Effects of altitude changes on mild-to-moderate closed-head injury in rats following acute high-altitude exposure

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Abstract. Mild-to-moderate closed-head injury (mmCHI) is an acute disease induced by high-altitudes. It is general practice to transfer patients to lower altitudes for treatment, but the pathophysiological changes at different altitudes following mmCHI remain unknown. The present study simulated acute high-altitude exposure (6,000 m above sea level) in rats to establish a model of mmCHI and recorded their vital signs. The rats were then randomly assigned into different altitude exposure groups (6,000, 4,500 and 3,000 m) and neurological severity score (NSS), body weight (BW), brain magnetic resonance imaging (MRI), brain water content (BWC) and the ratio of BW/BWC at 6, 12 and 24 h following mmCHI, and the glial fibrillary acidic protein levels were analysed in all groups. The results revealed that within the first 24 h following acute high-altitude exposure, mmCHI induced dehydration, brain oedema and neuronal damage. Brain injury in rats was significantly reversed following descent to 4,500 m compared with the results from 6,000 or 3,000 m. The results indicated that subjects should be transported as early as possible. Furthermore, avoiding large-span descent altitude was beneficial to reduce neurological impairment. The examination of brain-specific biomarkers and MRI may further be useful in determining the prognosis of high-altitude mmCHI. These results may provide guidance for rescuing high altitude injuries.

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Introduction

With the advancement in transportation options, millions of people travel to areas of high-altitude for recreational, sporting and military purposes. Thus, the occurrence of accidental injuries at high-altitudes has risen rapidly by 12.7% (1-3). Injuries from road traffic accidents maintained high mortality rates also at high-altitudes (4) and traumatic brain injury (TBI) obtained at high-altitudes are one of the most serious acute diseases (5). The main feature of high-altitude is hypobaric hypoxia, which often causes damage to the body, including high-altitude cerebral edema or high-altitude pulmonary edema, as a result, the central nervous system, circulation and respiratory system function impairment occurs (6). High-altitude cerebral oedema (HACE) and dehydration are common in individuals who are not acclimated to the altitude and can lead to mortality (7-9). TBI following abrupt exposure to higher altitudes can be more complicated than when observed at sea level. Mild-to-moderate closed-head injury (mmCHI) accounts for 81.53% of all TBI cases recorded in the higher regions of China (>3,000 m above sea level) (10). The majority of patients with mmCHI are conscious, exhibit concussions and mild brain contusions (11). In mild cases, certain patients are able to travel to nearby hospitals by themselves or be transported by medical staff. However, mmCHI cases at high-altitude are not well characterized. With inadequate knowledge of the pathophysiological changes in high-altitude mmCHI, clinicians often exclude mmCHI as a cause for TBI due to the high Glasgow Coma Score observed, which evaluate the extent of neurological damage (12). At present, no relevant guidelines about high-altitude mmCHI have been implemented (13,14).

In the present study, to simulate a rapid ascent to high-altitudes causing acute hypobaric hypoxia (AHH), 6,000 m above sea level was chosen as model high-altitude and hypobaric chambers were automatically adjusted depending on the pre-set pressures selected to simulate different high-altitude models in rats (extreme high-altitude,

5,500-8,850 m; medium high-altitude, 3,500-5,500 m; high-altitude, 1,500-3,500 m) (15). Weight-drop models were used to reproduce the physiopathology and the macro-and microscopic alterations in TBI, which were observed via imaging techniques and by examining neuronal damage markers to study new therapeutic approaches. The aim of the present study was to investigate the association between cerebral oedema, body weight (BW) and neurological function following acute high-altitude mmCHI, using magnetic resonance imaging (MRI) and glial fibrillary acidic protein (GFAP) immunohistochemistry to analyse whether transporting patients to different altitudes has an effect on injury outcome. The effects of rapid transport to lower altitudes on brain injury, pathophysiological association with altitude and the time window for most effective transport were further evaluated. The results of the current study may provide a reference for the clinical treatment of mmCHI at high altitude.

Materials and methods

Animals. A total of 162 male Sprague-Dawley rats (weight, 200±20 g; age, 7-8 weeks) were supplied by the Experimental Center for Medical Animals, Research Institute of Surgery, Daping Hospital (Third Military Medical University, Chongqing, China). Animals were allowed 2 days prior to experiments to acclimatize to the facilities and for training according to the neurological severity score (NSS) guidelines (16). All rats were housed under a controlled temperature (18˚C) with 12-h light/dark cycles and the range of atmosphere (Atm) was 970-1,000 Pascal (Pa). Rats had access to food and water *ad libitum*. Experiments were approved by the Ethics Committee of Third Affiliated Hospital and Research Institute of Surgery, Third Military Medical University (registration no. ChiCTR-RPC-15006770; Chongqing, China).

Preparation of animal models and grouping. The present study established models of AHH and simulated conditions of different altitudes. Rats were placed into a decompression chamber (Chongqing Key Laboratory of Vehicle Crash/Bio-impact and Traffic Safety, Institute for Traffic Medicine, Third Military Medical University, Chongqing, China) for exposure to an extreme high-altitude of 6,000 m at a velocity of 6.7 m/sec for 15 min, with -52 to -57 kPa and the corresponding oxygen content was 149 g/m^3 for 24 h. The chamber humidity was set to 40-50% and temperature was maintained at 18˚C with a 12-h light/dark cycle, in addition to free access to water and food. The CHI model was established via brain injury using a weight-drop device (17-19), modified for a pneumatic impact at a force of 0.8 MPa (6.67 m/sec; 955.6±16.35 N). Rats were anaesthetised with 2% isoflurane (1.5 l/min) using anaesthesia equipment (ZS-M; Beijing Zhongshi Di Chuang Technology Development Co., Ltd., Beijing, China) for ~2 min prior to injury. Animals that perished following the trauma or due to skull fractures were excluded from the present study. Cardiopulmonary resuscitation (CPR) was performed immediately when rats were found to have apnea following mmCHI. The oropharyngeal airway tube was placed immediately and chest compressions were performed until rats recovered spontaneous breathing. In the experimental study, uninjured control rats were simulated to ascend rapidly from plain (200 m above sea level) to plateau (6,000 m above sea level) and were then maintained in a continuous hypobaric environment with hypoxia at an altitude of 6,000 m for 48 h (n=6). mmCHI rats, following exposure to 6,000 m altitude for 24 h, were divided into groups according to altitude: Non-descending altitude group (ND; maintained at 6,000 m); D-4,500 m group (descent to 4,500 m; pressure: -42 to -47 kPa; oxygen content, 176 g/m^3); and D-3,000 m group (descent to 3,000 m; pressure, -30 to -35 kPa; oxygen content, 206 g/m^3). In order to observe changes in injuries over time, each mmCHI group was divided into three subgroups: 6, 12 and 24 h following descend (n=18/subgroup).

