Original Article

Pathological changes in the lung and brain of mice during heat stress and cooling treatment

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BACKGROUND: Heatstroke often leads to multiple organ dysfunction syndrome (MODS) with a death rate of 40% or a neurological morbidity of 30%. These high rates in patients with heatstroke are largely due to the progression of heat stress to MODS, resulting in no specific treatment available. This study aimed to develop a mouse model of heat stress and determine the pathological changes in the lung and brain during heat stress and cooling treatment.

METHODS: A mouse model of heat stress was established in a pre-warmed incubator set at $35.5 \pm 0.5^{\circ}\text{C}$ and with a relative humidity of $60\% \pm 5\%$. Rectal temperature was monitored, and at a temperature of 39°C , 40°C , 41°C , or 42°C , the mice were sacrificed. The remaining animals were removed from the incubator and cooled at an ambient temperature of $25 \pm 0.5^{\circ}\text{C}$ and a humidity of $35\% \pm 5\%$ for 12 or 24 hours at a temperature of 41°C or for 6 hours at a temperature of 42°C . The control mice were sham-heated at a temperature of $25 \pm 0.5^{\circ}\text{C}$ and a humidity of $35\% \pm 5\%$. The lungs and brains of all animals were isolated. Hematoxylin and eosin staining and light microscopy were performed to detect pathological changes.

RESULTS: All mice demonstrated a uniform response to heat stress. A low degree of heat stress induced marked pathological changes of the lungs. With the rise of the temperature to 42°C , progressively greater damage to the lungs with further congestion of the lung matrix, asystematic hemorrhage of alveolar space, abscission of alveolar epithelial cells, and disappearance of pulmonary alveolus tissue structure were detected. However, absorption of congestion and hemorrhage as well as recovery of pulmonary alveolus tissue structure was observed following cooling treatment at an ambient temperature. With a low degree of heat stress, the brain only showed moderate edema. Neuronal denaturation and necrosis were detected at a temperature of 42°C . Interestingly, the lesions in the brain were further aggravated at 42°C regardless of cooling treatment, but recovery was observed after cooling treatment at 41°C .

CONCLUSIONS: The pathological changes of the lungs and brain of mice showed distinctive lesions following heat stress and cooling treatment, and they were correlated with the time and duration of cooling treatment. The results of this study are helpful for further study of the mechanisms linking heatstroke.

KEY WORDS: Heat stress; Heatstroke; Cooling treatment; Lung; Brain; Pathological change; MODS

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INTRODUCTION

Despite adequate lowering of body temperature and intensive care, the mortality is as high as 10%-15% in patients with heatstroke. Heatstroke often leads to multiple

organ dysfunction syndrome (MODS) with a death rate of 40% or a neurological morbidity of 30%.^[1-3] These high rates in patients with heatstroke are largely due to the progression of heat stress to MODS. Yet there is no

specific treatment available.^[4,5]

Tissue damage is a common manifestation of heatstroke syndrome. Its complications include acute respiratory distress syndrome (ARDS), cerebral edema, disseminated intravascular coagulation (DIC), shock, rhabdomyolysis, renal failure, and hepatic dysfunction.^[6,7] The histopathology of injured tissue could reflect a common manifestation of MODS. To observe the pathological changes at the tissue level, we established a mouse heatstroke model, and cooling treatment was also applied to mimic clinical therapy. As clinical manifestations, pathological changes of the lungs and brain were assessed during heat stress and cooling recovery. The findings of this study help to investigate the mechanisms underlying heatstroke and MODS.

METHODS

Animals

Pathogen-free, 6-to 8-week-old male BALB/c mice were housed in barrier cages under controlled environmental conditions ($35\% \pm 5\%$ humidity at $25\text{ }^{\circ}\text{C}$) in the Experimental Animal Center of Southern Medical University, Guangzhou, China. The mice had free access to standard laboratory chow and water. All procedures dealing with the animals in this study were approved by the Animal Care and Use Committee of Southern Medical University before the experiment and were conducted under the Guidelines for Animal Care of Southern Medical University.

