Targeted temperature management at 36°C attenuates progression of inflammatory damages after hemorrhagic shock

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Targeted temperature management at 36°C attenuates progression of inflammatory damages after hemorrhagic shock

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It is my hope that this study, enriched with valuable data, will contribute significantly to the fundamental understanding of emergency medicine, thereby enhancing and advancing best practices through its findings from this animal research.

This dissertation is dedicated to my parents, who have imparted invaluable lessons throughout my life and guided me to this precious achievement. I am profoundly grateful for their dedication and sacrifice, which have made this accomplishment possible.

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ABSTRACT

Targeted temperature management at 36°C attenuates progression of inflammatory damages after hemorrhagic shock

Background: Hemorrhagic shock (HS) is a life-threatening condition associated with significant mortality and complications, necessitating advancements in therapeutic approaches. Despite current protocols, considerable HS-related mortality persists. This study explored the potential of targeted temperature management (TTM) in mitigating the inflammatory response and enhancing organ protection following HS. The involvement of key mediators, such as high mobility group box 1 protein (HMGB1), and the intricate pathophysiology of macrophage phenotype transformation were central to the hypothesis.

Methods: A rat model of HS was employed, with TTM interventions at 33 and 36 °C after fluid resuscitation. Survival rates, histopathology, apoptotic events, intracellular reactive oxygen species (ROS) levels, and cytokine expressions were assessed. Macrophage activation and polarization were analyzed through immunohistochemistry. Cytokine arrays were employed to profile the inflammatory milieu in kidney and lung tissues.

Results: Unfortunately, the application of TTM at 33°C increased deaths related to HS. TTM at 36 °C significantly improved overall survival rates, attenuated histological damage in lung and kidney tissues, and reduced serum lactate levels after HS. It also demonstrated a protective effect against apoptosis-mediated cell death and lowered intracellular ROS levels. TTM at 36 °C inhibited extracellular HMGB1 release and downregulated proinflammatory cytokines in both organs. Additionally, it modulated macrophage activation, suppressing the M1 phenotype while promoting M2 polarization. Cytokine array analysis revealed a significant reduction in proinflammatory cytokines with TTM at 36 °C.

Conclusion: This study provided comprehensive evidence supporting the protective benefits of TTM at 36 °C in the context of HS-induced injuries. The multifaceted positive effects encompass reduced mortality, histological damage, apoptosis, inflammation, and modulation of macrophage phenotypes. These findings suggest a potential therapeutic avenue for TTM in critical care following HS, paving the way for enhanced patient outcomes and decreased mortality.

Key words : hemorrhagic shock**;** targeted temperature management; high mobility group box 1 protein; apoptosis; inflammation; macrophage

1. INTRODUCTION

Hemorrhagic shock (HS) is a critical medical condition that can manifest in various clinical scenarios, including traumatic injury, gastrointestinal bleeding, complications during childbirth, and the rupture of aortic aneurysms [1]. Globally, HS poses a substantial public health challenge, resulting in an average of 2 million fatalities annually, of which approximately 1.5 million are attributed to traumatic injuries [2]. The traditional clinical management of HS emphasizes prompt hemorrhage control, intravenous blood product administration, blood pressure maintenance, and antifibrinolytic therapy [3]. Nevertheless, survivors of the initial HS frequently face severe complications, such as compromised organ reperfusion, delayed infections, immune system dysfunction, and an elevated risk of multiorgan failure (MOF) [4]. Approximately 40% of cases lead to single organ failure, with MOF impacting approximately 22% [5]. Additionally, traumatic major bleeding-induced coagulopathy, characterized by irregular coagulation processes due to trauma, further complicates the clinical condition [6]. Initially, this coagulopathy induces hypocoagulability, leading to excessive bleeding. Subsequently, it transitions into a hypercoagulable state associated with venous thromboembolism and MOF. These processes involve synergistic activation of endothelial cells, the immune system, platelets, and the clotting system, exacerbated by the “lethal triad” [7]. Furthermore, the commonly observed calcium deficiency in trauma plays a significant role in the pathophysiology of trauma-related coagulopathy, acidosis, and hypothermia. It should be considered as one of the components of the "diamond of death," akin to the lethal triad of trauma [8]. Unfortunately, despite potential advancements, nearly 20% of HS-related deaths persist as inevitable, highlighting limited progress in current treatment protocols [9].