Determination of BW, vital signs and NSS. Weights of rats were determined prior to being placed in the decompression chamber and at 24 h following exposure to high-altitude. Spontaneous breathing, level of consciousness and limb function following injury were observed and recorded. According to the NSS standards (19), scoring was performed 30 min following injury. The duration of coma and apnoea were further recorded. Following the initial scoring, rats in each group were evaluated and weighed again at 6, 12 and 24 h following descend.

Brain MRIscan. Rats in each group were randomly selected (n=6/group) and brain MRI scans were recorded at 6, 12 and 24 h following descend. Prior to MRI, rats were anaesthetised using the aforementioned method. Body temperature was continuously maintained at 37±0.5˚C and blood oxygen saturation and heart rate were monitored during the MRI scan. The 7.0T MRI (Bruker 7.0T/20 cm BioSpec-Avance system; Bruker Corporation, Ettlingen, Germany) was used to scan the brain tissues of rats through T2-weighted imaging [T2WI; rapid acquisition relaxation-enhanced (RARE) echo time, 30 msec; repetition time, 4,000 msec; spatial resolution: 256x256 matrix; field of view, 30x30 mm; slice thickness, 0.5 mm; interslice distance, 0 mm; RARE factor, 4], the field homogeneity across the brain was optimized and coronal scout images were obtained to orient the transverse slices throughout the brain region of interest (ROI).

Determination of brain water content (BWC). Injured rats in each group (n=6/group) were anesthetized with an intraperitoneal 1% sodium pentobarbital (v/v) injection (45 mg/kg) and brain tissues were collected following decapitation at 6, 12 and 24 h post-injury. The wet weight of each brain was measured using a precision electronic balance (BSA124S-CW; division value, d=0.1 mg; Sartorius AG, Göttingen, Germany). Tissues were then placed in a thermostatic oven and heated at 80˚C for 48 h to obtain a constant weight. In accordance with Elliot's formula (20), the percentage of BWC was calculated as follows: Water content (%)=[(wet weight-dry weight)/wet weight] x100%.

Morphological analysis. Injured rats of each group were randomly selected (n=6/group). Brain tissues of each group were collected at 24 h following mmCHI and fixed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) at 4˚C overnight. Samples were sectioned (10 μm) using a cryostat (Leica CM 1950; Leica Microsystems GmbH, Wetzlar, Germany) and

mounted on adhesion microscope slides. Pathological features of the transverse sections of brain tissues from the width of the lesion site were assessed by immunohistochemistry. Brain tissues slides were blocked for 1 h using 10% bovine serum albumin (Fraction V; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) at room temperature and 0.3% Triton X-100 and then incubated overnight at 4˚C with glial fibrillary acidic protein antibodies (GFAP; cat. no. YM3059; dilution, 0.5 μl/ml; ImmunoWay Biotechnology Company, Plano, TX, USA). Following washing with PBS, tissues were incubated for 1-2 h at room temperature with Alexa Fluor® 555 goat anti-rabbit IgG (cat. no. A27017; dilution, 2 μl/ml; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Following several PBS washes, nuclei were stained for 10 min at room temperature with DAPI Fluoromount-G (SouthernBiotech, Birmingham, AL, USA) and staining was detected via fluorescent microscopy (DM3000; Leica Microsystems GmbH). The number of positive cells was manually counted at 20x magnification and adjusted using image analysis software (Image-Pro plus 5.0; Media Cybernetics, Inc., Rockville, MD, USA) by two blinded, trained investigators. The ratio of positive cells was calculated as following: (Number of positive cells/total number of cells) x100%.

Statistical analysis. Factorial design with SAS9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The normal distribution of data (BWC and GFAP-positive cell levels) was measured and described using the mean ± standard deviation. The double-factor experimental design was compared between groups using two-way analysis of variance (ANOVA) with a Student-Newman-Keuls (SNK) post hoc test. Abnormal data distribution (NSS and BW) was measured and described using the median and quartile range (QR). The median value of NSS reduced used the double-factor experimental results were compared between groups using the Scheirer-Ray-Hare test and the Wilcoxon matched pairs signed-rank test. In addition, prior to using the SNK method, pair-wise comparisons of the primary data were sorted to generate new variables. The location of lesions in MRI images were determined using ImageJ (1.47v; National Institutes of Health, Bethesda, MD, USA) by tracing the area of quantification using the plug-in analysis tool. ROIs were segmented by manual selection or setting thresholds, then the area (cm^2) was calculated automatically according to the scale. Values of ROIs are presented as the mean ± standard deviation. Two-way ANOVA and post hoc Tukey's test were conducted to determine statistical significance using SPSS17.0 (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Vital signs and NSS. Following mmCHI, the vital sign analysis revealed that 57/126 rats exhibited either apnoea or seizure or both and that, of these 57 rats, 22 (38.6%) exhibited apnoea alone, 21 (36.8%) exhibited seizure alone and 14 (24.6%) exhibited both aponea and seizure. Rats underwent cardiopulmonary resuscitation (CPR) for 1-3 min until breathing resumed; one rat that perished due to failed CPR was excluded from the present study and was replaced with another rat that underwent the same group-specific procedures. The CHI procedure is the same as that of a previous study (21) and the observed mortality rate was 0.6% (1/162), which was consistent with data that has been previously reported (0-10%) (16,22). Mean coma duration was 13.13±10.4 min (n=162). NSS was determined (6±2.5) and limb dysfunction was not observed at 30 min following injury, which is in line with the reported effects of mmCHI (23). NSS analysis revealed that scores of injured rats in each group gradually improved over 24 h regardless of altitude changes. When comparing the time point effect using the Scheirer-Ray-Hare test between the groups, there was a consistent change in NSS score at the corresponding time points; however, the effect on the groups and the associations between the time points and the different groups was not statistically different. The results of different time point experiments on NSS revealed significant median reductions in NSS of 2.5 (QR, 1-5) at 12 h and 3 (QR, 1-4) at 24 h when compared with 1 (QR, 1-2) at 6 h ($H=10.29$; $P<0.05$; Fig. 1).