Heat stress protocol and cooling treatment

The mice were fasted 12 hours before the experiment but allowed to have water and libitum. After stabilization for 20 hours at an ambient temperature of $25 \pm 0.5\text{ }^{\circ}\text{C}$ with a humidity of $35\% \pm 5\%$, 48 mice were divided into two groups: control group ($n = 6$) and heat stress (HS) group ($n = 42$). The mice of the HS group were placed in a pre-warmed incubator at $35.5 \pm 0.5\text{ }^{\circ}\text{C}$ and a relative humidity of $60\% \pm 5\%$ in the absence of food and water. The mice in the control group were sham-heated at $25 \pm 0.5\text{ }^{\circ}\text{C}$ and a humidity of $35\% \pm 5\%$ for the time comparable to that of the HS group. Rectal core temperature was continuously monitored with a rectal thermometer.

The mice of the HS group were further assigned to one of the following 7 groups: $39\text{ }^{\circ}\text{C}$, $40\text{ }^{\circ}\text{C}$, $41\text{ }^{\circ}\text{C}$, or $42\text{ }^{\circ}\text{C}$ respectively, and groups in which animals were removed from the incubator and allowed to cool at an ambient temperature of $25 \pm 0.5\text{ }^{\circ}\text{C}$ and a humidity of $35\% \pm 5\%$ for 12 or 24 hours at $41\text{ }^{\circ}\text{C}$ or for 6 hours at $42\text{ }^{\circ}\text{C}$, respectively.

Histopathological analysis

The mice were anesthetized by intraperitoneal injection of urethane. The samples of the lungs and brains were excised, sectioned transversely or longitudinally, and fixed in 10% neutral-buffered formalin. The sections were then embedded in paraffin and stained with hematoxylin and eosin for microscopic evaluation at a magnification of $\times 200$. The extent of tissue injury was evaluated by two certified pathologists who knew nothing about the nature of the groups being assessed.

RESULTS

Thermal responses of mice to heat stress and cooling treatment

Thermal responses of mice during heat stress have been reported previously,^[8] i.e. the temperature reached $39\text{ }^{\circ}\text{C}$ followed by a decrease to $38\text{ }^{\circ}\text{C}$. After a slow rise over 3 hours, the temperature reached $42\text{ }^{\circ}\text{C}$. The temperature of the sham-heated animals didn't significantly change during the experiment. The mice at $42\text{ }^{\circ}\text{C}$ were dead, because the temperature further increased to $43\text{ }^{\circ}\text{C}$ in an incubator or at 6 hours after cooling treatment. Mice in the other groups survived for more than 24 hours.

Tissue histopathology

Histopathological investigation was performed and representative photomicrographs of the lungs and brains were taken (Figures 1 and 2).

Lungs

No significant abnormalities were detected in the lungs of control mice (Figure 1A). Pathological changes were observed at $39\text{ }^{\circ}\text{C}$, characterized by angiotectasis, congestion, and thickening of the lung matrix (Figure 1B). Progressively greater damage to the lungs with further congestion of the lung matrix, hemorrhage of the alveolar space, abscission of alveolar epithelial cells, and disappearance of pulmonary alveolus tissue structure were detected with the rise of a temperature to $42\text{ }^{\circ}\text{C}$ (Figure 1C–E). However, absorption of congestion and hemorrhage as well as recovery of pulmonary alveolus tissue structure was observed for a long time after cooling treatment (Figure 1F–H).

Brain

No significant abnormalities were noted in the brains of control mice (Figure 2A). At a low temperature of $39\text{ }^{\circ}\text{C}$ or $40\text{ }^{\circ}\text{C}$, the brain only showed moderate edema, characterized by vacant space surrounding the neurons

Figure 1. Hematoxylin and eosin staining of the lungs of mice following heat stress (original magnification $\times 200$). **A:** Normal lung; **B-E:** lungs of mice at 39 °C, 40 °C, 41 °C, or 42 °C; **F-H:** after recovery with cooling treatment for 6 hours at 42 °C (F) and 12 hours (G) or 24 hours (H) at 41 °C. No significant abnormalities were detected in A. Pathological changes in B were characterized by angiectasis, congestion, and thickening of the lung matrix. Greater damage to the lungs with further congestion of the lung matrix, asystematic hemorrhage of the alveolar space, abscission of alveolar epithelial cells (shown with black arrow in C), and disappearance of pulmonary alveolus tissue structure were detected in C-E. Absorption of congestion and hemorrhage as well as recovery of pulmonary alveolus tissue structure was observed in F-H for a prolonged time after cooling treatment.

