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The impact of high-altitude and cold environment on brain and heart damage in rats with hemorrhagic shock

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Abstract:

BACKGROUND: Hemorrhagic shock (HS) causes severe organ damage, worsened by high-altitude conditions with lower oxygen and temperatures. Existing research lacks specific insights on brain and heart damage under these conditions. This study hypothesizes that high-altitude and cold (HAC) environments exacerbate HS-induced damage in the brain and heart, aiming to improve treatment strategies.

MATERIALS AND METHODS: Twenty-four male Sprague-Dawley (SD) rats (200–250 g of weight) were randomly assigned into sham, HS + normal, HS + HAC (4,000 m), and HS + HAC (6,000 m). The HS model was established in SD rats (35% loss of total blood volume), and histopathological injuries of the brain and heart were detected using hematoxylin and eosin staining, Sirius red staining, and immunohistochemistry. Apoptosis of the brain and heart tissues was detected by terminal transferase-mediated dUTP nick end labeling (TUNEL) immunofluorescence staining. To determine the levels of tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (Mcp-1), BCL2-associated X (BAX), and myeloid cell leukemia-1 (Mcl-1) protein, western blotting assay was used.

RESULTS: The HAC environment induced pathological damage to the brain and heart and aggravated the degree of cardiac fibrosis in HS rats. However, it did not cause apoptosis of the brain and heart. In addition, it upregulated TNF-α, IFN-γ, Mcp-1, and BAX protein levels, but downregulated Mcl-1 protein levels ($P < 0.05$).

CONCLUSIONS: The HAC environment aggravated the degree of brain and heart damage in HS rats, which may be related to neuron nucleus pyknosis, myocardial fibrosis, and inflammatory and apoptosis activation.

Keywords:

Brain injury, heart injury, hemorrhagic shock, high altitude and cold

Introduction

Hemorrhagic shock (HS) is characterized by a rapid and massive loss of circulating blood, which exceeds the body's compensatory capacity. This often occurs in serious injuries or diseases.[1,2] Currently, trauma-related hemorrhage is the primary reason of death globally among individuals below 44 years old, resulting in around 1.5 million fatalities each year, of which HS is responsible for 30%–40%.[2] Severe shock and low blood volume can lead to tissue hypoperfusion, neuroendocrine stress responses, as well as coagulation activation. This results in metabolic disturbances, impaired organ function, and persistent hemodynamic instability.[3] The severity of organ damage

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is often influenced by environmental factors such as altitude and temperature.

The western region of China is located in high-altitude environment with most areas being above 3,000 m.[4,5] It is a vital military strategic location and home to many residents. The atmospheric pressure, oxygen concentration, and temperature here are lower than those in other regions, which may cause physiological or pathological changes in many people, such as dizziness, vomiting, gastrointestinal discomfort, and other adverse reactions.[6] Recently, in Western China, the incidence of traffic accidents has been continuously increasing.[7] A high-altitude environment can exacerbate the physiological and pathological changes associated with HS, leading to more severe organ damage and increased treatment difficulty for patients. Studies have reported that HS patients may suffer from acidosis, systemic inflammatory reactions, and hypoxic tissue damage at high altitude.[8,9] In addition, hypobaric and hypoxia can cause pulmonary vasoconstriction, increase pulmonary artery pressure and capillary permeability, and contribute to pulmonary edema and brain edema.[10] The infusion tolerance of HS patients with pulmonary edema in high-altitude areas was significantly reduced. Zhou *et al*. reported that acute exposure to high altitude (4,000 m) shortened the tissue oxygen supply and survival time after HS in rats.[11]

The mortality rate of HS patients has remained high since 2000. Although a significant portion of these deaths occur before hospitalization, there are also high mortality rates associated with subsequent multiple organ dysfunction and infection.[12] In high-altitude and cold (HAC) environments, the degree of organ damage caused by HS is greater, which can increase the treatment difficulty for patients. Therefore, it is crucial to explore the effects of HS on organ damage and related indicators for the clinical treatment of HS patients, particularly in combined HAC environments.[13] The brain and heart tissues are the organs with the highest oxygen consumption in the human body.[14] Studies have reported that in high-altitude hypoxia areas above 2,500 m, the heart, brain, and other important organs of the human body may undergo varying degrees of damage.[15,16] In this experiment, we aimed to explore the impact of the HAC environment on brain and heart damage in HS rats.

Materials and Methods

Animals

Twenty-four male Sprague-Dawley (SD) rats (200–250 g of weight) were maintained in identical conditions (temperature: 23°C ± 1°C; light: 12-h light/dark cycle) and received sufficient food and water. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Capital Medical University on October 14, 2021 (approval date and number: 2021/255).

Animal grouping and processing

Animals were randomly divided into sham, HS + normal, HS + HAC (4,000 m), and HS + HAC (6,000 m). There were six rats in each group, with three rats used for western blot detection, and the remaining three rats used for histological detection. In the HS + HAC group, animals were housed in a hypobaric chamber (Guizhou Fenglei Oxygen Tank Co. LTD) with a simulated high altitude of 4,000 m or 6,000 m for 3 days before modeling, and the chamber temperature was maintained at 5°C.[17,18] The process of making the HS model and the maintenance of the HS state were carried out under normal pressure and oxygen environment. The experimental design is shown in Figure 1a.