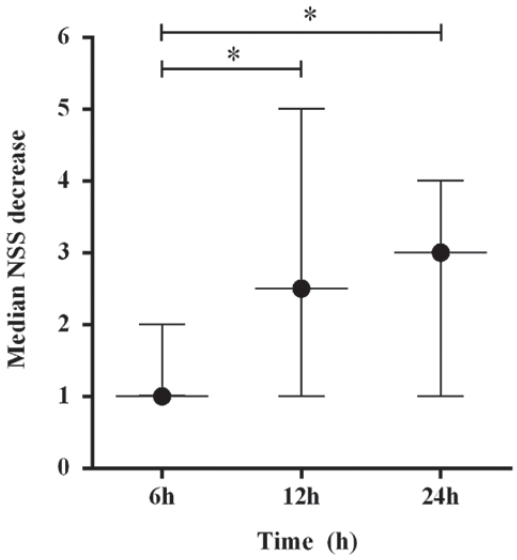

Figure 1. NSS continuously decreases over time in rats with mild-to-moderate closed head injury. Scheirer-Ray-Hare test results revealed a decrease in NSS for experimental groups; differences between initial NSS and NSS determined at varying time points is reported. NSSs were not significantly different between the groups, but at different time points. No association between time points and groups were observed. *$P<0.05$. NSS, neurological severity score.

BWchanges. Following exposure to AHH for 24 h and prior to injury, the median BW of rats decreased from 215.0 g (QR, 205.3-221.2 g) to 190.0 g (QR, 182.8-200.6 g). Results of Wilcoxon matched pairs signed-rank test demonstrated that the mean BW of rats significantly decreased by 23.9 g (QR, 12.3-26.4 g) at 24 h, accounting for 9.90% (QR, 2.00-15.30%) of the initial BW ($P<0.05$; Fig. 2A). The strongest change in BW was observed in the ND group following mmCHI. The BW loss for each group at each time point when compared with the initial weight was as follows: ND [6 h, 15.42% (QR, 10.00-20.00%); 12 h, 14.83% (QR, 13.50-16.60%); 24 h, 20.20% (QR, 17.60-21.20%)]; D-4,500 m [6 h, 15.58% (QR, 14.00-18.10%); 12 h, 15.56% (QR, 9.60-17.80%); 24 h, 17.87% (QR, 20.90-13.50%)]; and D-3,000 m [6 h, 10.08% (QR, 6.90-12.80%); 12 h, 14.08% (QR,

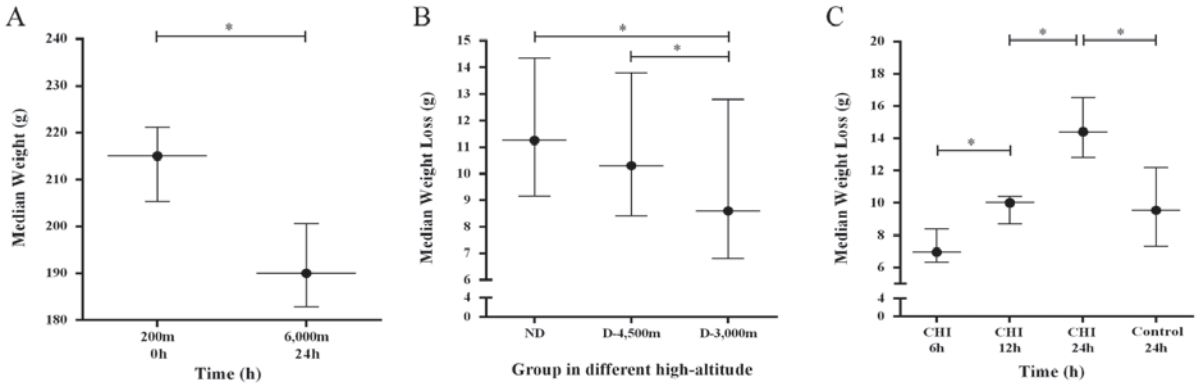

Figure 2. BW decreases over the time for rats in the high-altitude groups. (A) Wilcoxon matched pairs signed-rank test was applied to compare the BW of rats; The median weight from 200 m ascended to 6,000 m and exposure for 24 h was 23.9 (26.4-12.3) g, the difference was statistically significant, *P<0.05. (B) Median weight loss of rats with mmCHI in the various experimental groups; the difference to the initial BW is reported. *P<0.05. (C) Weight loss over time with the results for the groups combined. The control describes the median weight loss of rats not injured following acute high-altitude exposure at 48 h; all other animals were exposed to acute high-altitude for 24 h prior to injury and subsequent descend. *P<0.05. Plain, 200 m above sea level; Plateau, 6,000 m above sea level exposure for 24 h; BW, body weight; mmCHI, mild-to-moderate closed head injury; ND, non-descending altitude; D-4,500 m, descent to 4,500 m following injury; D-3,000 m, descent to 3,000 m following injury.

