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RESEARCH ARTICLE

Effect of helium preconditioning on neurological decompression sickness in rats

Rongjia Zhang,[1]* Yongchao Yu,[2]* Anatol Manaenko,[3]* Hongda Bi,[4]* Ning Zhang,[1]* Ling Zhang,[5] Ting Zhang,[1] Zhouheng Ye,[1] and Xuejun Sun[1]

[1]*Department of Naval Aeromedicine, Faculty of Naval Medicine, Second Military Medical University, Shanghai, People's Republic of China;* [2]*Department of Cardiac Surgery, Changhai Hospital, Second Military Medical University, Shanghai, People's Republic of China;* [3]*Department of Neurology, University of Erlangen-Nuremberg, Erlangen, Germany;* [4]*Department of Plastic Surgery, Changhai Hospital, Second Military Medical University, Shanghai, People's Republic of China; and* [5]*Department of Medical Genetics, Second Military Medical University, Shanghai, People's Republic of China*

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Zhang R, Yu Y, Manaenko A, Bi H, Zhang N, Zhang L, Zhang T, Ye Z, Sun X. Effect of helium preconditioning on neurological decompression sickness in rats. *J Appl Physiol* 126: 934–940, 2019. First published January 17, 2019; doi:10.1152/japplphysiol.00275.2018.—Decompression sickness (DCS) occurs because of an excessively rapid and extensive reduction of the ambient pressure. Bubble-induced spinal cord ischemia is generally considered as a part of neurological DCS pathogenesis. Because helium preconditioning (HPC) recently demonstrated beneficial properties against ischemic damage, we hypothesized that HPC may decrease the neurological deficits of DCS in rats. Seventy-five male Sprague-Dawley rats were divided into a non-HPC group ($n = 25$) and a HPC group ($n = 25$) and 25 naive animals that were euthanized for histological examination ($n = 5$) or anesthetized for baseline somatosensory evoked potential (SSEP) recordings ($n = 20$). To induce DCS, rats were compressed with air to a pressure of 709 kPa for 60 min and decompressed at a rate of 203 kPa/min. HPC was administered as three episodes of 79% helium-21% oxygen mixture inhalation for 5 min interspersed with 5 min of air breathing. We found that HPC resulted in significantly decreased DCS incidence and delay of DCS onset. HPC also improved animal performance on the grip test after decompression and significantly ameliorated decompression-induced decrease of platelet number. Furthermore, the incidence of abnormal SSEP waves and histological spinal lesions was significantly reduced by HPC. We conclude that HPC can decrease the occurrence of DCS and ameliorate decompression-induced neurological deficits.

NEW & NOTEWORTHY Helium preconditioning ameliorates decompression-induced neurological deficits in rats. Helium breathing before air dives may prevent neurological deficit and attenuate symptoms after decompression.

decompression sickness; helium preconditioning; neurological deficit; somatosensory evoked potential; spinal cord injury

INTRODUCTION

Decompression sickness (DCS) occurs because of an excessively rapid and extensive reduction of the ambient pressure (2). DCS manifestations range from simple joint pain to severe injury, including cardiopulmonary failure and neurological deficits. Neurological deficits, such as sensory abnormalities, difficulty in walking, and paraplegia, are attributed to spinal cord injury (SCI). The spinal cord white matter is therefore considered a major target organ for DCS (16). Although there is no definitive etiology of neurological DCS (14), bubbles in the tissue and blood vessels after decompression are purported to be responsible for decompression-induced neurological symptoms (1, 2). Bubbles may play a role in decompression-induced SCI in two possible ways (44): First, intravascular or extravascular bubbles may directly cause mechanical damage to blood vessels and decrease spinal cord perfusion, resulting in neurological damage (13, 30). Second, bubbles may also initiate biochemical cascades such as leukocyte adhesion, platelet aggregation, and complement activation, which in turn lead to ischemic damage (12, 46). Although the significance of ischemia in SCI pathogenesis after fast decompression is still debated (29), several publications have clearly demonstrated that spinal cord ischemia is responsible for the neurological deficits (7, 15). The beneficial effects of treatments for ischemia have also been shown in animal models of DCS (44).