The intricate pathophysiology of systemic inflammatory response syndrome highlights the crucial roles of the inflammatory response, innate immune response, and apoptotic cell death in the development of hypovolemic MOF following major trauma [10-12]. Cellular apoptosis is a significant contributor to this process, and targeted temperature management (TTM) has proven effective in reducing the occurrence of apoptotic events [13, 14]. High mobility group box 1 protein (HMGB1), a molecule vital for the structural organization of DNA in eukaryotic cells, serves as a key mediator in the innate immune response [15]. Released during injury incidents, HMGB1's interactions with

inflammatory factors amplify the inflammatory response, potentially contributing to tissue damage following hypovolemic shock.

Macrophages, whether in the bloodstream or distributed throughout various organs and tissues, serve as the initial defense against diseases [16]. They play a pivotal role in regulating both innate and adaptive immunity, maintaining tissue balance, supporting angiogenesis, and contributing to metabolic processes. What defines macrophages is their remarkable diversity and adaptability, allowing them to exhibit a wide range of characteristics. These traits are well-coordinated responses to stimuli from the local tissue environment or microbial agents and their byproducts. Macrophages are classified into two primary groups based on their gene expression profiles: classically activated (M1) and alternatively activated (M2). The transformation of macrophage phenotypes from M1 to M2 is a critical factor in the onset, progression, and resolution of numerous inflammatory diseases [17, 18].

The use of TTM to regulate core body temperature is a crucial strategy for neuroprotection in post-resuscitation care. Temperature management options include targeted hypothermia, typically involving the reduction of core body temperature within the range of 32–36 °C, and targeted normothermia (NT), aiming to maintain core body temperature below 37.7 °C to prevent fever during treatment [19-21]. TTM has proven effective in improving both survival rates and neurological outcomes, benefiting not only individuals who have experienced cardiac arrest but also adult patients with acute liver failure and infants with hypoxic–ischemic encephalopathy. Since the early 2000s, TTM has become a widely accepted standard of care for patients with cardiac arrest. Moreover, TTM has been introduced into clinical practice for cardiovascular surgery, the treatment of severe traumatic brain injury, near-drowning, ischemic stroke, neonatal hypoxic–ischemic encephalopathy, and spinal cord injury [22, 23]. However, inducing hypothermia has few absolute contraindications even in patients with cardiac arrest, such as medical conditions associated with potentially excessive risks, including severe sepsis, refractory hypotension despite multiple vasopressors, intracranial hemorrhage, and severe hemorrhage leading to exsanguination [24]. Moreover, past studies have explored the potential role of induced hypothermia in traumatic HS. Experimental research has indicated that hypothermia can modulate immune and inflammatory responses [25].

Our hypothesis proposed that TTM would have the potential to ameliorate the inflammatory response in vital organs by limiting the extracellular release of HMGB1 and classical activation of M1 macrophages. Additionally, we anticipated that TTM would facilitate the transformation of M2

macrophages in a rat model of HS, potentially leading to enhanced organ protection during hypovolemic injuries. Hence, this study aimed to investigate the protective benefits of TTM, advancing therapeutic strategies that alleviate severe organ dysfunction, enhance patient outcomes, and ultimately reduce mortality resulting from HS-induced injuries.

2. MATERIALS AND METHODS

2.1. Animal preparation

Healthy, adult male Wistar rats weighing 360-380g and aged between 12 and 13 weeks were procured from a single-source breeder at Orientbio (Seongnam, Republic of Korea). Prior to experimentation, the animals were kept under controlled conditions. The temperature was maintained at 21±2°C with a relative humidity of 50±5%. The light/dark cycle was set to 12-hour intervals, with light intensity between 150-200 lux measured at 40-80 cm above ground level, using racks with shaded tops. Noise levels were kept below 60 dB, and the airflow rate was maintained at 13-18 cm/sec. The pressure was regulated to be slightly positive, with a pressure differential of +2 for the housing room, +1 for the animal corridor, and 0 for the external environment (2.5-3 mmHO pressure differential considered as +1). The animals were given an acclimation period of seven days before the start of the experiment. All experiments and animal care strictly adhered to guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Yonsei University Health System (2019-0106) and the National Institutes of Health.

