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Acute high-altitude exposure shortens survival after uncontrolled hemorrhagic shock in rats

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ABSTRACT

Background: Uncontrolled hemorrhage (UH) remains the most common cause of death on the battlefield. This study examined the pathophysiological characteristics of UH in rats acutely exposed to high altitude.

Material and methods: Rats raised at sea level were randomly divided into two groups. Rats in the high-altitude group were exposed to hypobaric hypoxia in a hypobaric chamber (simulating 4000 m above sea level) for 2 d and then were performed a hemorrhagic shock protocol in the hypobaric chamber. Rats that underwent the same hemorrhage procedure at sea level were used as control. Anesthetized rats were bled to maintain their mean arterial pressure at 45 mmHg for 1 h. The distal quarter of the tail was amputated to allow free blood loss. After 1 h, the tail cut was ligated to induce hemostasis. mean arterial pressure, acid–base balance, blood loss, and survival were recorded. Rats were killed, and tissues were obtained for histological analysis.

Results: Rats in the high-altitude group suffered less uncontrolled blood loss, more severe acidosis (lower pH and base excess), and inferior tissue oxygen supply (lower oxygen saturation and higher arterial lactate concentration) during the hemorrhage periods compared with the control group. Survival rates were significantly lower in the high-altitude group than those in the control group ($P < 0.05$), which was consistent with the results of pathological tissue injury.

Conclusions: In this rat model of hemorrhagic shock, acute high-altitude exposure resulted in decreased UH but more serious hemorrhagic shock injuries than that at sea level.

Introduction

Hemorrhagic shock is considered one of the greatest threats to an injured soldier's life.[1] In modern warfare, multiple military conflicts are likely to occur at higher altitudes. The borders between countries can be partly located in mountainous regions and could be potential areas of combat. Depending on military requirements, soldiers usually stationed at sea level may be required to deploy to higher altitudes with little or no time for physiological acclimation. People acutely exposed to high altitudes undergo physiological and pathological changes because of hypobaric hypoxia. When a person who

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has not acclimatized is injured and is in hemorrhagic shock at high altitude, exacerbation of the injury is different to that at sea level.

Although hemorrhagic shock occurring at sea level is well studied,[2,3] research on hemorrhagic shock at high altitude is relatively scarce. Preliminary studies have reported that hemorrhagic shock occurring at high altitude deeply exacerbated tissue oxygen supply and induced more serious acidosis compared with that at sea level.[4] An experimental study reported that rats acutely exposed to high altitude were less tolerant to blood loss than those at sea level.[5,6] There is no research on uncontrolled hemorrhage (UH), which remains the most common cause of potentially preventable death on the battlefield,[7,8] occurring at high altitude, reported in the literature. And little attention has been given to the effects of the high-altitude environment on tissue damage caused by hemorrhagic shock despite the hemodynamic response and physiological changes of hemorrhagic shock at high altitude having been studied.[9]

The present study investigated the effects of hypobaric hypoxia on blood loss and pathophysiological changes associated with uncontrolled hemorrhagic shock in rats. It is thought that the results may provide initial suggestions for the treatment of UH at high altitudes.

Material and methods

Ethical considerations

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences, Beijing, China. The research protocol adhered to the guide for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering.

The hypobaric chamber

We used a temperature-controlled hypobaric chamber ($25 \pm 2^{\circ}C$; Medical Science Laboratory Module on Simulation of Multifactor and Complex Environment, Peking, China) that included a closed cabin (spatial volume, length × width × height: $6 \times 3 \times 2.2$ m^3) and a buffer compartment (spatial volume, length × width × height: $1.5 \times 3 \times 2.2$ m^3). The pressure in the closed cabin was set to mimic an altitude of 4000 m, with an atmospheric partial pressure of oxygen of 100 mmHg and an atmospheric pressure of 468 mmHg, and could be maintained for up to 1 mo. The investigators accessed the closed cabin through the buffer compartment to perform the experiments.

Animals and experiment groups

Healthy male Wistar rats (260-300 g; Vital River, Beijing, China) with access to food and water *ad libitum* were randomly assigned to the high-altitude group and the control group ($n = 10$ per group). In the high-altitude group, rats were exposed to hypoxia in the hypobaric chamber for 48 h before being subject to an uncontrolled hemorrhagic shock protocol in the hypobaric chamber. In the control group, the same hemorrhagic shock procedure was performed in the laboratory at an altitude of ~50 m.

Animal operation

Rats were anesthetized by intraperitoneal injection of 50-mg/kg sodium pentobarbital (Peking Chemical Agent, Peking, China) and placed in the supine position, breathing spontaneously, on a warming pad (TMS-202; Softron, Peking, China) maintained at $37 \pm 0.1^{\circ}C$. Heparin (400 U/kg; Chinese Medicine Group Chemical Agent, Peking, China) was administered intravenously to inhibit coagulation. The right femoral artery was cannulated for blood pressure monitoring. The left femoral artery was cannulated to achieve bleeding using a syringe pump (LZS-AJ10; Softron) and to sample the arterial blood for blood gas analysis.

Experimental protocol

The experimental protocol is shown in Figure 1. After surgical preparation, rats were allowed to stabilize for 15 min. After baseline measurements were taken, blood was withdrawn from the left femoral artery at a rate of 0.4 mL/min until mean arterial pressure (MAP) reached 45 mmHg. Blood was withdrawn as required at a rate of 0.3 mL/min to maintain MAP at 45 ± 2 mmHg. After 1 h of controlled hemorrhage, the experimental time was set to zero (shock, time = 0 min), and the distal quarter of the tails of rats were amputated (measured from the tip of the tail) for 1 h of UH, which has been described previously with some modification.[10-12] The volume of uncontrolled blood loss was recorded (UH, time = 60 min). After this study, the tail cut was ligated to induce hemostasis, and further observations were recorded for 1 h (time = 120 min). Immediately after the death of the rats, brain, lung, liver, and kidney tissues were obtained for histological analysis.

Measurement of MAP and arterial blood gases

MAP was continuously monitored using a multiple-channel recorder (MP150 Research System; Biopac System, Montreal, Canada) throughout the experiment. Arterial blood (0.1 mL) was withdrawn for blood gas analysis using an ABL90 (Radiometer, Copenhagen, Denmark) at baseline, shock, and UH. Measurements included pH, base excess (BE), arterial partial pressure of carbon dioxide ($PaCO_2$), bicarbonate (HCO_3^-), arterial partial pressure of oxygen (PaO_2), arterial oxygen saturation (SaO_2), lactate concentration (Lac), hemoglobin concentration (Hb), and hematocrit (Hct).

Histological analysis

Tissues fixed in 10% neutral buffered formalin were embedded in paraffin and sectioned into 4-6 μm slices. After deparaffinization and dehydration, slices were stained with hematoxylin and eosin and observed under optical microscopy.

Brain injury was evaluated according to cortical and hippocampal damage (score, 0-4). Lung, liver, and kidney damage were evaluated according to our previous method and were scored independently by two pathologists who were blinded to