The hemorrhagic shock model

After anesthetized, the rats' limbs were fixed, and catheters were inserted into their right femoral arteries for bloodletting and blood pressure monitoring. The bloodletting volume was calculated as 35% of the total blood volume, which was derived from, total blood volume (ml) = rat weight (mg) × 7%.[19,20] The HS model was deemed successful when the mean arterial pressure (MAP) dropped below 25 ± 2 mmHg as shown in Figure 1b.[21] The rats were maintained at this MAP level for 45 min. After the shock phase, rats were resuscitated with a lactated Ringer solution infusion at three times the volume of shed blood for 30 min. During the observation phase, maintenance fluid of lactated Ringer solution at 1 ml/kg/h was administered. Rats were sacrificed 240 min after HS for sampling. The body temperature of rats was tested by the rectal probe (BAT-10, Physitemp Instruments Inc, Clifton, NJ) and maintained by a heating pad (37°C ± 0.5°C).[21]

Histopathological and immunohistochemical analysis

After anesthetizing the rats, we performed perfusion through the left ventricle with 40 ml of saline to clear the blood, followed by perfusion with 40 ml of 4% paraformaldehyde to fix the tissues. Then, the brain and heart tissues were extracted and immediately fixed in 4% paraformaldehyde for an additional 24 h. After

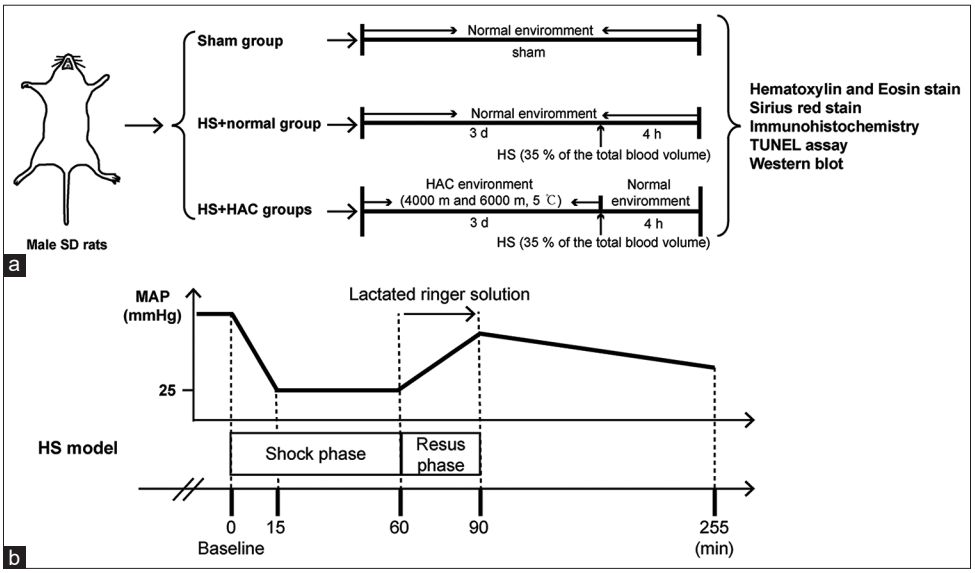

Figure 1: Schematic presentation of the experimental design. (a) Rats grouping and processing. In this study, 24 adult male Sprague-Dawley rats were randomly divided into four groups: sham group, hemorrhagic shock (HS) + normal group, HS + high-altitude and cold (HAC) (4000 m) group, and HS + HAC (6000 m) group. In the sham group, only a laparotomy was performed. In the HS group, all rats' catheters were placed in the right femoral artery for bloodletting. The bloodletting was 35% of each rat's total blood volume. In the HS + HAC group, all rats were placed in a hypobaric chamber at a simulated high altitude of 4000 m and 6000 m (the chamber temperature is 5°C) for 3 days before modeling. Four hours after the HS model, all rats were anesthetized for further test $n = 6$. (b) Experimental protocol for the HS model. The MAP dropped to 25 ± 2 mmHg in 15 min and maintained for 45 min. After the shock phase, rats were resuscitated with an infusion of lactated Ringer solution at three times the volume of shed blood for 30 min. During the observation phase, maintenance fluid of lactated Ringer solution at 1 ml/kg/h was administered. Rats were sacrificed 240 min after HS for sampling. HS: Hemorrhagic shock, HAC: High altitude and cold, SD: Sprague-Dawley

fixation, the tissues were embedded in paraffin and cut into 4 μm thick slices. To assess tissue structure and cell density, hematoxylin and eosin (HE) staining was used. To assess the degree of myocardial collagen fiber deposition, Sirius red staining was used as previously described.[19] The tissue slices panoramic scanning system (PANNORAMIC MIDI, 3DHISTECH Company, Hungary) was used to capture images. Tissue damage was quantified using previously published scoring criteria.[20] For each group of sections, we selected five visual fields for statistical analysis.