2.2. Experimental rat model of HS

Rats were anesthetized with a 5% isoflurane mixture along with nitrous oxide (0.7 L/min) and oxygen (0.3 L/min). Blood pressure measurement involved the following steps: Endotracheal intubation using a 16-G Angio catheter (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and maintenance of anesthesia with 1.5% isoflurane in a mixture of 80% nitrous oxide and 20% oxygen. Mechanical ventilation (tidal volume, 2.3 mL; respiratory rate, 50 cycles/min) was performed using a rodent ventilator (SAP-830/AP; CWE, Inc, Ardmore, PA, USA). The left inguinal area was shaved, and local anesthesia was administered using a 0.2% bupivacaine solution. Subsequently, the left femoral artery was exposed, distally ligated with 4–0 black silk, and proximally clamped with a micro clip. A small incision was made between the microclip and the ligature using microscissors. During the removal of the microclip, a polyethylene-50 tube (Scientific Commodities Inc., Lake Havasu City, AZ, USA) was inserted and advanced to the inguinal ligament level through the femoral artery. Arterial blood pressure was measured by connecting a catheter to

the left femoral artery and then to the Lifewindow LW9x multi-parameter physiologic monitoring device (Digicare Biomedical Technology, Inc., Boston Beach, FL, USA) via a 25 IU/mL heparin-filled invasive blood pressure transducer (Utah Medical Products, Inc., Midvale, UT, USA). The invasive blood pressure transducer was positioned at heart level. After obtaining a stable and clear arterial waveform from the left femoral artery, the catheter was secured with 4–0 black silk. Body temperature and end-tidal carbon dioxide levels were continuously monitored using a rectal thermoprobe and capnography on the Lifewindow LW9x multi-parameter physiologic monitor to ensure the rat's physiological condition remained constant throughout the entire procedure. The systolic pressure, diastolic pressure, and calculated mean arterial pressure (MAP) were recorded three minutes after completing the procedure. Similarly, an angiocatheter was inserted into the right femoral artery for blood withdrawal, transfusion, and normal saline infusion, and securely fixed for the experiment.

2.3. Experiment protocol

The objective was to rapidly achieve and strictly maintain a target MAP of 38 ± 1 mmHg for 60 minutes. This was accomplished by carefully withdrawing and reinfusing blood using syringe pumps, potentially through a right femoral artery angiocatheter if required. Shed blood, treated with heparin, was kept at a controlled warm temperature with regular stirring. Total blood volume was estimated based on the participants' weight. After inducing HS for one hour, animals were promptly resuscitated to achieve a MAP of 95 mmHg through blood transfusion and normal saline infusion.

In the sham and HS without TTM groups, the core temperature was maintained as uncontrolled. In the TTM groups with target temperatures of 33 and 36 °C, external surface cooling commenced immediately after resuscitation and 5 minutes later, achieved by placing ice packs on the whole body. The targeted temperatures of 33 and 36 °C were rigorously maintained at 33.0 ± 0.2 and 36.0 ± 0.2 °C, respectively. Body temperature was monitored and maintained using surface cooling ice packs, a temperature monitoring system, and a feedback-controlled heating pad (HB 101; Harvard Apparatus, Holliston, MA, USA) throughout the surgical procedure. To prevent shivering induced by TTM, all animals were intramuscularly injected with vecuronium (0.9 mg/kg). Subsequently, in the TTM groups, rapid cooling was employed to reduce the rectal temperature to either 33 or 36 °C, maintaining this temperature for 3 hours using ice packs. Afterward, a careful rewarming process

was initiated, gradually raising the temperature to 37.5 °C over 1 hour, utilizing heating pads and packs.

Initially, we assessed survival rates among the HS, HS + TTM at 33 °C, and HS + TTM at 36 °C groups. Unexpectedly, TTM at 33 °C was associated with significantly increased mortality after HS, leading to its exclusion from the study. To evaluate the protective effects of TTM at 36 °C, experimental rats were randomly divided into four groups: sham, sham + TTM at 36 °C, HS, and HS + TTM at 36 °C, each consisting of 5 rats. Sacrifices were carried out at various time points, with the sham group sacrificed 4 hours after the experiment's completion; the TTM group, after temperature management; and the HS group, 4 hours after resuscitation. Subsequent to the experimental procedure, macrophage staining was conducted at the 24-hour time point. After the experiments, we used isoflurane for euthanasia, adjusting the concentration to 5% with a vaporizer. We continued the exposure to isoflurane for at least 15 minutes after respiratory arrest, and extended it for an additional one minute after breathing had stopped, ensuring a humane and effective euthanasia process [26].