10.50-16.70%); 24 h, 14.90% (QR, 12.10-18.80%)]. BW changes following mmCHI with abnormal distribution were analysed using the Scheirer-Ray-Hare test at 6, 12, and 24 h following mmCHI; the difference between the final and initial BW for each group at different time points was calculated. BW comparisons of each group following injury revealed that the median BW loss in the D-3,000 m group [8.6 g (QR, 6.8-12.8 g)] was decreased compared with the ND group [11.15 g (QR, 9.15-14.35 g)] and the D-4,500 m group [10.3 g (QR, 8.4-13.8 g)]. It was demonstrated that weight loss significantly changed following mmCHI at different high-altitudes (H=6.96; P<0.05; Fig. 2B). Over time there significant changes in weight loss were observed for the injured rats of the ND, D-4,500 and D-3,000 m groups, with 6.95 g (QR, 6.3-8.4 g) between 0-6 h, 10.0 g (QR, 8.7-10.4 g) between 0-12 h, and 14.4 g (QR, 12.8-16.5 g) between 0-24 h (H=36.43; P<0.001; Fig. 2C). In the control group, comprised of rats that were not injured following acute high-altitude exposure, changes in BW between 0-24 h were 9.55 g (QR, 7.3-12.2 g), which was significantly decreased compared with the injured animals over the same period (P<0.05; Fig. 2C). No association between the various injury groups at different time point were determined with regards to the median BW (H=6.58; P=0.721).

BWC. BWC continuously increased in the ND group following mmCHI and it continuously decreased in the D-4,500 m and the D-3,000 m groups. A two-way ANOVA revealed no significant differences in BWC among the different groups or the time points (Fig. 3A). The present study calculated the ratio of BWC to BW (‰). Two-way ANOVA demonstrated that following mmCHI, BWC/BW increased in the ND group over time (6 h, 4.304±0.206‰; 12 h, 4.482±0.193‰; and 24 h, 4.884±0.217‰). In the D-4,500 and D-3,000 m groups at 6 h following injury, the BWC/BW was 4.389±0.215 and 3.987±0.275‰, respectively, and peaked at 12 h following injury with 4.548±0.264 and 4.451±0.224‰, respectively. In the following measurement at 24 h following mmCHI, the BWC/BW decreased in the D-4,500 and D-3,000 m groups (4.183±0.228 vs. 4.414±0.395‰). All changes exhibited by the various groups were statistically significant (F=7.22; P<0.05). When comparing the different time points following mmCHI at high-altitudes, results revealed that at 6 h the BWC/BW for D-3,000 m was the lowest and the difference was statistically significant compared with the ND and the D-4,500 m groups (P<0.05). At 12 h following mmCHI, no significant differences were observed between the groups. At 24 h following injury, the highest ratio was observed in the ND group, which was significantly increased compared with the D-3,000 and D-4,500 m groups (P<0.05). Additionally, an association between the different time-points and various groups was determined (F=8.68; P<0.05; Fig. 3B).

MRI analysis. To evaluate the diagnostic value of MRI on determining the level of brain damage in rats with mmCHI following AHH, brain tissues of rats were examined at 6, 12 and 24 h following injury at varying altitudes using T2WI. The MRI scan in T2WI revealed that the signals of cortex and white matter were uniform and there was no cerebral oedema, contusion or haemorrhage in the cortex of the impact region. Furthermore, it did not identify subarachnoid haemorrhages (SAH) or intraventricular haemorrhage (IVH) (data not shown). The region of the corpus callosum (CC) produced high signals, attributed to an increase in the BWC in these tissues. The lateral ventricles (LV) were markedly dilated at 6 h following injury in each group. Furthermore, the area of the ROIs were analyzed (Fig. 4A and B).

Two-way ANOVA revealed that time (F=211.376; P<0.001), altitude (F=206.061; P<0.001) and interactions between time and altitude (F=69.561; P<0.001) had significant effects on CC swelling. At 6 h post-mmCHI, CC swelling was significantly higher in the ND group (0.11±0.01 cm^2; P<0.05) compared with the D-3,000 m group (0.10±0.01 cm^2). However, there were no significant differences between the D-4,500 m group (0.10±0.01 cm^2) and the ND or D-3,000 m group. At 12 h post-mmCHI, CC swelling was significantly lower in the D-4,500 m group (0.03±0.01 cm^2; P<0.05) compared with the ND (0.12±0.01 cm^2) or D-3,000 m groups (0.12±0.01 cm^2). At 24 h post-mmCHI, CC swelling was significantly higher in the