To further explore the degree of cardiac injury, transforming growth factor (TGF)-immunohistochemistry (IHC) was used to detect the degree of cardiac fibrosis. Three low-power fields of each group were examined. The average optical density value was analyzed and calculated using ImageJ software (National Institutes of Health, Bethesda, MD) as a semi-quantitative parameter index.

TUNEL assay

We used a TUNEL assay kit (Roche, Mannheim, Germany) to detect cell apoptosis in the brain and heart tissues.[22] The tissues were cut into 10-μm sections, deparaffinized, rehydrated, and then permeabilized with proteinase K solution. The sections were incubated with the TUNEL reaction mixture at 37°C for 60 min in the dark, following the kit instructions. After incubation, the sections were rinsed with phosphate-buffered saline and counterstained with 4′,6-Diamidino-2′-phenylindole (DAPI). Finally, the stained sections were observed under a confocal microscope, where apoptotic cells appeared green and nuclei were blue. Five randomly selected sections at ×400 magnification were photographed and analyzed using ImageJ software. (http://rsb.info.nih.gov/ij/macros/toolsets National Institutes of Health, Bethesda, Maryland, United States).

Western blot

Extracted whole protein from the heart and brain tissues of rats was used to detect the expression levels of inflammatory and apoptosis proteins. Primary antibodies used included rabbit anti-rat β-actin (1:1,000; CST, Boston, Mass, USA), tumor necrosis factor-α (TNF-α) (1:1,000; CST), interferon-gamma (IFN-γ) (1:1000; Abcam, Cambridge, MA, USA), monocyte chemoattractant protein-1 (Mcp-1) (1:1000; Abcam), BCL2-associated X (BAX) (1:1000; CST), and myeloid cell leukemia-1 (Mcl-1) (1:1000; CST). The secondary antibody used was HRP-conjugated anti-rabbit secondary antibody (1:2000; Thermo). Membrane reacted with an enhanced chemiluminescence substrate (Thermo, Waltham, MA, USA). The membrane was imaged using an Odyssey dual-color infrared fluorescence imaging system (LICOR, US), and the resulting images were analyzed using ImageJ software.

Statistical analysis

The data were analyzed using GraphPad Prism

version 6.0 (GraphPad Software, San Diego, CA, USA) and presented as mean ± standard error of the mean from each independent experiment. The normality of data was determined using the Shapiro–Wilk test. A Student's *t*-test (for two groups) was performed for data points from normally distributed populations, while a one-way analysis of variance followed by a Tukey *post hoc* test was used for data from more than two groups. $P < 0.05$ was considered statistically significant for all analyses.

Results

High-altitude and cold environment causes pathological damage to the brain and heart in hemorrhagic shock rats

Studies have reported that the body temperature of rats can change in cold and hypoxic environments.[23] As shown in Figure 2a, after 3 days of exposure to the HAC environment, the body temperature of rats decreased significantly ($P < 0.01$). Nevertheless, there