Although helium has been considered a biologically inert gas, recent work has shown the potential biochemical actions of helium (5, 25, 43) and several short helium breathing episodes, known as helium preconditioning (HPC), can reduce ischemic damage in cardiac (20, 21), brain (28, 38), liver (48), and intestinal (9) tissues. Although it has been reported that breathing of helium-oxygen mixtures after decompression provides benefits for spinal cord DCS (8, 24, 27) and affects the evolution of gas bubbles that have formed during and after decompression (22, 23), these studies did not address any effects of helium breathing before a dive and the potential biochemical actions of helium. Since hypoxia is generally considered a part of DCS pathogenesis and HPC may have beneficial properties for the prevention of hypoxia-induced injury, we hypothesized that helium-oxygen mixture breathing before a dive may have benefits for DCS.

In the present study, we explored the effectiveness of HPC (breathing of helium-oxygen mixture before a dive) in a model of neurological DCS in rats. The effects of HPC on the onset of DCS symptoms and survival rate were evaluated. Grip test, somatosensory evoked potential (SSEP) recordings, blood cell

* R. Zhang, Y. Yu, A. Manaenko, H. Bi, and N. Zhang contributed equally to this work.

Address for reprint requests and other correspondence: X. Sun, Dept. of Naval Aeromedicine, Second Military Medical Univ., 800 Xiangyin Rd., Shanghai, People's Republic of China (e-mail: sunxjk@hotmail.com).

counting, and histological examinations of spinal cord were also employed. To the best of our knowledge this is the first study investigating the effects of HPC on experimentally induced DCS.

METHODS

Animals and experimental protocol. All procedures and methods for this study were approved by the Animal and Ethics Review Committee at the Second Military Medical University and performed in accordance with the relevant guidelines and regulations. Seventy-five male Sprague-Dawley rats (300–328 g; Shanghai Slac Laboratory Animal) were used and were housed in an air-conditioned (23–25°C) room on a 12:12-h light-dark cycle with free access to a pelleted rodent food and water. Animals were randomly divided into two groups: In the Non-HPC group, 25 animals were exposed to hyperbaric air followed by fast decompression to induce DCS without HPC intervention. In the HPC group, 25 animals were subjected to three 5-min episodes of HPC (Fig. 1) followed by compression and decompression as described for the Non-HPC group. In addition, 5 naive animals were euthanized for histological examination and another 20 animals were only anesthetized for baseline SSEP recordings (the Sham group).

Hyperbaric exposure and helium preconditioning. The animals were placed in a 5.3-liter transparent hyperbaric rodent chamber (type RDC 150-300-6; Second Military Medical University) to receive HPC and hyperbaric exposure or hyperbaric exposure alone. To induce DCS, animals were compressed with air to a pressure of 709 kPa at a rate of 101 kPa/min, kept at pressure for 60 min, and then rapidly decompressed to ambient pressure at a rate of 203 kPa/min (47). Soda lime was added to the chamber and fresh gas flow was maintained in order to avoid carbon dioxide retention. HPC was administered as three episodes of mixed (79% helium-21% oxygen) inhalation (5 min each episode) interspersed with three 5-min washout periods using air at 101 kPa (Fig. 1), similar to other studies (19, 36). To avoid the possibility of helium-induced hypothermia (6), all gas mixtures used in our experiments were warmed by a heating bath before HPC. Animal rectal temperatures were recorded by a meter 30 min before hyperbaric exposure, before hyperbaric exposure, and after rapid decompression.

Symptom observation. After decompression, rats were transferred into individual cages. A dedicated investigator blinded to the treatment monitored the onset of DCS symptoms for 30 min. Symptoms of DCS included walking difficulties, forelimb and/or hindlimb paralysis, abnormal breathing patterns, convulsions, or death (11). Animals were diagnosed as having DCS when one or more of these symptoms appeared.

Grip tests. For the grip test, a beam (50 cm in length and 3 cm in diameter) was pulled taut between two vertical supports and elevated 2 m above a platform. At 15 min and 30 min after decompression, the animals were placed on the beam between the supports and evaluated for 30 s. The evaluations were scored by two blinded observers as follows: 0 = forelimb and hind legs seize the string firmly and climb freely; 1 = hind legs can seize the string and keep the body balanced but cannot climb; 2 = hind legs cannot seize the rope, but the rat does not fall from the string; 3 = fall from the string during the observation period (31, 42).

