WILDERNESS & ENVIRONMENTAL MEDICINE, **27,** 355–363 (2016)

ORIGINAL RESEARCH

Pathophysiologic Determination of Frostbite Under High Altitude Environment Simulation in Sprague-Dawley Rats

Jie Hu, MD; Hua Li, MD; Xiliang Geng, MD; Lin Jiao, MD; Hongping Song, MD; Lin Lou, MD; Mingke Jiao, MD

From the Department of Echocardiography, Affiliated Traditional Chinese Medicine Hospital, Xinjiang Medical University, Urumqi, China (Drs Hu and Li); the Department of Medical Engineering, Urumchi General Hospital of Lanzhou Military Region, Urumchi, China (Drs Geng, Jiao, Lou, and Jiao); and the Department of Ultrasound, Xijing Hospital, The Fourth Military Medical University, Xi'an, China (Dr Song).

Objectives.—Pathophysiologic changes of frostbite have been postulated but rarely understood, especially the changes caused by chilly high altitude environment. Therefore, we investigated the pathophysiologic changes of high altitude frostbite (HAF) caused by cold and hypoxia.

Methods.—Forty Sprague-Dawley rats were randomly divided into 5 equal groups, namely, control, superficial HAF (S-HAF), partial-thickness HAF (PT-HAF), full-thickness HAF (FT-HAF), and partial-thickness normal frostbite (PT-NF) groups. The S-HAF, PT-HAF, and FT-HAF groups were fed under hypobaric hypoxic conditions simulating an altitude of 5000 m. Then, the PT-NF, S-HAF, PT-HAF, and FT-HAF models were constructed by controlling the duration of the direct freezing by liquid nitrogen. Animal vital signs were measured after the operation, and histopathologic changes were observed after routine hematoxylin and eosin staining. In addition, the microcirculation of frostbite tissues was monitored and compared by contrast ultrasonography during wound healing.

Results.—The S-HAF, PT-HAF, and FT-HAF groups showed significant differences in the microcirculatory and histopathologic changes in the various tissue layers ($P < .05$). In addition, combined cold and hypoxia caused more damage to frostbite tissue than pure cold. The circulation recovery of HAF models was significantly slower relative to NF models ($P < .05$).

Conclusions.—A safe and reproducible HAF model was proposed. More important, pathophysiologic determination of HAF provided the foundation and potential for developing novel and effective frostbite therapies.

Key words: pathophysiology, high altitude frostbite, high altitude environment, microcirculation, contrast ultrasonography

Introduction

Frostbite is the acute freezing of tissues caused by exposure to harsh, low-temperature environments.[1,2] Different populations suffer from severe cold damage.[3,4] Frostbite is the most common cold injury of outdoor workers undergoing activities in high altitude and frigid regions.[5,6] Moreover, frostbite injuries usually occur among cold-storage depots as a result of accidental misuse of medical cold devices.[7] Soldier deaths caused by frostbite are common at subzero environments during wartime.[4] Frostbite causes pain, productivity loss, high expenditures, and potential for permanent disability.[8,9] However, few studies have indicated allegedly successful treatment of cold injuries in small experiments or case reports.[10–16] Screening of new and effective therapies using convenient nonhuman animal experiments is essential. Thus, some of the current studies have observed the pathophysiologic changes of frostbite models and showed important developments.[10,17–19] However, the pathophysiologic determination of frostbite was incomplete for the design of frostbite models. Some frostbite models were restricted by the applied techniques to a designated part of animals, such as ear and leg.[20–22] In addition, the depth of frostbite was also limited to some certain tissue layers for different reasons.[17,18] The most important factor was the

Corresponding author: Mingke Jiao, MD, Department of Medical Engineering, Urumchi General Hospital of Lanzhou Military Region, 359 Youhao Road, Urumchi, China 830000 (e-mail: 82161659@qq.com).

Submitted for publication December 2015.
Accepted for publication March 2016.

fact that current frostbite pathophysiologic models were formulated by only considering the direct injury of cold, but injury from combined cold and tissue hypoxia was ignored.[17–22] However, frostbite frequently occurs among high altitude climbers, which results not only from severe cold but also from tissue hypoxia.[5,23,24] Hypoxia and cold cause increases in blood packed-cell volume, viscosity, and small vessel blockage, which are the main contributors to frostbite injuries.[25,26] Therefore, more suitable frostbite models and pathophysiologic determination should be implemented to elucidate clearly and completely high altitude frostbite (HAF) pathophysiology and obtain suitable reference for clinical practical application.