2.4. Histopathology

The lung and kidney tissues were fixed in 4% paraformaldehyde and processed using standard procedures for paraffin embedding. Following this, the sections underwent hematoxylin and eosin (H&E) staining for histological evaluation, and images were captured using a suitable imaging system. The system used for slide scanning was the Axioscan 7 (Carl Zeiss, GmbH, Göttingen, Germany), and the software used for capturing images was ZEN 2.3 Lite (Carl Zeiss, GmbH, Göttingen, Germany). Lung damage was evaluated using a score adapted from Bilsborough et al. [27]. The Endothelial, Glomerular, Tubular, and Interstitial (EGTI) histology scoring system was used for kidney damage [28]. For histological evaluation, five slides each of lung and kidney tissues per animal were processed from the four experimental groups: Sham, Sham + TTM, HS, and HS + TTM, resulting in a total of 40 slides per tissue type across all groups.

2.5. Terminal Deoxynucleotidyl Transferase (TdT)-Mediated dUTP Nick End Labeling (TUNEL) Assay

To detect apoptotic cells, we performed the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay using the DeadEndTM Fluorometric TUNEL system (G3250; Promega, Madison, WI, USA), following the manufacturer's instructions. From each animal, we selected and stained one slide. The hypo-perfused area in the stained sections was identified using a confocal microscope (LSM780; Carl Zeiss AG, Oberkochen, Germany). Average values of TUNEL-positive cells were derived from the stained sections. For the TUNEL assay, five slides each of lung and kidney tissues per animal were processed from each of the four experimental groups: Sham, Sham + TTM, HS, and HS + TTM, resulting in a total of 40 slides per tissue type across all groups.

2.6. Detection of Intracellular Levels of Reactive Oxygen Species (ROS)

We utilized the cell-permeant stain 2',7'-dichlorofluorescein diacetate (DCFDA) from Abcam (ab113851; Cambridge, UK) to evaluate intracellular levels of reactive oxygen species (ROS). The process involved deparaffinizing the sectioned slide, applying 25 μM of DCFDA to the slide, and incubating it for 45 minutes at 37 °C in a light-restricted environment. Following DCFDA staining, the generation of ROS within the cells was observed using a confocal microscope (LSM780; Carl Zeiss AG). In the presence of ROS, DCFDA undergoes oxidation, leading to the formation of 2',7'-dichlorofluorescein, emitting green fluorescence. This green fluorescence, indicative of intracellular ROS, cannot pass through the cell membrane. Quantification of fluorescence intensity was performed using the MetaMorph microscopy automation and image analysis software (Molecular Devices, San Jose, CA, USA), as fluorescence intensity correlates with intracellular ROS levels. ROS detection involved processing five slides each of lung and kidney tissues per animal from the Sham, Sham + TTM, HS, and HS + TTM experimental groups, totaling 40 slides per tissue type across all groups.

2.7. Immunohistochemistry Analysis

Tissue sections, with a thickness of 4 μm, were precision-cut using a microtome (Leica, Wetzlar, Germany). These sections underwent permeabilization with a 0.1% Triton X-100 buffer, followed by blocking using 3% goat serum (or 2.5% horse serum) in phosphate-buffered saline (PBS) for 1 hour at room temperature. Subsequently, the samples were incubated overnight with primary antibodies. Antibodies used for immunofluorescence analyses included rabbit anti-CD68 (1:250;

ab125212; Abcam), mouse anti-inducible nitric oxide synthase (iNOS) (1:2000; ab49999; Abcam), mouse anti-CD206 (1:50; sc-70586; Santa Cruz Biotechnology Inc., Dallas, TX, USA), and rabbit anti-HMGB1 (1:100; ab18256; Abcam). Following incubation, the samples were washed and incubated for 2 hours at 37 °C with secondary fluorescent antibodies, namely goat anti-rabbit IgG Alexa-fluor 488 (1:200; 31402; Invitrogen, Carlsbad, CA, USA) and mouse IgGκ binding protein-PE (1:50; sc-516141; Santa Cruz Biotechnology Inc.). Finally, the samples were mounted using ProLongTM Diamond Antifade Mountant with DAPI (p36962; Invitrogen) and observed using a confocal microscope (LSM780; Carl Zeiss AG). Immunohistochemistry analysis included processing five slides each of lung and kidney tissues per animal from the Sham, Sham + TTM, HS, and HS + TTM experimental groups, resulting in 40 slides per tissue type across all groups. HMGB1 staining encompassed 40 slides, while macrophage staining utilized an additional 40 slides per tissue type across all groups.