SSEP recordings. Lumbar SSEP recordings were selected to investigate spinal cord function after rapid decompression (Shanghai Haishen Medical Electronic Instrument). Animals were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (8 mg/kg) 30 min after decompression, and a thermostatically heated pad was used to keep animal body temperature at 37°C. To eliminate electric interference, the heating pad was disconnected during recordings (35). The stimuli employed for the SSEP recordings were electrical square-wave pulses (0.1 ms, 4 Hz) delivered via a disposable monopolar needle with cable electrodes. The needle electrodes were placed subcutaneously between the medial malleolus and Achilles tendon of each hind paw to stimulate the peroneal nerve. Recording electrodes were placed transcutaneously over the L_{1-2} intervertebral spaces. Subcutaneous electrodes positioned between the ilial crests served as the reference for L_{1-2} recordings. An additional electrode below the tail root served as a common ground (see Fig. 4*A*). Impedance of the recordings and reference electrodes was maintained below 5 kΩ. The band-pass filter was between 30 and 3,000 Hz, and recordings of SSEP were amplified 5,000-fold, digitized at a sampling rate of 200 kHz, and then transferred to a computer. Analysis time was 15 ms, and 500 repetitions per trial were averaged. Before being accepted, each averaged recording was replicated and superimposed to verify reproducibility of waveform, latency, and amplitude.

Blood cell tests. For blood tests, 20 μl of blood was taken from the tip of the tail. An equivalent volume of 2 mM EDTA was added to avoid coagulation. Blood was taken before the experiment (basal level) and 60 min after decompression (Fig. 1). Red blood cells, leukocytes, and platelets were determined by an automated procedure in the Department of Inspection, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University.

Histology. Trimmed at cervical, thoracic, and lumbar levels, spinal cord specimens were divided into three parts accordingly, and each part was again divided into two blocks. The blocks were then fixed in 10% buffered formalin solution, embedded in paraffin, sectioned at 5-μm thickness, and stained with Luxol fast blue or toluidine blue. Sections were examined with a computer image analysis system (Smart Scape; Furi Science & Technology, Shanghai, China).

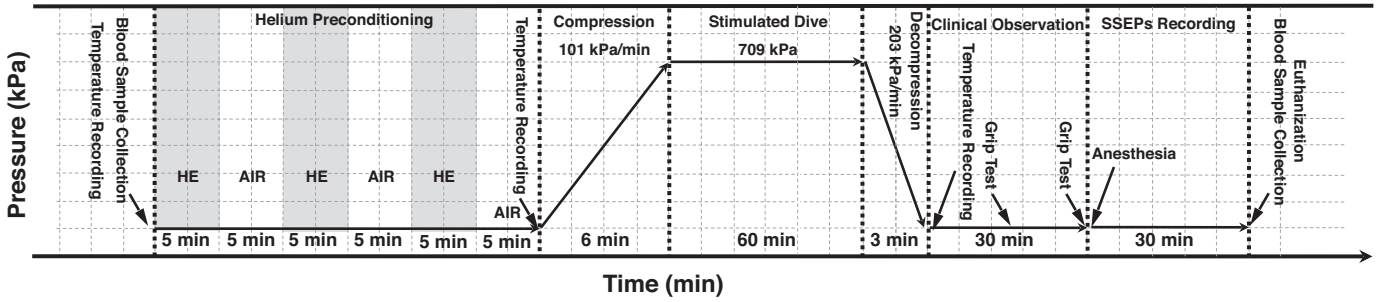

Fig. 1. Experimental design of helium preconditioning (HPC) in decompression sickness (DCS). After blood collection, animals received HPC consisting of 3 episodes of helium-oxygen (79%–21% mixture, 5 min each) interspersed with three 5-min episodes of room air inhalation. Animals were compressed with air to 709 kPa at the rate of 101 kPa/min, kept at that pressure for 60 min, and then rapidly decompressed at the rate of 203 kPa/min. After decompression, rats were observed for DCS symptoms for a 30-min period and tested neurologically with the grip test at 15 and 30 min. Thirty minutes after decompression animals were anesthetized and somatosensory evoked potential (SSEP) monitoring was performed. At the end of the experiment, blood samples were collected again and histological examination was started. Animal rectal temperatures were recorded 30 min before hyperbaric exposure, before hyperbaric exposure, and after rapid decompression.

Statistical analysis. DCS incidence and survival rate were compared between different groups by χ^2-test. Development of DCS and death were displayed by the Kaplan-Meier method and then compared with the log-rank test. Results of the grip test and incidence of SSEP abnormalities/histological spinal lesions were analyzed by Fisher's test. Blood platelet count was analyzed with a Wilcoxon test in matched comparisons and a Mann-Whitney test in different groups. Temperature data were analyzed with one-way analysis of variance. A P value of <0.05 was considered statistically significant.