Figure 2. Hematoxylin and eosin staining of the brains of mice after heat stress (original magnification $\times 200$). **A:** Normal lung; **B-E:** lungs of mice at 39 °C, 40 °C, 41 °C, or 42 °C; **F-H:** after recovery with cooling treatment for 6 hours at 42 °C (F) and 12 hours (G) or 24 hours (H) at 41 °C. No significant abnormalities were noted in the brains of control animals (A). At 39 °C or 40 °C (B and C), the brain only showed moderate edema, characterized by vacant space surrounding the neurons and capillaries (black arrows in C). Neuronal denaturation and necrosis (black arrows in E) were detected at 41 °C, and this was more evident at 42 °C (D and E). Aggravated brain lesions were characterized by gliocyte disaggregation (black arrow in F) and more obvious edema and neuron necrosis after cooling treatment at 41 °C or, especially, 42 °C (F-H).

and capillaries (Figure 2 B and C). Neuronal denaturation and necrosis were detected when the temperature reached 41 °C, and they became more obvious at 42 °C (Figure 2 D-E). Interestingly, brain lesions were further aggravated with gliocyte disaggregation and more obvious edema and neuron necrosis after the temperature reached 41 °C or, especially, 42 °C, even after cooling treatment at ambient temperature (Figure 2 F-H).

DISCUSSION

Heatstroke is a life-threatening illness characterized by a rapidly increasing core body temperature

(temperature >40 °C) and central nervous system abnormalities such as delirium, convulsions, and coma after exposure to a high ambient temperature (classical or nonexertional heatstroke) or strenuous exercise (exertional heatstroke). Current reports suggest that pathophysiological responses to heatstroke may not be due to the immediate effects of heat exposure but to a systemic inflammatory response syndrome (SIRS) induced by thermal injury. The high mortality observed after heatstroke is thought to be secondary to the occurrence of multi-organ dysfunction.^[9-11] Frequently encountered complications include ARDS, cerebral edema, seizures, DIC, shock, rhabdomyolysis, renal failure, and

hepatic dysfunction.^[12,13]

Leon et al^[9] observed lesions in the duodenum, spleen, and kidney in mice after heat strain, but there were no significant abnormalities in the liver, brain, heart, lung, or large intestine. In heat stress in baboons, Bouchama et al^[14] found that tissue damage of varying degree was present in the liver, brain, and gut. These studies only detected pathological changes at a single time point in heatstroke animals. In our study we determined pathological changes after heatstroke and cooling recovery.

To investigate predictors of MODS in patients with heatstroke, Varghese et al^[7] found that respiratory failure (85.7%) was common, and more than three quarters of such patients required mechanical ventilation. Kibayashir et al^[15] found pulmonary edema and acute microvascular lung injury after heat stress at a single time point. In our study, obvious pathological changes and progressively greater damage to the lungs during heat stress, absorption of congestion and hemorrhage and recovery of pulmonary alveolus tissue structure were also found for a prolonged time after passive cooling at ambient temperature. These changes were consistent with the clinical manifestations of patients with heatstroke, whose pulmonary function was usually impaired but recovered quickly.

According to the definition of heatstroke, the pathological changes of the central nervous system (CNS) are essential to the understanding of its pathophysiological mechanisms. However, there is little information about the occurrence of brain lesions. In our study, injury to the brain appeared even at 39 °C, and this was further aggravated with a rise of the temperature regardless of cooling treatment. The findings were consistent with the clinical manifestations of heatstroke patients who present with CNS dysfunction and neurological morbidity.

In conclusion, our study provides evidence for lesions in the lungs and brain after heat stress, including those after a cooling recovery. These changes are correlated with the timing and duration of cooling treatment, and help to investigate the relationship between heatstroke and MODS. The limitations of this study include no data obtained from a model of exertional heat stroke, nor determination of the relationship between organ function and the observed tissue damage, which may provide substantial information about the pathophysiology of heatstroke.

Conflicts of interest: The authors declare that there are not any financial interests or potential conflicts of interest relating to the study.

Contributors: Liu ZF proposed and wrote the study. All authors contributed to the intellectual content and approved the final version.

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