2.8. Enzyme-Linked Immunosorbent Assay (ELISA) for Lactate Detection

To obtain rat serum, blood was collected 4 hours after resuscitation or post-TTM at 36 °C. The collected blood was transferred into Z Serum Sep Clot Activator tubes (Greiner Bio-One, Kremsmunster, Austria) with a volume of 1 mL, followed by centrifugation at 3,000 rpm for 15 minutes. Lactate concentrations were quantified using a lactate enzyme-linked immunosorbent assay (ELISA) kit (MBS755975; MyBioSource, San Diego, CA, USA) specifically designed for rats.

2.9. Real-Time Polymerase Chain Reaction (RT-PCR)

After obtaining lung and kidney tissue from rats, specific primers for genes, including glyceraldehyde-3-phosphate dehydrogenase, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, were designed using PrimerQuest (IDT, Skokie, IL, USA). RNA isolation from the tissue samples was performed using the Hybrid-R kit (305-010; GeneAll Biotechnology, Seoul, Republic of Korea). Single-stranded complementary DNA (cDNA) was synthesized using the PrimeScript 1st strand cDNA synthesis kit (RR037A; Takara Bio, Tokyo, Japan) with 500 ng of total RNA. Subsequently, quantitative polymerase chain reaction (PCR) was conducted on an ABI 7500 system (Applied Biosystems, Foster City, CA, USA) using SYBR-green (Q5602-005; Gendepot, Katy, TX, USA). For RT-PCR analysis, tissues from four animals per group for both lung and kidney were utilized from the four experimental groups: Sham, Sham + TTM, HS, and HS + TTM. This approach

ensured robust sampling across the different conditions studied. The expression levels of TNF-a, IL-1b, IL-6, and GAPDH were assessed using duplicate repeat experiments, enhancing the reliability and consistency of the gene expression measurements.

2.10. Cytokine Array

To construct a cytokine array using a Proteome Profiler array, we obtained tissue samples from the right kidney and lower lung. Tissue homogenization was performed using a homogenizer (FastPrep-24 5G; MP Bio, Seoul, Republic of Korea) in PBS with a protease inhibitor (P3100-005; GenDepot), followed by protein quantitation using a BCA kit (23225, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. The Rat Cytokine Array Panel A (ARY008; R&D Systems, Minneapolis, MN, USA) was utilized for the Proteome Profiler array, following the manufacturer's instructions. Blots were visualized using the ECLTM Western Blotting Analysis System (GE Healthcare, Chicago, IL, USA) and LAS 4000 mini (GE Healthcare). Analysis of the array results involved quantization of the blots using HLImage++ (Western Vision software, Salt Lake City, UT, USA).

2.11. Statistical Analysis

The results of all experiments are expressed as mean ± standard deviation (SD). Survival data were analyzed using the Mantel–Cox log-rank test. Statistical analyses were conducted using either an unpaired t-test or one-way analysis of variance (ANOVA), followed by Bonferroni post-hoc tests for multiple group comparisons. A significance level of $p < 0.05$ was considered. Post-hoc statistical power analyses were performed for each aspect, including RT-PCR, immunohistochemistry, ROS detection, cytokine array, ELISA assay, and TUNEL assay, using either an unpaired t-test or ANOVA. To ensure the identification of distinct differences with a sample size of five animals per group, and assuming an alpha value of 0.05, the mean and SD were calculated. Post-hoc power calculations revealed that all sets of immunohistochemistry and TUNEL assays had statistical power values exceeding 80%, which was considered sufficient for detecting significant differences.

3. RESULTS

3.1. TTM at 36 °C improves overall survival and protects against tissue damage in both kidneys and lungs after HS

The experiment was conducted as shown in Figure 1a. To compare the effects of TTM at both 33 and 36 °C after HS, we initiated TTM at these temperatures after restoring normal blood pressure after fluid resuscitation for HS. The mean time taken to achieve a target MAP of 38 mmHg was 11.4 ± 2.5 min and the target MAP was maintained for 60 minutes. The target core temperatures of either 33.0 ± 0.2 or 36.0 ± 0.2 °C were reached within 24.5 ± 2.8 and 21.0 ± 3.2 minutes, respectively. Following initiation, the TTM induction model maintained the targeted temperature (36 °C) for a duration of 3 hours, including the cooling period, and then underwent sequential rewarming over 1 hour to achieve a normothermic state of 37.5 °C. In contrast, the non-TTM induction model prevented hypothermic status for a total of 4 hours (Fig. 1b).