RESULTS

DCS incidence and survival rate. A total of 50 rats were subjected to clinical observation after decompression. HPC significantly decreased incidence of DCS (Fig. 2*A*; 18/25 and 11/25, $P = 0.045$) and significantly increased the time before onset of DCS symptoms (Fig. 2*B*; $P = 0.039$) compared with the Non-HPC group. No significant difference in the time of onset of death was found between the Non-HPC group and the HPC group (Fig. 2*C*; $P = 0.529$).

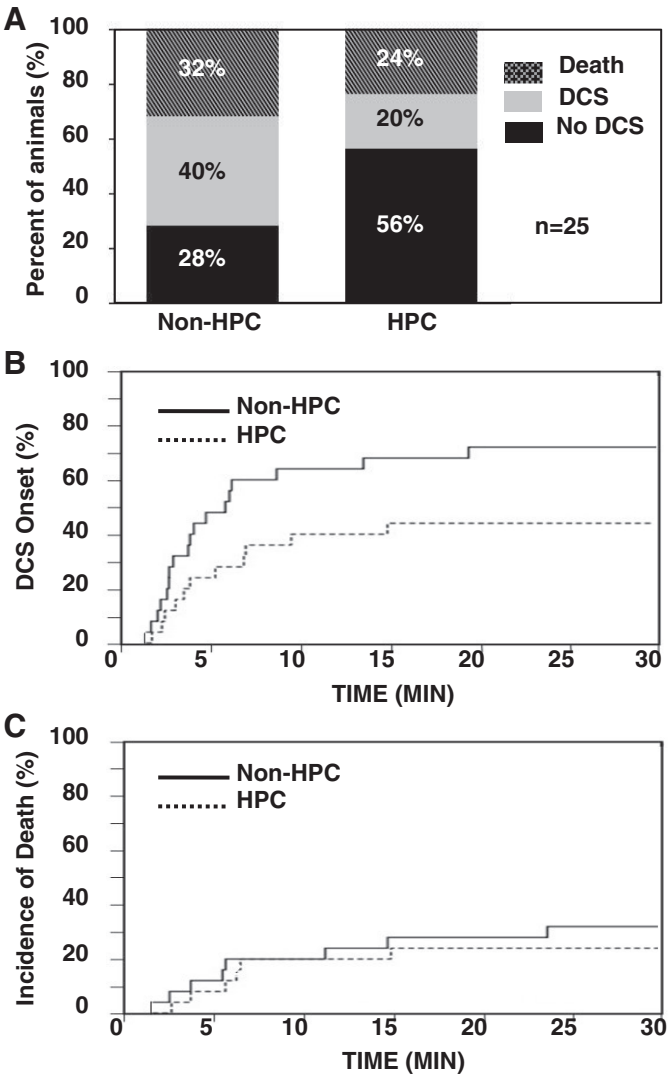

Fig. 2. Incidence of decompression sickness (DCS) and death in helium preconditioning (HPC) and Non-HPC rats after decompression. *A*: incidence of DCS and death in Non-HPC and HPC groups during 30-min period after decompression. Death, DCS symptoms culminating in death; DCS, DCS symptoms excluding death; No DCS, no clinical symptoms after decompression. *B*: the HPC group had significantly delayed onset time of DCS compared with the Non-HPC group. *C*: no effect of HPC on the time of onset of death was found.

Body temperature and grip tests. No significant change in body temperature was observed between the HPC and Non-HPC groups at all designated time points (Fig. 3*A*; $P = 0.891$, $P = 0.949$, $P = 0.483$, respectively). HPC significantly improved the performance of animals suffering from DCS in the grip test at 15 min after decompression (Fig. 3*B*; 12/17 and 6/19, $P = 0.044$). HPC also showed significant tendency toward improvement of performance in the test at 30 min (Fig. 3*B*; 11/17 and 6/19, $P = 0.049$).

SSEP recordings. In the Sham group, waveform morphology of the evoked potentials was remarkably consistent at each recording site and was similar to that described in previous publications (26, 47). At L_{1-2}, an initial sharp positive deflection (P1) was followed by a higher-amplitude negative peak (N1) that was frequently but not uniformly superseded by a lower-amplitude positive wave (P2), as can be seen in Fig. 4*B*. The amplitude exhibited wide variability between the animals, but the latencies had narrow standard deviations. SSEP recordings were successfully obtained from those animals that survived the experimental conditions. Abnormal SSEP components were calculated when peaks could not be identified (Fig. 4*B*) or their latency was more than two standard deviations from the mean of the Sham group as determined by two blinded observers (26); 47.4% of survival rats (9/19) in the HPC group had one or more abnormalities in their SSEP recordings, which was significantly lower than the Non-HPC group (88.2%, 15/17) (Fig. 4*C*; $P = 0.014$).