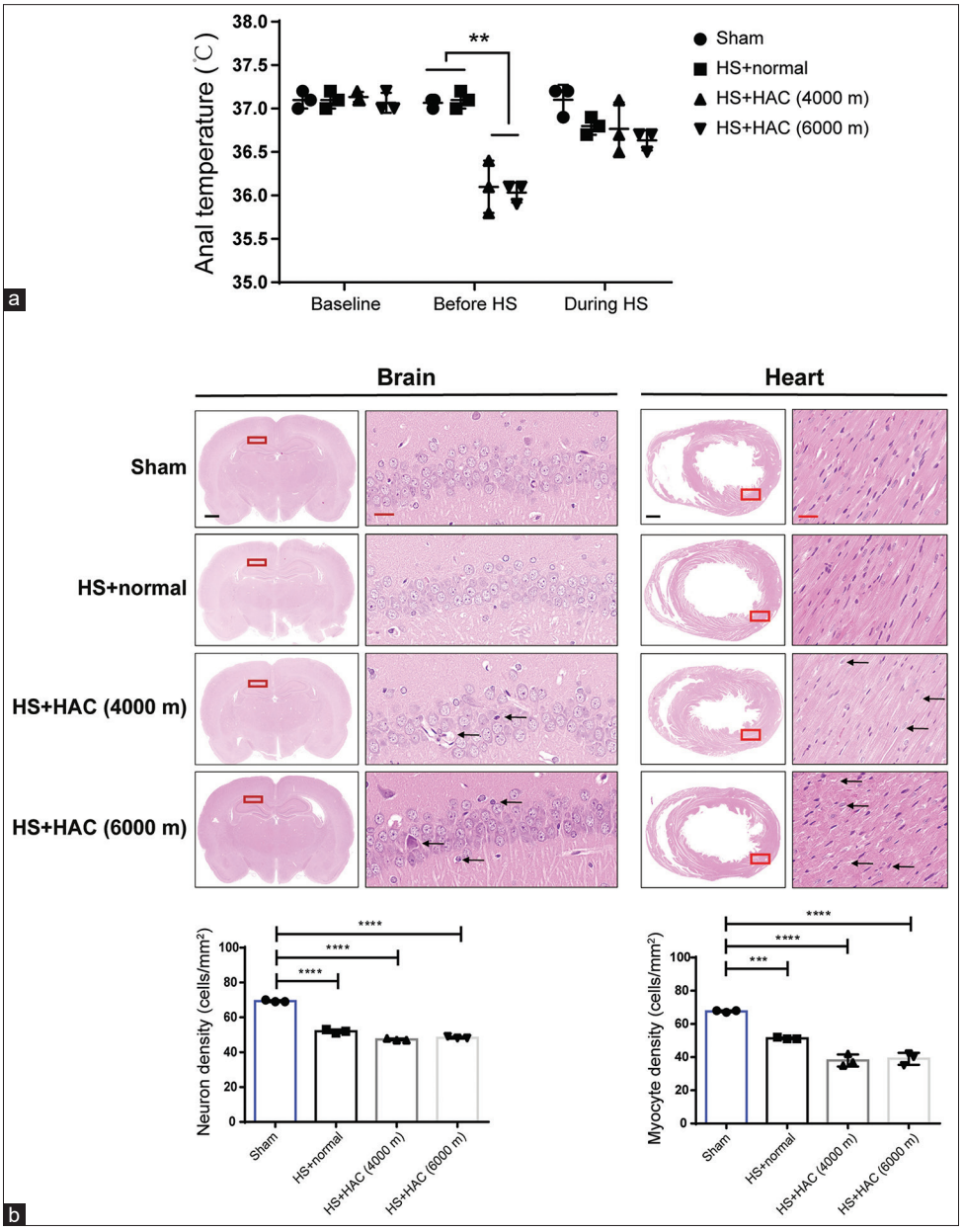

Figure 2: HE staining of the brain and heart tissues in different groups. (a) The body temperature of rats in different groups. Data are expressed as mean ± standard error of the mean (SEM) $n = 3$. $^{**}P < 0.01$. (b) Representative cases of HE staining in the brain and heart. The pictures on the left showed the overall representation of the brain and cardiac coronal sections. Scale bar = 1000 μm. The pictures on the right showed an enlarged view of the hippocampus CA1 region and the left ventricle right anterior wall. The arrow indicates the location of the tissue lesion. Scale bar = 50 μm. Data are expressed as mean ± SEM $n = 3$. $^{****}P < 0.0001$, $^{***}P < 0.001$. HS: Hemorrhagic shock, HAC: High altitude and cold

was no significant difference when comparing the two groups of high altitude.

To evaluate the effect of HAC conditions on brain and heart injuries in HS rats, the HE staining assay was used. As shown in Figure 2b, the sham group exhibited a complete brain tissue structure with orderly arranged neurons in the hippocampus. Compared to the sham group, no pathological changes were observed in HS + normal condition. However, the HS + HAC group showed unevenly arranged cells and neuron nucleus pyknosis in the hippocampus region. Meanwhile, the neuron density was markedly decreased in the HS group. These results indicated damage to the heart tissue.

In the heart tissue, the sham group showed a complete and orderly myocardial structure with no pathological changes in HS + normal condition. In contrast, both the HS and HS+HAC groups exhibited damage to myocardial fibers. However, there were no differences observed between the HS and HS+HAC groups (Figure 2b), indicating abnormalities in both groups.

Overall, these findings suggested that the HAC environment caused pathological injury to the brain and heart tissues in HS rats.

High-altitude and cold environment aggravates the degree of cardiac fibrosis in hemorrhagic shock rats

Cardiac fibrosis was assessed using Sirius red stain and IHC. As shown in Figure 3, the cardiac tissue of the sham group had a complete morphology, with continuous myocardial cells and no collagen fiber deposition cells. In contrast, the myocardial tissue of the HS group had a blurry boundary and enlarged tissue gap. The HS + HAC group exhibited broken myocardial fibers and extensive collagen fiber deposition. The pathological score showed that HS + HAC (6,000 m) significantly increased the score compared with other groups.

In Figure 4a, the cardiac tissue of the sham and HS + normal groups had no TGF-β positive cells. However, in the HS + HAC (6,000 m) group, the myocardial tissue exhibited TGF-β positive cells. In Figure 4b, analysis of the optical density value showed that compared to the sham and HS + normal groups, the TGF-β level was significantly increasing in the HS + HAC (6,000 m) group, $P < 0.05$. The above findings indicated that the HAC environment can exacerbate cardiac fibrosis in HS rats.

High-altitude and cold environment did not induce cell apoptosis in the brain and heart of hemorrhagic shock rats

To evaluate apoptotic of brain and heart tissue in HS rats, the TUNEL staining was used. As results shown in Figure 5, no apoptosis cells were found in the brain and heart tissues of the sham or HS group. This indicated that HS did not induce apoptosis in the brain and heart tissues.