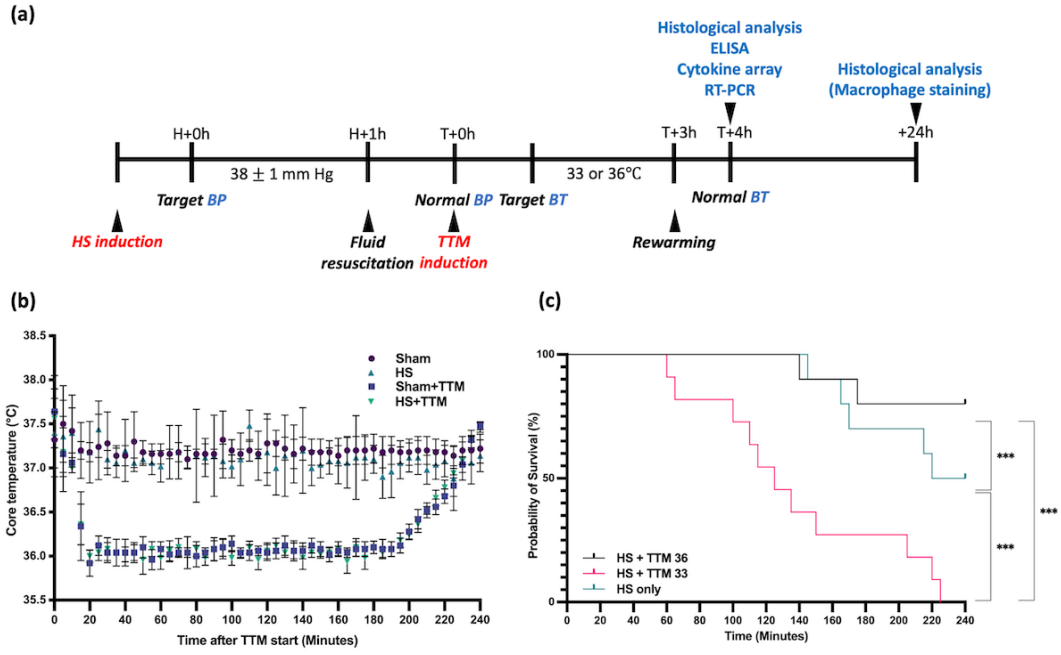

Fig. 1. Experiment design

a. Experimental schedule. b. Changes in rat body temperature after targeted temperature management (TTM) induction (n = 5 in each group). c. Kaplan–Meier survival plot (***p < 0.001), comparing hemorrhagic shock (HS), HS + TTM at 33 °C, and HS + TTM at 36 °C. Unexpectedly, treatment with TTM at 33 °C significantly increased deaths related to HS, leading to its exclusion from the study.