Blood cell counts. Comparison of blood cell numbers in blood of animals before and after decompression showed that the fast decompression caused significant decrease in the number of platelets (HPC, $P = 0.003$; Non-HPC, $P = 0.003$), leukocytes (HPC, $P = 0.007$; Non-HPC, $P = 0.001$), and red blood cells (HPC, $P = 0.007$; Non-HPC, $P = 0.01$). HPC significantly ameliorated decompression-induced decrease in the number of platelets (Fig. 5; $P = 0.036$), but no statistically significant differences between the two groups were found in the decreased numbers of leukocytes ($P = 0.974$) and red blood cells ($P = 0.948$).

Histological examination. By gross observation of the spinal cord, injuries to the lumbar segment were clearly observed in some animals after rapid decompression (3 in HPC group and 7 in Non-HPC group), which indicates that those animals suffered severe DCS (Fig. 6*A*). In Luxol fast blue-stained spinal sections, axonal degeneration (areas with diminished staining, as shown in Fig. 6*B*) were also seen in rats suffering severe DCS only. Alterations in gray matter of the spinal cord were investigated by toluidine blue staining of spinal cord sections. No changes between naive and decompressed animals were observed in the gray matter of spinal cord stained with toluidine blue (Fig. 6*C*). All of these histological findings are in accordance with previous studies (24, 37, 47), and thus we characterized the decompression-induced histological SCI by the injuries observed during the gross observation and the degenerated axons of the white matter. Abnormal histological SCI lesions were observed in 15.7% of survival rats (3/19) in the HPC group, which was significantly lower than the Non-HPC group (10/17) (Fig. 6*D*; $P = 0.014$).

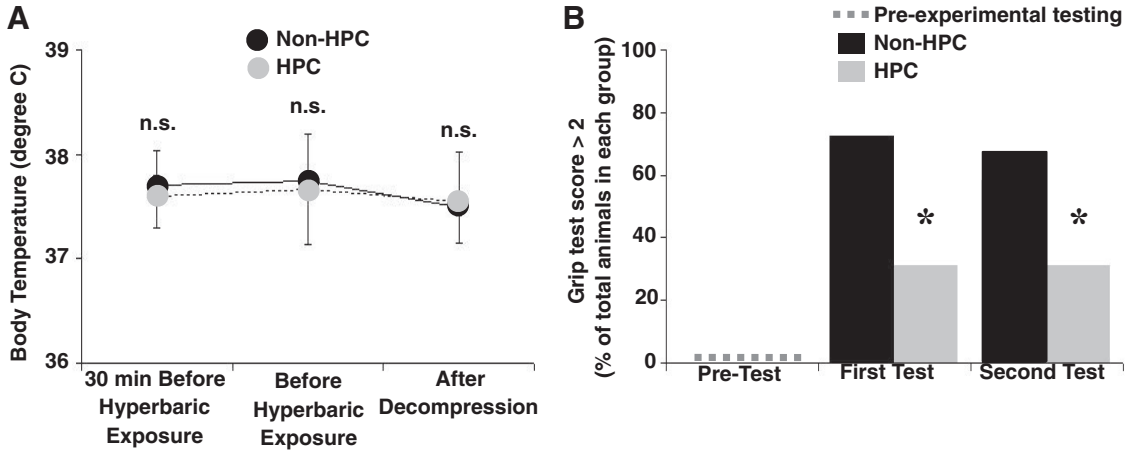

Fig. 3. Effect of helium preconditioning (HPC) on body temperature and grip test performance. *A*: no significant change in body temperature was observed between the HPC and Non-HPC groups 30 min before hyperbaric exposure, immediately before hyperbaric exposure, and after rapid decompression. n.s., Not significant. *B*: compared with the Non-HPC group, the HPC group showed significantly ameliorated neurological deficits in animals suffering from decompression sickness (DCS) at 15 min and showed a significant tendency toward improvement of performance at 30 min after fast decompression. $*P < 0.05$ between groups.

DISCUSSION

While hyperbaric oxygen remains the gold standard for treatment of DCS, standard hyperbaric oxygen treatment may be delayed because of the remote location of diving or flying events that result in decompression injury. The large logistical footprint of recompression facilities and the cost of equipment and staffing necessarily limit the number of locations available. To avoid the need for compression after decompression, non-recompression strategies for prevention or mitigation of DCS have long been sought by researchers in the flying and diving communities.