High-altitude and cold environment activated inflammation and apoptosis in the brain and heart tissues of hemorrhagic shock rats

Inflammation-related proteins (TNF-α, IFN-γ, and Mcp-1)

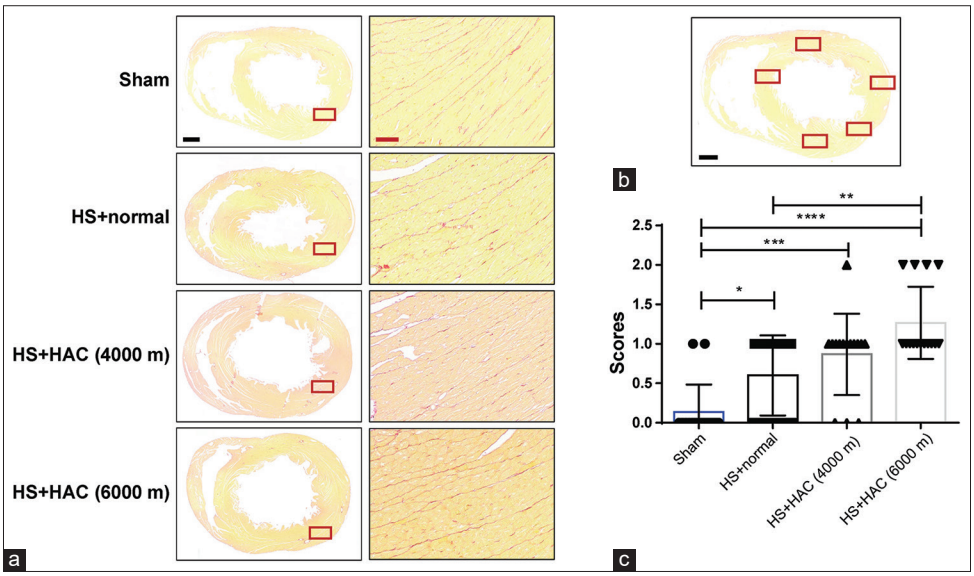

Figure 3: Sirius red staining of the heart tissue in different groups. (a) Representative cases of Sirius red staining in the heart. The pictures on the left showed the overall representation of cardiac coronal sections. Scale bar = 1000 μm. The pictures on the right showed an enlarged view of the right anterior wall of the left ventricle. Scale bar = 50 μm. (b) The red box shows five quantitative visual fields. (c) Bar graph shows the pathological score. Each group consisted of three mice, and five fields were counted for each mouse. Therefore, the bar scatter plot displays 15 data points. Data are expressed as mean ± standard error of the mean. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$ versus sham; **$P < 0.01$ versus hemorrhagic shock + normal. HS: Hemorrhagic shock, HAC: High altitude and cold

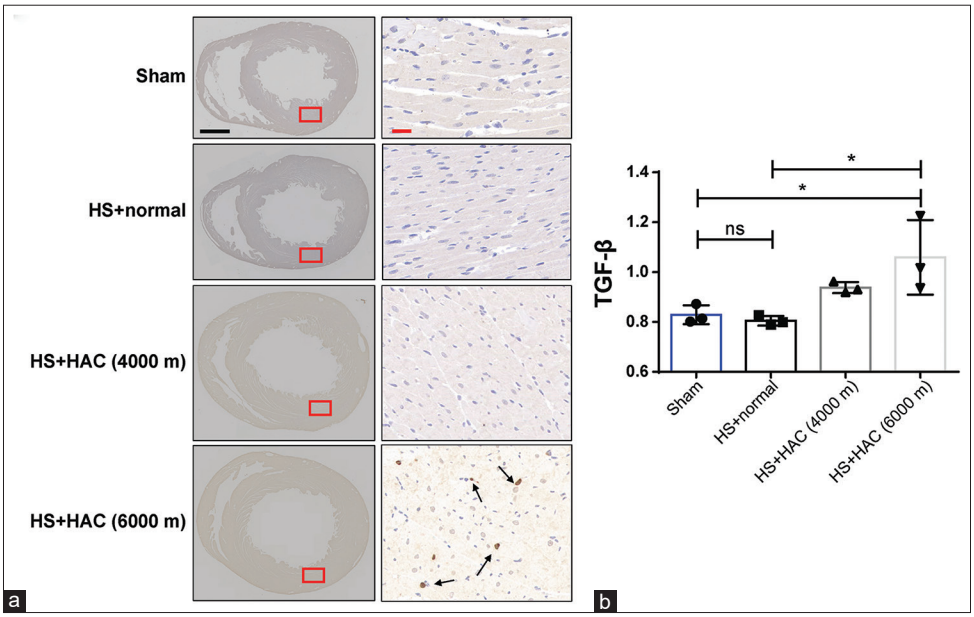

Figure 4: Immunohistochemistry of the heart tissue in different groups. (a) Representative cases of transforming growth factor-β (TGF-β) in the heart. The picture on the left showed the left ventricle, and the picture on the right showed the right anterior wall. The arrows indicated TGF-β-positive staining signals. (b) Bar graph shows the expression level of TGF-β in the heart. Data are expressed as mean ± standard error of the mean $n = 3$. *$P < 0.05$ versus sham; *$P < 0.05$ versus hemorrhagic shock + normal. HS: Hemorrhagic shock, HAC: High altitude and cold