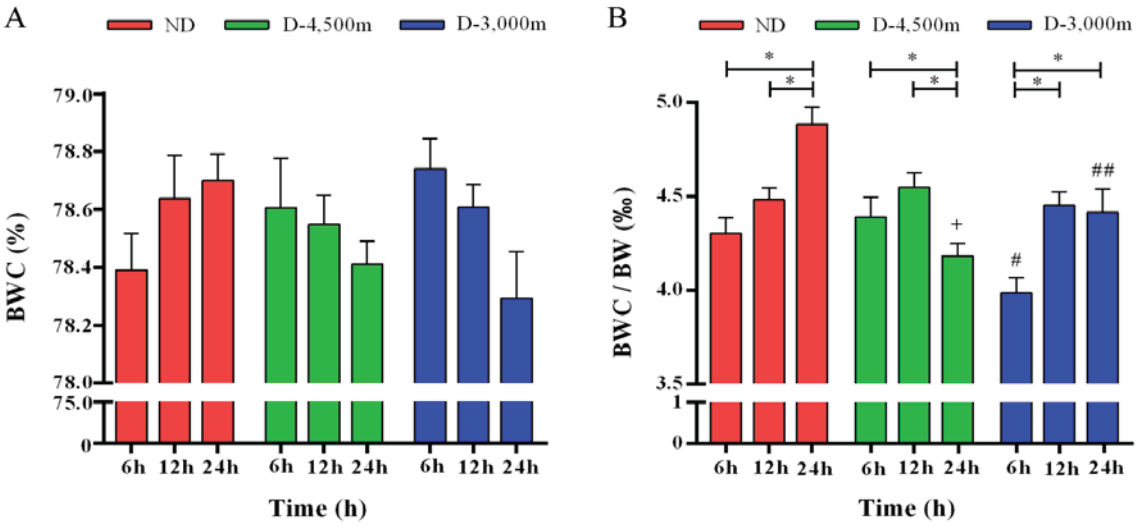

Figure 3. BWC is associated with BW changes overtime in rats with mild-to-moderate closed head injury exposed to high-altitudes. (A) BWC in various groups over time did not change significantly. No association between time and altitude was observed. (B) The ratio of BWC/BW (‰) significantly changed over time in the various groups, *P<0.05. Differences between the altitude groups were significant at 6 and 24 h following injury: $^{+}$P<0.05 vs. ND 24 h; $^{\#}$P<0.05 vs. ND 6 h; $^{\#\#}$P<0.05 vs. ND 24 h (n=6/group). P<0.05 was considered to indicate a statistically significant difference. BWC, brain water content; BW, body weight; ND, non-descending altitude; D-4,500 m, descent to 4,500 m following injury; D-3,000 m, descent to 3,000 m following injury.

D-3,000 m group (0.05±0.01 cm^2; P<0.05) compared with the D-4,500 m group (0.02±0.01 cm^2) and results in the altitude groups were significantly decreased compared with the ND group (0.08±0.01 cm^2; P<0.05). Tukey's post hoc tests were applied to identify significance for P≤0.05 and the following was observed: CC swelling was significantly lower at 24 h following mmCHI compared with 12 h in the ND group. In the D-4,500 m group CC swelling was significantly higher at 6 h compared with 12 or 24 h. For the D-3,000 m group, levels of CC swelling were significantly lower at 6 h compared with 12 h and significantly higher at 6 h compared with 24 h following mmCHI (Fig. 4C).

In addition, two-way ANOVA revealed that time (F=123.760; P<0.001), altitude (F=753.681; P<0.001) and interactions between time and altitude (F=188.876; P<0.001) had significant effects on LV dilation. At 6 h following mmCHI, LV dilation was significantly higher in the ND group (0.46±0.03 cm^2; P<0.05) compared with the D-4,500 m (0.41±0.02 cm^2; P<0.05) or D-3,000 m groups (0.40±0.01 cm^2; P<0.05). At 12 h post-mmCHI, LV dilation was lower in the D-4,500 m group (0.21±0.02 cm^2; P<0.05) compared with the ND (0.52±0.02 cm^2) or D-3,000 m groups (0.51±0.01 cm^2). At 24 h post-mmCHI, LV dilation was significantly higher in the D-3,000 m group (0.32±0.03 cm^2; P<0.05) compared with the D-4,500 m group (0.16±0.01 cm^2), and significantly lower in the D-3,000 m group compared with the ND group (0.52±0.01 cm^2; P<0.05). Tukey's post hoc tests were applied to identify significance for P≤0.05 and the following was observed: In the ND group, LV dilation was significantly lower at 6 h compared with 12 or 24 h following mmCHI. In the D-4,500 m group, LV dilation was significantly lower at 12 h compared with 6 h and significantly higher at 12 h compared with 24 h following mmCHI. LV dilation was significantly lower at 6 h compared with 12 h and significantly higher at 6 h compared with 24 h following mmCHI in the D-3,000 m group (Fig. 4D).

GFAP immunohistochemistry. To determine the effect of high-altitudes on reactive astrogliosis following AHH mmCHI *in vivo*, the present study conducted immunofluorescence staining and discovered that GFAP-positive astrocytes were observed in injured brains at 24 h post-mmCHI. Quantitatively, the ratio of GFAP-positive astrocytes in the D-3,000 m group (45.32±4.17%; P<0.05) was significantly higher compared with the D-4,500 m group (36.26±3.55%) and significantly decreased compared with the ND group (56.88±5.62%; Fig. 5A and B).

Discussion

Abrupt exposure to higher altitudes (>3,500 m above sea level) can cause acute mountain sickness and can lead to complications, including HACE, high-altitude pulmonary oedema, reduced body heat metabolism in plateau, increased evaporation, and significant dehydration, which in turn lead to increased difficulty in treatment of TBI (24,25). To the best of our knowledge, no previous reports have described the neurological, pathophysiology and imaging characteristics of the acute phase of mmCHI at varying high-altitudes.

In the present study, the neurological function of rats was evaluated using the NSS, which was developed to define the clinical condition of rats following trauma. In contrast to previous research (26), the present results revealed that there was a higher proportion of respiratory depression and seizure following mmCHI at high-altitudes, which maybe caused by partial pressure changes in brain tissues due to significantly decreasing oxygen levels (27). The mortality rate following injury was 0.6%, and absence of breathing recovery was determined as the cause of death. No significant difference was identified between the different altitude groups in the NSS evaluation. However, the overall trend of injury changes exhibited varying patterns at different time points. Neurological function recovered slowly during an