A novel HAF model was developed in the present study to determine the pathophysiology of frostbite. In particular, HAF in this paper means the frostbite was caused by cold at a simulated altitude equivalent to approximately 5000 m above sea level. The HAF models were completed by causing superficial (S-HAF), partial-thickness (PT-HAF), and full-thickness (FT-HAF) frostbite injury in the dorsum of Sprague-Dawley rats under cold and hypoxic environment simulation. We observed and determined the pathophysiology of accidental HAF in this paper. Vital signs, as well as microcirculatory and histologic changes, were observed systematically and compared using contrast ultrasonography and light microscopy.

Material and Methods

ANIMALS

A total of 40 Sprague-Dawley rats (3-month-old males weighing 275 ± 10 g) were obtained from the Animal Center of the Urumqi General Hospital of Lanzhou Military Region. All uses of animals and experimental procedures followed the guiding principles of the Institutional Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals, Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23) and the Urumqi General Hospital Institution Review Board. The rats were allowed to adapt to the hypoxia and low-pressure conditions for 1 week before testing. Animals were housed in a large hypobaric chamber (DYC-3013M, Urumqi General Hospital of Lanzhou Military Region, Urumqi, China) with controlled temperature (23° ± 2°C), humidity (50% ± 10%), pressure (54.1 ± 1.0 kPa), and light-dark cycles (9:00 AM–9:00 PM with lights on). Significantly, 54.1 ± 1.0 kPa is the atmospheric pressure one expects at approximately 5000 m (17,000 feet) above sea level. The rats received standard chow and clean tap water ad libitum from an automated watering system during the entire experiment.

EXPERIMENTAL DESIGN

A total of 40 rats were equally divided (n = 8) and randomly assigned to 5 groups, as follows: control, S-HAF, PT-HAF, FT-HAF, and PT-normal frostbite (NF) groups. The HAF groups (except the control and the PT-NF groups) were acclimated to a hypoxic and low-pressure environment for 1 week. Blood samples were collected from the tail vein to evaluate the hemorheology and inspected for any disease signs. The rats in the HAF and NF groups were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg body mass). We selected the dorsum to freeze to produce frostbite although different parts of the animals could be frozen to form frostbite in our study. Theoretically, the chill could easily reach different layers because of the thin tissue layers in the dorsum. The dorsum was shaved, with a 35-mm diameter, and the operative site was prepared aseptically before freezing after adequate anesthesia.

The cold-injury equipment used custom-built liquid nitrogen penetration. Liquid nitrogen was stored in an aluminum alloy can with an air sandwich layer. The can was sealed with a resin plug, which consisted of an intake orifice and a gas leak bolt. The container surface was covered with a silicon dioxide aerogel flexible heat insulation film to protect the operator. Air was pumped into the aluminum alloy container by a micropump through plastic pipes to create a pressure gradient, which allowed the liquid nitrogen to penetrate the aluminum alloy connection. A mesh-hole construction and asbestos stopped the container bottom.

The skin of the rat dorsum was made to touch the aluminum alloy mesh upon freezing, which ensured that the liquid nitrogen directly froze the rat tissues. Liquid nitrogen penetration flow was controlled by loosening or tightening the gas leak bolt (Figure 1). The freezing duration was varied to control the frostbite degree.

The freezing duration was limited to 5, 15, and 28 seconds to generate S-HAF, PT-HAF, and FT-HAF, respectively. To compare the HAF group with the NF group, the same process was performed to generate PT-NF with the freezing time of 15 seconds. The different effective freezing durations for inducing replicable HAF models have been studied based on the injury that reached the epidermis, dermis, and muscle layer. Regarding the thickness or depth of the frostbite, the following were observed: S-HAF injury extends to the epidermis; PT-HAF injury extends through the epidermis into the dermis; and FT-HAF injury extends through the dermis into muscle and even bone. The results were confirmed by histologic observation in previous experiments. After surgery, rats were housed individually and fed under normal temperature (23° ± 2°C) and pressure (91.5 ± 0.1 kPa,