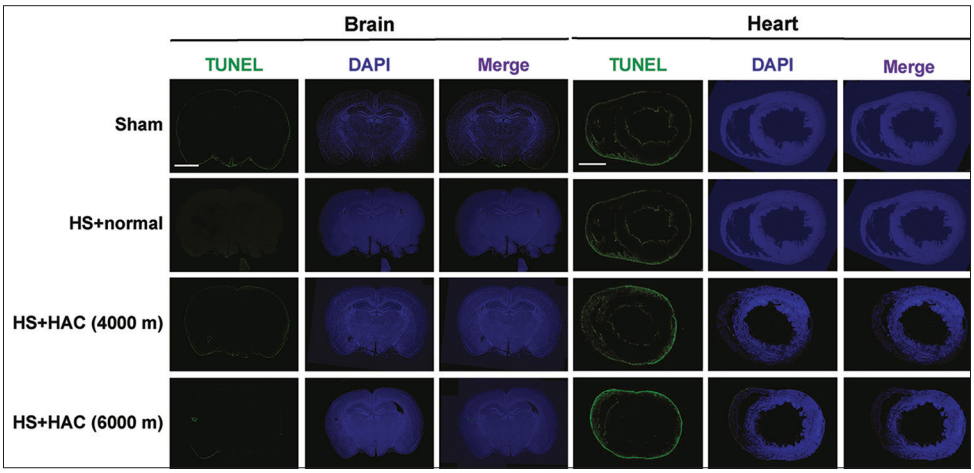

Figure 5: TUNEL staining of the brain and heart tissues in different groups. The pictures showed the overall representation of the brain and heart sections. Green labeled TUNEL-positive cells, blue labeled DAPI. Scale bar = 100 μm $n = 3$. HS: Hemorrhagic shock, HAC: High altitude and cold

and apoptosis-related proteins (BAX and Mcl-1) levels were measured using western blot analysis. In brain tissue, compared to the group of sham, there was no change in HS + normal conditions, but Mcp-1 and BAX levels were significantly upregulated, and Mcl-1 level was significantly downregulated in HS + HAC condition. In addition, compared with the group of HS + normal, IFN-γ, Mcp-1, and BAX levels were also significantly upregulated ($P < 0.05$) in the HS + HAC (6,000 m) group [Figure 6].

In the heart, compared with the group of sham, TNF-α level was upregulated in the HS + normal group ($P < 0.05$), and TNF-α, IFN-γ, and BAX levels were significantly increased ($P < 0.01$) in the HS + HAC group. In addition, compared to the HS + normal group, the IFN-γ protein level was also significantly increased ($P < 0.05$) in the HS + HAC group [Figure 7].

These results demonstrated that inflammation and apoptosis were not activated in the brain and heart tissues of HS rats under normal environmental conditions. However, these processes were activated in the HAC environment, indicating that the HAC environment can exacerbate the injury induced by HS.