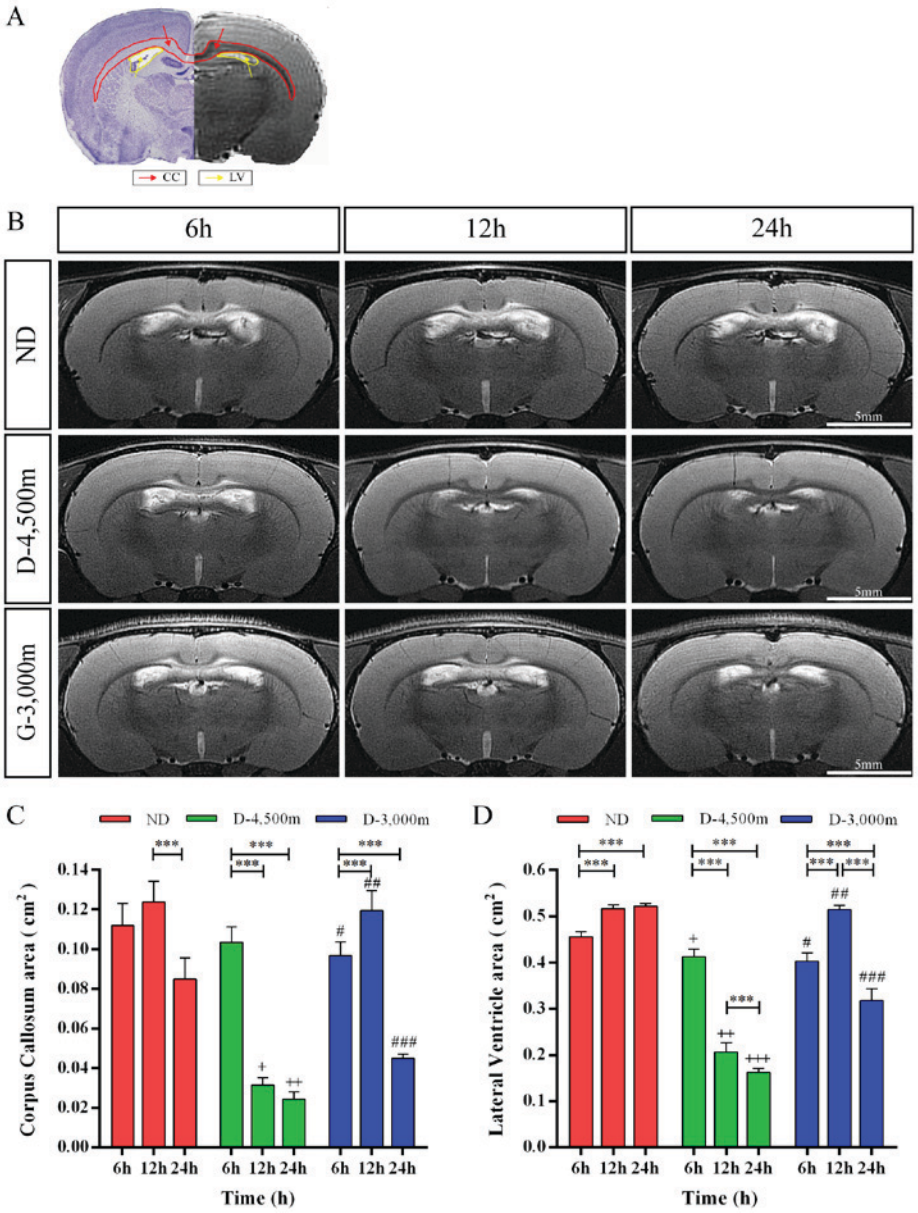

Figure 4. ROIs vary between time points for various altitude groups of rats with mmCHI. (A) Haematoxylin-eosin staining and coronal T2 imaging revealed the location of ROIs in the normal brain aiding the calculation of the area of the CC (red) and the LV (yellow). (B) Time-dependent changes in the T2-weighted anatomical MRI of various groups following AHH mmCHI at 6, 12 and 24 h; a hyper-intense signal covering the CC and LV dilation were observed in the brains. Scale bar, 5 mm. CC and LV changes overtime in rats with mild-to-moderate closed head injury exposed to high-altitudes. (C) The effect of time in each group on CC swelling, ***P<0.001; Differences among groups on CC swelling: $^{+}$P<0.05 vs. ND and D-3,000 m 12 h; $^{++}$P<0.05 vs. ND and D-3,000 m 24 h; $^{\#}$P<0.05 vs. ND 6 h; $^{\#\#}$P<0.05 vs. D-4,500 m 12 h; $^{\#\#\#}$P<0.05 vs. ND and D-4,500 m 24 h. (D) The effect of time effects in each group on LV dilation.***P<0.001; Differences among different groups on CC swelling: $^{+}$P<0.05 vs. ND 6 h; $^{++}$P<0.05 vs. ND and D-3,000 m 12 h; $^{+++}$P<0.05 vs. ND and D-3,000 m 24 h; $^{\#}$P<0.05 vs. ND 6 h; $^{\#\#}$P<0.05 vs. D-4,500 m 12 h; $^{\#\#\#}$P<0.05 vs. ND and D-4,500 m 24 h. ROIs, regions of interest; AHH, acute hypobaric hypoxia; mmCHI, mild-to-moderate closed head injury; MRI, magnetic resonance imaging; CC, corpus callosum; LV, lateral ventricle; ND, non-descending altitude; D-4,500 m, descent to 4,500 m following injury; D-3,000 m, descent to 3,000 m following injury.

acute period, which maybe due to increased disturbance in consciousness and respiratory depression following mmCHI at high-altitudes. Rats resumed breathing and regained conscious and the neurological function further recovered, when promptly provided with CPR and maintenance of the airway. This suggested that the NSS may have limitations at high-altitude. Hypoxia is not conducive to the recovery of neurological function, but cerebral ischaemia and hypoxia are the final common pathways of secondary brain damage (28); therefore, early restoration of respiration and continuous supplemental oxygen were important in the treatment of mmCHI at high-altitudes. Neurological recovery at 24 h and altitude were not directly associated with NSS.