Helium is an inert element that belongs to the family of noble gases. Recent studies have shown that helium may have beneficial properties for the prevention of hypoxia-induced injury to several tissues (5, 9, 20, 21, 28, 38, 44, 48). These organs can be protected against ischemia-reperfusion injury by subjecting them to three episodes of helium-oxygen inhalation interspersed with 5 min of air inhalation. Since hypoxia is generally considered a part of DCS pathogenesis (7, 15, 45), we chose a similar HPC protocol to investigate the effects of HPC on neurological DCS in rats.

In the present study, 72% of animals subjected to the simulated dive with fast decompression had symptoms of DCS within 30 min of decompression. This is consistent with a previous publication using a similar protocol of DCS induction (11). We found that HPC significantly decreased the incidence of DCS and increased the time before DCS symptom onset. Previous studies suggested that helium inhalation after decompression is a promising approach to treating neurological DCS (8, 24, 27), and our results indicate the potential application of helium before compression in providing benefits for neurological DCS.

The development of neurological deficits is a major consequence of DCS. In our study, neurological deficits were evaluated by the grip test, which is a well-established test used in animal models of DCS (3, 45). We found that grip test performance at 15 and 30 min after decompression could be significantly improved by HPC. To further investigate the

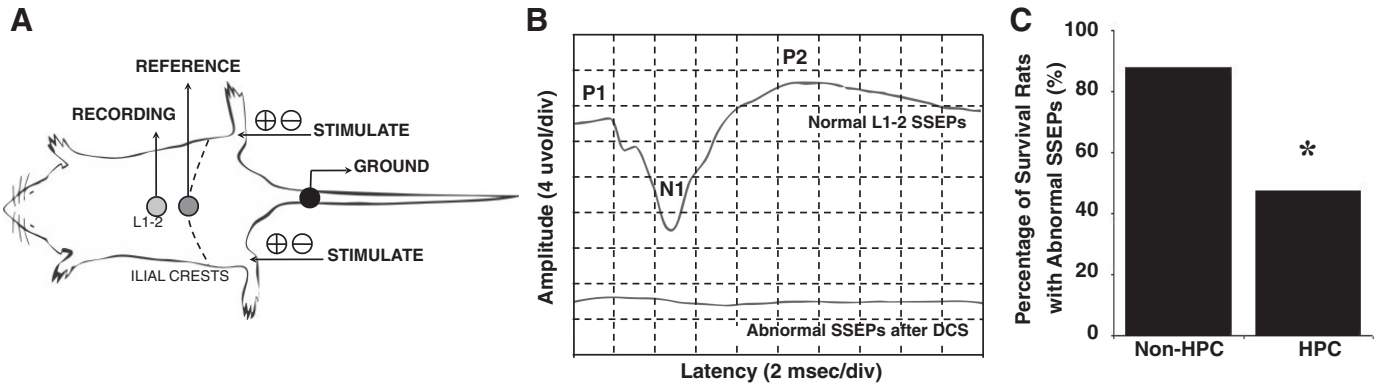

Fig. 4. Effect of helium preconditioning (HPC) on spinal cord function evaluated by somatosensory evoked potential (SSEP) recordings after decompression. *A*: general scheme of electrode connections of the rat for SSEP recordings. Stimulation electrodes were placed on the hind paws for the peroneal nerve. The responses were recorded from electrodes at lumbar ($L_{1–2}$) intervertebral spaces. Reference electrodes were positioned between the ilial crests. A common ground electrode was placed below the tail root. *B*: normal and abnormal SSEP recordings from $L_{1–2}$. Each recording was replicated to verify reproducibility. *C*: % of survival rats with abnormal SSEP recordings. $*P < 0.05$ between groups.

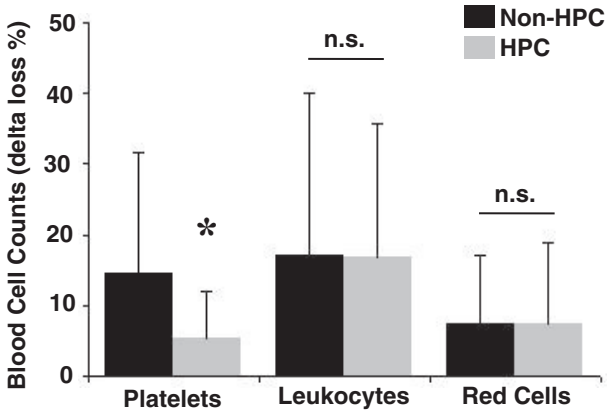

Fig. 5. Helium preconditioning (HPC) ameliorated decompression-induced decrease in number of platelets: % of blood cell consumption after decompression from baseline for Non-HPC and HPC groups of rats. Decompression induced significant decrease of platelets, leukocytes, and red blood cells. HPC attenuated decompression-induced decrease of platelets. $*P < 0.05$ between groups. n.s., Not significant.

