Towne JE, Harrod KS, Krane CM, Menon AG. Decreased expression of aquaporin (AQP)1 and AQP5 in mouse lung after acute viral infection. *Am J Respir Cell Mol Biol*. 2000;22(1):34-44. doi:10.1165/ajrcmb.22.1.3818
16. Jiao G, Li E, Yu R. Decreased expression of AQP1 and AQP5 in acute injured lungs in rats. *Chin Med J (Engl)*. 2002;115(7):963-967.
17. Leach RM, Rees PJ, Wilmshurst P. Hyperbaric oxygen therapy. *BMJ*. 1998;317(7166):1140-1143. doi:10.1136/bmj.317.7166.1140
18. Basnyat B. High altitude cerebral and pulmonary edema. *Travel Med Infect Dis*. 2005;3(4):199-211. doi:10.1016/j.tmaid.2004.06.003
19. Liu LH, Cheng B. Observation on the clinical effect of hyperbaric oxygen in treating 90 cases of high altitude pulmonary edema. *J Mil Surg in Southwest China*. 2008;10:49-50.
20. Duan J, Zhang X, Liu H. Impact of pulmonary-artery pressure for hyperbaric oxygenation (HBO) treatment of high altitude pulmonary edema (HAPE) on the spot of extreme altitude. *J Qing Hai Med Col*. 2004;25:301-303.
21. Pablos MI, Reiter RJ, Chuang JI, et al. Acutely administered melatonin reduces oxidative damage in lung and brain induced by hyperbaric oxygen. *J Appl Physiol (1985)*. 1997;83(2):354-358. doi:10.1152/jappl.1997.83.2.354
22. Speit G, Bonzheim I. Genotoxic and protective effects of hyperbaric oxygen in A549 lung cells. *Mutagenesis*. 2003;18(6):545-548. doi:10.1093/mutage/geg028
23. Li Z, Gao C, Wang Y, et al. Reducing pulmonary injury by hyperbaric oxygen preconditioning during simulated high altitude exposure in rats. *J Trauma*. 2011;71(3):673-679. doi:10.1097/TA.0b013e3181f5b073
24. Su X, Song Y, Jiang J, Bai C. The role of aquaporin-1 (AQP1) expression in a murine model of lipopolysaccharide-induced acute lung injury. *Respir Physiol Neurobiol*. 2004;142(1):1-11. doi:10.1016/j.resp.2004.05.001
25. Cremona G, Asnaghi R, Baderna P, et al. Pulmonary extravascular fluid accumulation in recreational climbers: a prospective study. *Lancet*. 2002;359(9303):303-309. doi:10.1016/s0140-6736(02)07496-2
26. Gabry AL, Ledoux X, Mozziconacci M, Martin C. High-altitude pulmonary edema at moderate altitude (< 2,400 m; 7,870 feet): a series of 52 patients. *Chest*. 2003;123(1):49-53. doi:10.1378/chest.123.1.49
27. Nikfarjam M, Cuthbertson CM, Malcontenti-Wilson C, Muralidharan V, Millar I, Christophi C. Hyperbaric oxygen therapy reduces severity and improves survival in severe acute pancreatitis. *J Gastrointest Surg*. 2007;11(8):1008-1015. doi:10.1007/s11605-007-0175-2
28. Chen HM, Shyr MH, Ueng SW, Chen MF. Hyperbaric oxygen therapy attenuates pancreatic microcirculatory derangement and lung edema in an acute experimental pancreatitis model in rats. *Pancreas*. 1998;17(1):44-49.
29. Balkan A, Balkan M, Yasar M, et al. Pulmonary protective effects of hyberbaric oxygen and N-acetylcysteine treatment in necrotizing pancreatitis. *Physiol Res*. 2006;55(1):25-31. doi:10.2310/6650.2004.03038
30. Engebretsen BJ, Irwin D, Valdez ME, O'Donovan MK, Tucker A, van Patot MT. Acute hypobaric hypoxia (5486 m) induces greater pulmonary HIF-1 activation in hilltop compared to Madison rats. *High Alt Med Biol*. 2007;8(4):312-321. doi:10.1089/ham.2007.1031
31. Harris ZL, Ridge KM, Gonzalez-Flecha B, Gottlieb L, Zucker A, Sznajder JI. Hyperbaric oxygenation upregulates rat lung Na, K-ATPase. *Eur Respir J*. 1996;9(3):472-477. doi:10.1183/09031936.96.09030472
32. Jain KK. *Textbook of Hyperbaric Medicine*. Edited by Hogrefe & Huber Publishers; 2004:334-335.
33. Wada K, Miyazawa T, Nomura N, Tsuzuki N, Nawashiro H, Shima K. Preferential conditions for and possible mechanisms

of induction of ischemic tolerance by repeated hyperbaric oxygenation in gerbil hippocampus. *Neurosurgery*. 2001;49(1): 160-166; discussion 166-167. doi:10.1097/00006123-20010 7000-00025

34. Bartsch P. High altitude pulmonary edema. *Respiration*. 1997; 64(6):435-443. doi:10.1159/000196720
35. Nielsen S, King LS, Christensen BM, Agre P. Aquaporins in complex tissues. II. Subcellular distribution in respiratory and glandular tissues of rat. *Am J Physiol*. 1997;273(5): C1549-1561. doi:10.1152/ajpcell.1997.273.5.C1549
36. Herrera M, Hong NJ, Garvin JL. Aquaporin-1 transports NO across cell membranes. *Hypertension*. 2006;48(1):157-164. doi:10.1161/01.HYP.0000223652.29338.77
37. Blanco YC, Farias AS, Goelnitz U, et al. Hyperbaric oxygen prevents early death caused by experimental cerebral malaria. *PLoS One*. 2008;3(9):e3126. doi:10.1371/journal.pone.0003126