Previous studies have demonstrated that in early hypoxia, humans and animals adapt to a hypoxic environment via reduced water intake, a modest decrease or increase in urine production, and a gradual increase in perspiration to increase blood oxygen content as soon as possible (29,30). In these integrated factors, decreased water intake is an essential measure for animals to adapt to a hypoxic environment during early stages and it further is the main reason for weight loss. The present study confirmed that decreases in BW were significantly lower following acute

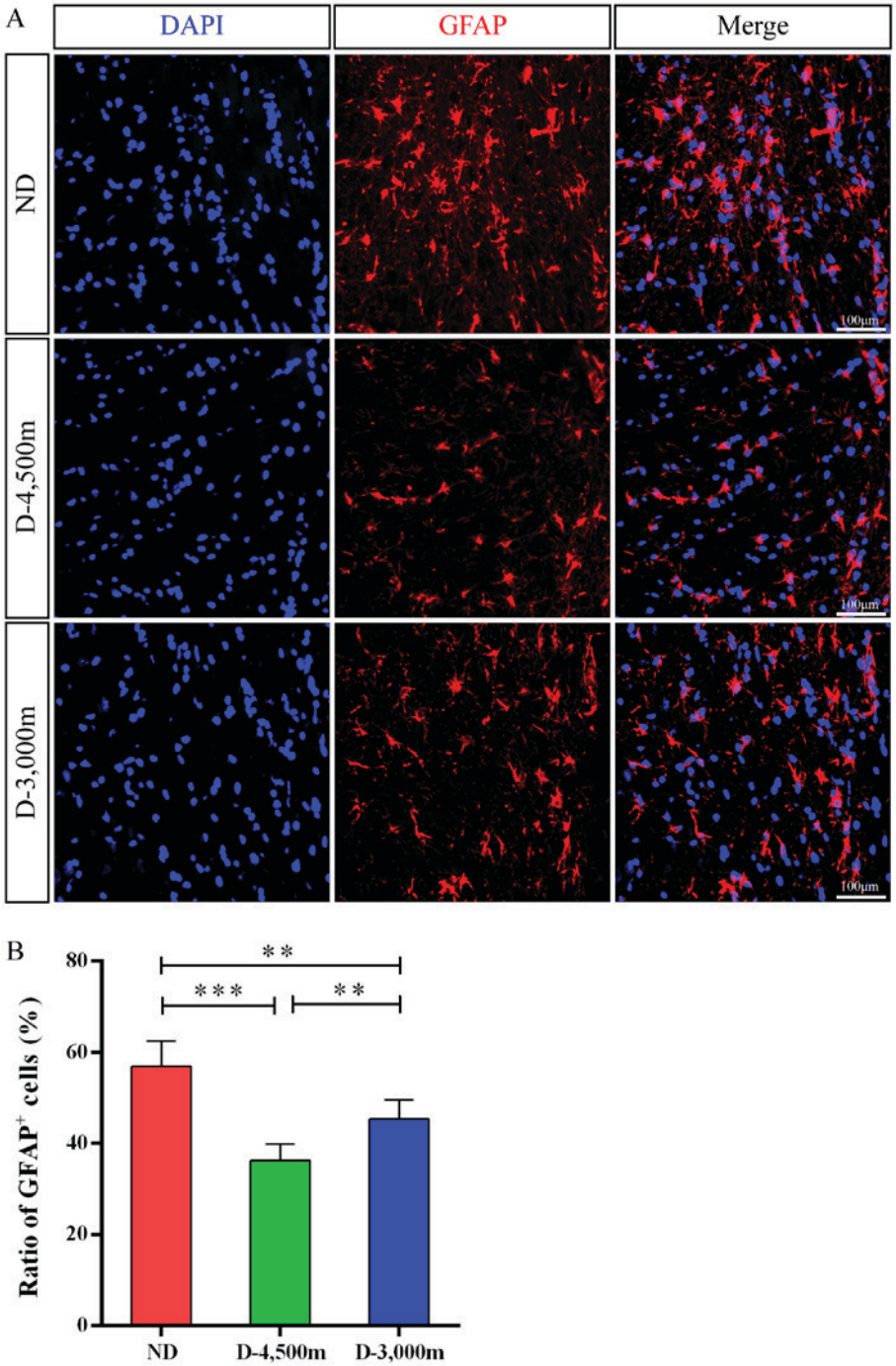

Figure 5. GFAP$^+$ cell number changes in the brains of rats with mmCHI at 24 h following injury. (A) Representative images for GFAP$^+$ cells (red) revealed the distribution of astrocytes in the injured brain among the ND, D-4,500 and D-3,000 m groups at 24 h following TBI. Nuclei were counterstained with DAPI (blue); scalebar, 100 μm. (B) GFAP$^+$ cells in the injured brain for the ND, D-4,500 and D-3,000 m groups at 24 h post-TBI (n=6/group). ***P<0.001 vs. D-4,500 m group; **P<0.01 vs. D-3,000 m group. GFAP, glial fibrillary acidic protein; mmCHI, mild-to-moderate closed head injury; TBI, traumatic brain injury; ND, non-descending altitude; D-4,500 m, descent to 4,500 m following injury; D-3,000 m, descent to 3,000 m following injury.

high-altitude exposure for 24 h compared with rats at sea level. Subsequently, rats that underwent mmCHI lost an increased amount of weight compared with healthy individuals at high-altitude. Following high-altitude injury, rats that rapidly descended to lower altitudes lost the least amount of weight. In addition, previous studies have demonstrated that rats were less active and had a reduced desire for food and water following AHH injury (31,32). This suggested that changes in BW following brain injury were closely associated with the hypoxic environment and may be due to excessive stress reactions during head injury, including hyperventilation, acute dieresis, metabolism decreases and a decline in food and water intake (33).