effects of HPC on spinal cord function after DCS, SSEP monitoring was also performed. Measured change in the SSEP recordings is a valid method of evaluating possible decompression insults to the spinal cord and has been successfully employed in decompression research in animals (26, 47) and in the clinical evaluation of acute DCS in humans (32). In our experiment, we found that HPC can significantly improve the spinal conductive dysfunction in rats with experimentally induced DCS, which is in agreement with our previous findings of grip test performance, suggesting beneficial properties of HPC in a neurological DCS rodent model.

We observed a significant decrease in the number of blood cells (platelets, leukocytes, and red blood cells) in both the Non-HPC and HPC groups compared with baseline level, and we found a significant tendency for HPC to ameliorate decompression-induced decrease of blood platelets only, as others have reported (32, 41). The change in platelet count is usually attributed to change in clotting activity following exposure of the collagen under bubble-damaged vascular endothelial cells or to direct interactions between bubbles and platelets (17, 18). The postdive decrease of platelets has also been used as a predictor of DCS severity (40). Thus the tendency for HPC to ameliorate decompression-induced decrease of blood platelet number suggests a potential mechanism for the beneficial effect of HPC on DCS observed in this experiment. In addition, the fall in leukocyte count after DCS is usually attributed to diapedesis (33), and the formation of red cell aggregates appears to be associated with flow stasis (39). In our experiment, we did not find significant differences between the Non-HPC and HPC groups in the decrease of leukocytes and red blood cells, which suggests that leukocyte and red blood cell counts are not as strongly correlated with DCS severity as platelet count (39).

In accordance with previous studies (24, 37, 47), red lesions of the lumbar spinal cord can be found in some animals after decompression, and foci of demyelination and spongiosis in the white matter regions of the spinal cord can also be observed in rats suffering from severe DCS in our experiment. Myelin degeneration is considered to be a response to neuronal degeneration, and spongiosis is a result of lesions such as splitting of the myelin sheath, separation of extracellular space, and swelling of neuronal and glial cell processes (10). We found that the incidence of spinal cord lesions in rats with experimentally induced DCS is significantly decreased after HPC, further suggesting that the decreased incidence of spinal cord lesions in HPC rats is consistent with the reduced severity of neurological deficits in these rats.

Helium is suitable for use in humans because of its favorable characteristics including lack of hemodynamic side effects (34). Helium has a lower density compared with oxygen and nitrogen and thus reduces airway resistance and promotes airflow through the lungs. Helium is not toxic, and, to date, inhalation of normoxic helium-oxygen gas under normobaric conditions has not been found to cause any adverse effects. Therefore, HPC before decompression seems to be a promising approach to preventing neurological DCS. However, the spe-