Discussion

The study examined the effects of the HAC environment

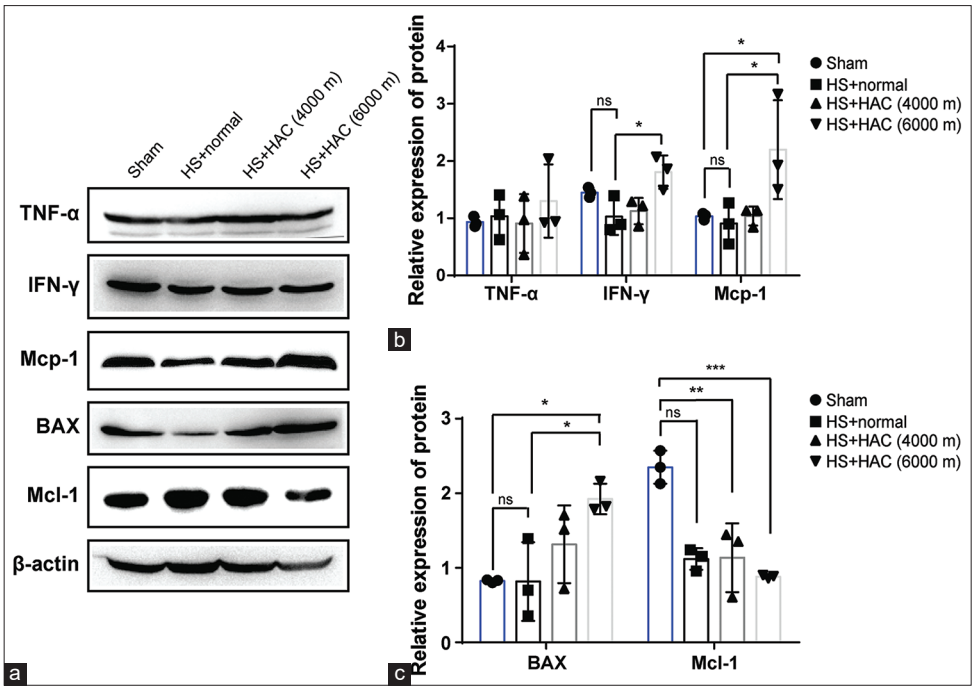

Figure 6: The expression levels of tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), monocyte chemoattractant protein 1 (Mcp-1), BCL2-associated X (BAX), and myeloid cell leukemia-1 (Mcl-1) protein in the brain tissue. (a) The expression of TNF-α, IFN-γ, Mcp-1, BAX, and Mcl-1 protein were analyzed by western blot. (b) The relative quantification of western blot analysis of TNF-α, IFN-γ, and Mcp-1 protein in different groups. (c) The relative quantification of western blot analysis of BAX and Mcl-1 protein in different groups. Data are expressed as mean ± standard error of the mean $n = 3$. $^{*}P < 0.05$, $^{***}P < 0.001$ versus sham; $^{*}P < 0.05$, $^{**}P < 0.01$ versus hemorrhagic shock + normal. HS: Hemorrhagic shock, HAC: High-altitude and cold, TNF-α: Tumor necrosis factor-α, IFN-γ: Interferon-gamma, Mcp-1: Monocyte chemoattractant protein-1, BAX: BCL2-associated X, Mcl-1: Myeloid cell leukemia-1

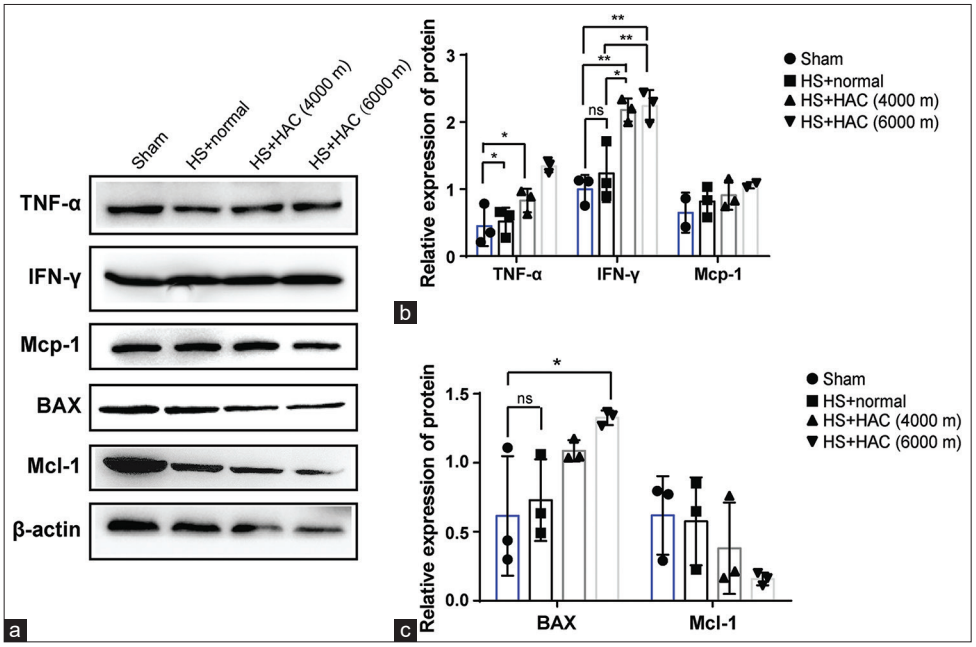

Figure 7: The expression levels of tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), monocyte chemoattractant protein-1 (Mcp-1), BCL2-associated X (BAX), and myeloid cell leukemia-1 (Mcl-1) protein in heart tissue. (a) Expression of TNF-α, IFN-γ, Mcp-1, BAX, and Mcl-1 proteins were analyzed by western blot in the heart tissue. (b) The relative quantification of western blot analysis of TNF-α, IFN-γ, and Mcp-1 protein in different groups. (c) The relative quantification of western blot analysis of BAX and Mcl-1 protein in different groups. Data are expressed as mean ± standard error of the mean $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$ versus sham; $^{*}P < 0.05$, $^{**}P < 0.01$ versus hemorrhagic shock + normal. HS: Hemorrhagic shock, HAC: High altitude and cold, TNF-α: Tumor necrosis factor-α, IFN-γ: Interferon-gamma, Mcp-1: Monocyte chemoattractant protein 1, BAX: BCL2-associated X, Mcl-1: Myeloid cell leukemia-1

on brain and heart damage in HS rats. All findings suggest that the HAC environment can induce pathological damage to the brain and heart, and exacerbate cardiac fibrosis in HS rats. However, the

HAC environment did not cause cell apoptosis in the brain and heart of HS rats. Interestingly, it can activate inflammation and apoptosis in the brain and heart tissues of HS rats. Specifically, in the brain, the HAC environment upregulated IFN-γ, Mcp-1, and BAX protein levels, and downregulated Mcl-1protein level; in the heart, it upregulated IFN-γ and BAX protein levels.

Severe trauma resulting in excessive blood loss can cause HS, which can damage several organs in the human body. Shock particularly affects vital organs such as the brain and heart.[24,25] During the early stages of shock, patients may experience inadequate perfusion to the brain and heart, leading to symptoms such as tachycardia, nervousness, inattention, and rapid breathing.[13,26] When individuals are exposed to a unique environment of high altitude hypoxia and cold, the damage caused by HS to the human body can be exacerbated, leading to more challenging treatments.[11] Studies have reported that at high altitudes above 2,500 m, low pressure, and low oxygen can cause myocardial cell damage, oxidative stress reactions, and vascular injury in brain tissue.[27] This is the primary cause of altitude heart disease, acute mountain sickness, and altitude cerebral edema.[28-30] Our results indicated that the HAC environment can exacerbate the damage to brain and heart tissue in HS rats.