Certain studies have demonstrated that altitude hypoxia is responsible for acute mountain sickness (34,35). It can generate HACE, which may be more serious and can be fatal following TBI (36). In the present study, the absolute BWC of rats

following mmCHI at varying high-altitudes was not significantly different among the groups. However, the BWC/BW ratio was significantly different at the various altitudes and times. The analysis demonstrated that within 24 h following mmCHI at high-altitudes, the BWC/BW ratio in the ND group was still increasing, while the ratio at 6 h in the D-3,000 m group was the lowest, which maybe due to low levels of weight loss observed among the rats that rapidly descended to 3,000 m following injury. This indicated that BWC may be associated with BW changes and altitude. Although there was a reduction in the degree of weight loss and improved neural function recovery following injury at decreased altitude, the BWC was not reduced. Compared with the absolute BWC, the relative BWC, referring to the BWC/BW ratio, maybe more informative of the actual cerebral oedema. Previous studies have demonstrated that brain oedema occurs for the first time following 24 h of closed head injury in low-altitude areas and peaks on days 5-8 (37-39). The ratio of the rats that descended to altitudes of D-4,500 m and D-3,000 m reached a peak at 12 h following injury prior to ratios starting to decline. At 24 h, the BWC/BW ratio of the D-4,500 m group was significantly lower compared with the D-3,000 m group. This suggested that there may be a correlation between BWC/BW, times and altitude levels following mmCHI at high-altitudes. The brain oedema following TBI at high-altitudes occurred earlier and was more severe compared with that observed in areas of lower altitude.

Although brain MRI has been used in various studies of simulated high-altitude (40,41), the present study first investigated the dynamic change of brain MRI during 24 h at varying high-altitudes following AHH mmCHI. The MRI scan in T2WI revealed that the model of head injury was due to mmCHI. In patients with mmCHI, MRI findings are often accessible and include diffuse axonal injury (DAI) lesions and subarachnoid haemorrhages (SAH) (42). The pathology of DAI is characterized histologically by microscopic axonal damage observed in the parasagittal white matter and grey-white matter junctions of the cerebral cortex and in the CC and brainstem (43). Ventricular dilation is a frequent phenomenon in patients with TBI and can present in follow-up examinations and develop into post-traumatic hydrocephalus (PTH) (44). Various studies have indicated that SAH and intraventricular haemorrhage (IVH) may be associated with hydrocephalus development following TBI, which can result in acute ventricular dilation at 24 h following TBI (45,46). As a result of the tearing of subependymal veins that line ventricular cavities, IVH has been associated with severe injuries (47). The mechanisms of ventricular dilation are still not well understood. In the current study, the location of CC exhibiteds ignificantly higher signalling and the LVs were enlarged markedly in injured rats at 6 h following AHH. This result indicated that there was an axonal injury in mmCHI following AHH. In addition, mmCHI following AHH resulted in the earlier occurrence of acute hydrocephalus, but MRI scanning did not identify SAH or IVH in our study. In the control group, no such observations were made; indicating that CC swelling and acute ventricular dilatation of mmCHI may be closely associated with AHH. PTH may cause increased intracranial pressure, which has been recognized as an indicator of poorer outcomes following TBI (48). The dynamic brain MRI manifestations were more gradually improved by hypobaric-hypoxia conditions (slowly descending altitude) compared with rapid reoxygenation (rapidly descending altitude) and continuous hypoxia (maintained at extreme high-altitude) in mmCHI following AHH at 12 and 24 h.

Exposure to a hypobaric environment following TBI increases the neuroinflammatory response to injury and the severity of secondary brain injury (49). Various studies have indicated that increased local tissue GFAP immunoreactivity is a sensitive indicator of neuronal injury and the increase in GFAP immunoreactivity is a sensitive marker of reactive astrocytosis (50,51). Additionally, GFAP levels increase when cerebral tissues are damaged due to trauma (52). GFAP is an early diagnostic indicator of TBI and a sensitive indicator of mortality following TBI (53). GFAP is further the major protein of glial intermediate filaments in astrocytes and is often used as a hallmark of astrocyte reactivity (54). An increase in GFAP expression is a feature of various pathological conditions of the central nervous system (55). Although the NSS in the current study was similar following mmCHI at 24 h in the various groups, levels of GFAP-positive cells varied. Levels of GFAP-positive cells following continuous hypoxia were the highest in the ND group, however, in the other two groups, gradual reoxygenation produced lower levels in the D-4,500 m group compared with rapid reoxygenation in the D-3,000 m group. Furthermore, levels of GFAP-positive cells reflected on the degree of neuronal damage under mmCHI hypobaric-hypoxia. Due to the different oxygen contents at different altitudes, continuous hypobaric-hypoxia following TBI significantly aggravated secondary brain injury and rapid improvement of hypobaric and hypoxic conditions following AHH TBI was not conducive to neurological recovery.

In conclusion, increasing attention should focus on the initial 24 h of mmCHI following AHH exposure. Subjects may benefit from being transported at the earliest possible time and avoiding large-span descent altitude was beneficial to reduce neurological impairment. Brain-specific biomarkers and MRI results may further the understanding altitude mmCHI and can be translatable to clinical practice. Further studies are required in order to understand the precise mechanisms and long-term effects of brain damage caused by mmCHI in rats following AHH. Levels of inflammation and mechanisms of secondary brain injury following exposure to AHH need to be investigated further.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

HW and XZ designed the current study, performed the experiments, analyzed the data and wrote the paper. HX and ZL performed the experiments, administered food and anaesthesia to animals, and contributed experimental equipment. MR and PW designed the current study and performed the experiments. MG, YL and YZ anlyzed and searched for literature. HZ and MX designed the current study, provided experimental equipment, analysed the data and presented results. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experiments were approved by the Ethics Committee of Third Affiliated Hospital and Research Institute of Surgery, Third Military Medical University (registration no. ChiCTR-RPC-15006770; Chongqing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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