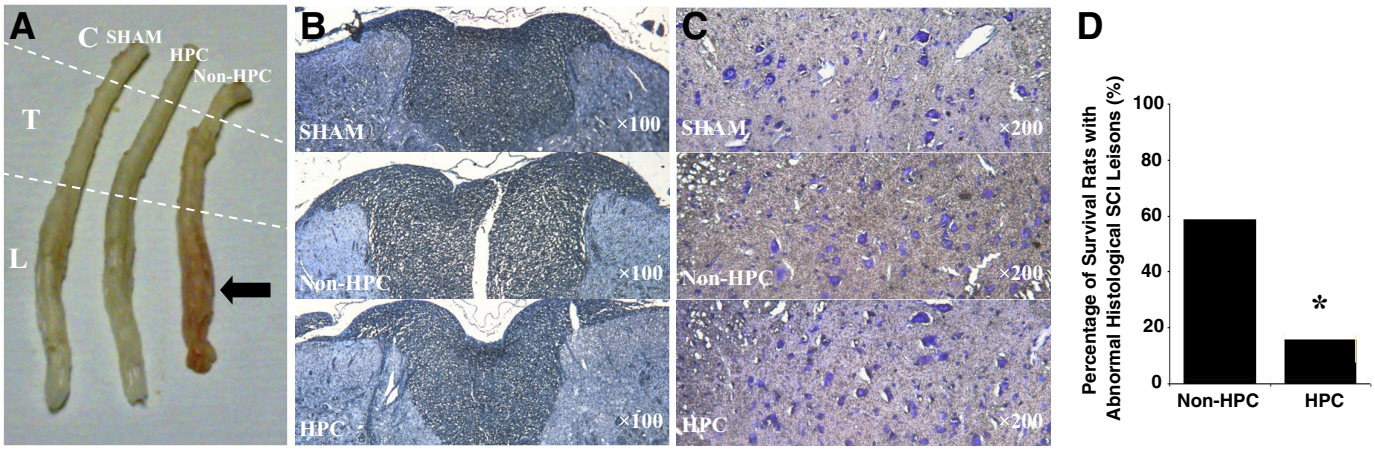

Fig. 6. Histological examination of spinal cords of animals suffering from decompression sickness (DCS). *A*: compared with the Sham group, 58.8% of DCS survival rats suffered severe DCS, which presented as minor lesions (arrow) in the lumbar spinal cord. However, in 84.3% of DCS survival rats after helium preconditioning (HPC) these spinal lesions cannot be observed. C, cervical; T, thoracic; L, lumbar. *B*: microscopic examination of Luxol fast blue-stained lumbar spinal cord. Axonal degeneration (areas of diminished staining) was observed in white matter of spinal cord of rats suffering from severe DCS (magnification ×100). *C*: microscopic examination of toluidine blue-stained lumbar spinal cord. No obvious changes were observed in gray matter of the spinal cord among the group (magnification ×200). *D*: % of survival rats with abnormal histological spinal cord injury (SCI) lesions. $*P < 0.05$ between groups.

cific mechanisms responsible for HPC's actions remain poorly understood. Previous findings have shown that helium inhalation provides neuroprotection in rats subjected to transient middle cerebral artery occlusion by producing hypothermia (6). In our experiment, HPC still provided effective protection against DCS while animal body temperature was controlled, indicating that hypothermia is not the only method of producing helium-induced neuroprotection. Apart from hypothermia, helium-induced denitrogenation may be another potential cause of reduced decompression damage in HPC rats. Breathing of helium before a dive may reduce postdive N_2 supersaturation achieved by predive N_2 washout, thus leading to reduced free gas (circulating bubbles-venous gas emboli) formation and fewer blood/tissue-bubble interactions and related cellular, tissular, and inflammatory changes. We cannot exclude the possibility because gas phase presence monitoring was not used in our study. However, it was reported previously that 20-min oxygen breathing at 1 atmosphere absolute before a hyperbaric exposure failed to show protection against DCS in rats (45). Another experiment also reported that denitrogenation with 45-min 100% oxygen prebreathing at sea level did not attenuate symptoms of neurological deficits after decompression in rats (4). Considering that the cumulative time of helium breathing in our experiment was only 15 min, it seems that helium prebreathing-related N_2 washout was not the main reason for the neuroprotection by HPC from DCS. In addition, previous studies also reported that the repeated interruption of helium-oxygen breathing with short air breathing periods in HPC profile was important during HPC-induced liver (48) and intestine (9) protection; thus we cannot deny that those repeated gas interruptions may also contribute to the beneficial actions against DCS induced by HPC.

Finally, responses that are due to the different physiological gas exchange properties of the gases and other responses that are due to the partitioning or binding of the gases in hydrophobic pockets of proteins should be differentiated. For example, the beneficial effects of helium breathing on in vivo gas bubble resolution are putatively due to the different permeabilities of helium and nitrogen in tissue and the influences of those differences on the kinetics of tissue-bubble gas exchange when breathing gases are switched after decompression (22, 23, 27), whereas HPC results are thought to be caused by direct gas-protein interactions (48). The mechanisms underlying helium-induced protection from neurological DCS need further investigation.

In conclusion, DCS incidence and neurological deficits can be significantly reduced by preconditioning with helium-oxygen mixture inhalation interspersed with repeated air exposure in rats. The described technique of HPC has promise as a preventative measure to reduce neurological DCS.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.Z., H.B., N.Z., L.Z., T.Z., Z.Y., and X.S. conceived and designed research; R.Z., Y.Y., and N.Z. performed experiments; R.Z., Y.Y., and A.M. analyzed data; R.Z. and A.M. prepared figures; R.Z. and A.M. drafted manuscript; R.Z., A.M., H.B., and X.S. edited and revised manuscript; R.Z., Y.Y., A.M., H.B., N.Z., L.Z., T.Z., Z.Y., and X.S. approved final version of manuscript.

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