The present study provides evidence that the HAC environment can worsen cardiac fibrosis in HS rats. Cardiac fibrosis involves an excessive buildup of extracellular matrix (ECM) proteins, particularly collagen, as a pathological reaction to various cardiac injuries.[19] In this study, using Sirius red staining and IHC, we observed that the myocardial tissue of HS rats displayed broken myocardial fibers and extensive collagen fiber deposition in the normal environment, indicating the presence of cardiac fibrosis. In addition, TGF-β-positive cells were found in the heart of the HS + HAC (6,000 m) group, indicating that the HAC environment exacerbated the degree of cardiac fibrosis. The above results align with prior studies which have documented this effect.[31] The research of those authors reported that high-altitude environments can increase oxidative stress and promote the activation of several molecular pathways of promoting fibrosis, for instance, TGF-β, connective tissue growth factor, and matrix metalloproteinases.[32] TGF-β is a key regulator of fibrosis that stimulates ECM production, inhibits ECM degradation, and promotes myofibroblast differentiation, all of which play a central role in the development of cardiac fibrosis.[33] We suggest that the HAC environment may worsen cardiac fibrosis through the activation of TGF-β signaling.

Studies have shown that the cell oxidative stress response is enhanced when hypoxia occurs in the brain and heart tissues, resulting in an accumulation of reactive oxygen species (ROS) in cells.[34] High levels of ROS can affect cell viability, inhibit migration and invasion abilities, promote cell apoptosis, and eventually cause damage to the brain and heart. BAX and Mcl-1 proteins belong to the Bcl-2 gene family. BAX overexpression can form a dimer with Bcl-2 antagonizes its protective effect, inducing cell death. Mcl-1 can bind to the BH3 domain of BAX/Bak, inhibit the formation of BAX/Bak dimers, and develop an anti-apoptosis effect.[35] In the study, there was no difference of BAX and Mcl-1 proteins in the brain and heart tissues between the sham and HS + normal groups. However, in the HAC environment, the expression of BAX was upregulated, and Mcl-1 was downregulated in the brain and heart tissues of HS rats. Although we did not observe apoptosis signals in the brain and heart tissues of HS rats through TUNEL staining, the changes of BAX and Mcl-1 in the HS + HAC group indicated that the HAC environment may induce apoptosis in the brain and heart tissues of HS rats.

Inflammation was high in the HAC environment likely due to enhanced oxidative stress and immune response activation.[36,37] Hypoxia-induced oxidative stress can release pro-inflammatory cytokines and chemokines, exacerbating tissue damage. However, neuronal apoptosis remained low despite the hypoxia. This may be due to a transient adaptive mechanism where cells activate protective pathways to survive the acute hypoxic insult.[38] Clinically, understanding this balance is crucial; interventions that modulate the inflammatory response and protect against ROS-induced damage could improve outcomes in HS patients under HAC conditions.[39] This highlights the importance of timely intervention and potential therapeutic targets, such as inflammatory mediators and apoptosis regulators, to mitigate brain and heart damage in these patients.

It has been reported that the inflammatory response is an important factor regulating hypoxic-ischemic injury in the brain and heart tissue.[40-42] Tumor necrosis factor (TNF-α) is produced by activated mononuclear macrophages and promotes the differentiation of inflammatory cells.[43] Recombinant human IFN-γ, a type II interferon, can recruit inflammatory cells, induce tissue damage, and directly affect tissue cells, leading to cell activation, damage, and apoptosis.[44] Monocyte chemoattractant protein-1 (Mcp-1) is a crucial proinflammatory factor that can specifically chemotactically activate monocytes/macrophages and plays an important role in many inflammation-related diseases.[45] Our results indicate that HS did not activate the inflammatory responses of the brain and heart in the short term under a normal environment. However, in the HAC environment, the inflammatory response was triggered, characterized by increased IFN-γ and Mcp-1 levels. Overall, the HAC environment can activate the

response of inflammatory molecules and cells in the HS rats, leading to damage to the brain and heart.

Conclusions

The HAC environment aggravated the degree of brain and heart damage in HS rats, potentially leading to damage to the brain and heart. Although our study provides valuable insights into the effects of the HAC environment on HS-induced brain and heart injury, it has several limitations. First, we focused exclusively on brain and heart damage, inflammation, and apoptosis, without exploring other potential effects of HAC environments on different organs or systems. Second, the study utilized a small sample size of 24 male SD rats, which may not fully represent the variability seen in larger populations. Finally, the study did not investigate the long-term effects of HAC environments on HS-induced damage, limiting our understanding of chronic outcomes. Future research should aim to address these gaps to provide a more comprehensive understanding.

Author contributions

CR and XJ: study design, conceptualization. JX, WY, NL, SL, XW, and CG: literature search, experimental implementation, data acquisition. JX and FYL: statistical analysis. CR and JX: manuscript writing. KJ: revision manuscript. All authors have approved the final manuscript.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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