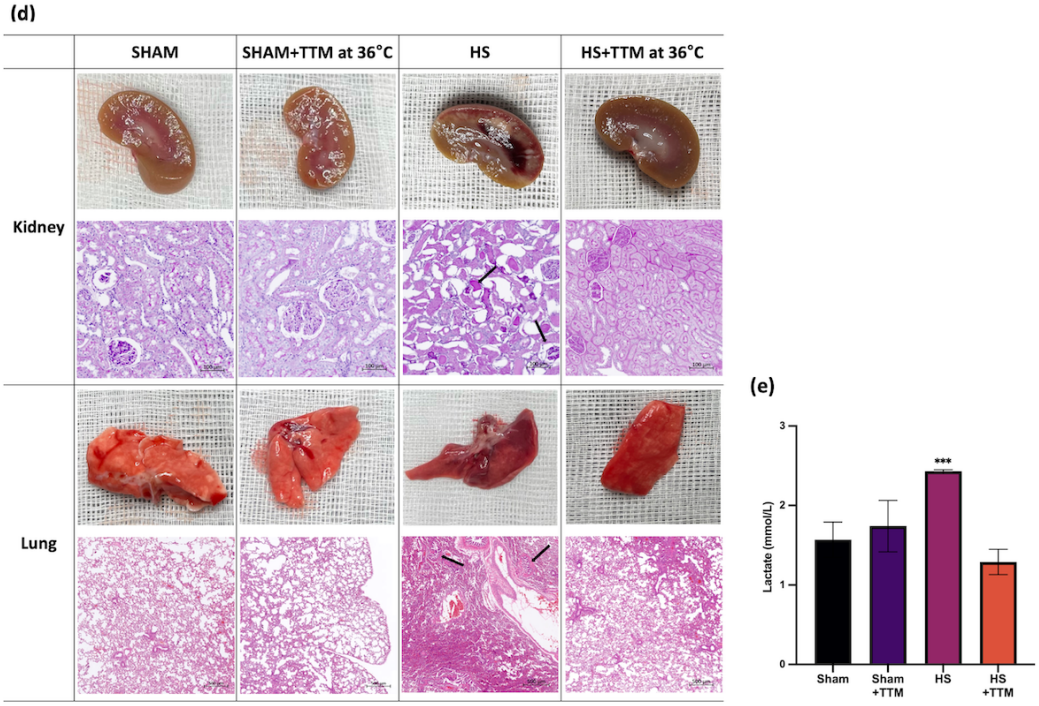

Fig. 1 (Cont.). d. Gross histopathological characterization in rats, achieved through gross necropsy and hematoxylin–eosin (H&E) staining. e. Quantification of serum lactate level by enzyme-linked immunosorbent assay (ELISA) (n = 4), ***p < 0.001, comparing HS with and without TTM at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Results are presented as mean ± SD. Abbreviations: HS, hemorrhagic shock; H, hemorrhagic shock maintenance; TTM, targeted temperature management; T, targeted temperature management induction, BP, blood pressure; BT, body temperature; ELISA, enzyme-linked immunosorbent assay; RT-PCR, real-time polymerase chain reaction

We investigated whether TTM at both 33 and 36 °C enhances survival, assessed 240 minutes after HS. Our findings revealed a significant difference in the survival rates between the three groups ($p < 0.001$) (Fig. 1c), indicating that TTM at 36 °C improves overall survival after HS. For survival analysis, among a total of 10 rats assigned to the TTM group at 33°C, all the rats died within 225 minutes of TTM, with no individuals completing the experiment. This stands in contrast to the 2 rats out of 10 in the TTM group at 36°C, showing a statistically significant difference in survival rates ($P < 0.001$). Regarding the survival analysis results, TTM at 33°C significantly increased mortality compared to TTM at 36°C and the uncontrolled body temperature group (both $p < 0.001$ respectively). Therefore, the TTM at 33°C group was excluded from subsequent experiments. Morphological changes in lung and renal cells were observed through gross necropsy and H&E staining (Fig. 1d). Rat lung subjected to HS exhibited suspected pulmonary edema and hyperemia (reddish color in gross necropsy). HS induced significant histological lung damage (Fig. 1d). This was evident in H&E staining, which revealed fibrous tissues in alveolar spaces with alveolar septa infiltration, atelectasis hemorrhage, necrosis of alveolar tissues, and edema. Conversely, TTM-treated rats showed less severe alveolar damage in the lung sections. Histological scores assessing the proportion of lung injury, the proportion of collapsed or irregular alveoli, hyperplasia of bronchial epithelial cells, metaplasia of club cells, mucus plugging, and infiltration of inflammatory cells were 13.8 ± 1.8 and 7.8 ± 1.5 for HS with and without TTM, respectively ($p < 0.001$). Similarly, rat kidneys exposed to HS showed hyperemic changes (blackish color in gross necropsy) (Fig 1d). HS-induced renal injury manifested as tubular epithelial cell swelling, necrosis, and desquamation. In contrast, TTM-treated rats exhibited a reduction in renal tubular injury (Fig 1d). EGTI scores were 12.8 ± 1.1 and 7.6 ± 0.9 for HS with and without TTM, respectively ($p < 0.001$). As lactate levels indirectly reflect tissue perfusion, we conducted an ELISA to measure serum lactate levels 4 hours after restoring normal blood pressure, following fluid resuscitation for HS. As anticipated, lactate levels increased after HS, but this rise was significantly attenuated by TTM at 36 °C (Fig. 1e).

3.2. TTM at 36 °C mitigates apoptotic-mediated cell death in the kidney and lung after HS

To confirm the association between TTM at 36 °C and apoptosis-mediated cell death, rats under TTM at 36 °C exhibited significantly lower numbers of TUNEL-positive cells than those under uncontrolled body temperature in both kidneys and lungs 4 hours after HS (Fig. 2).

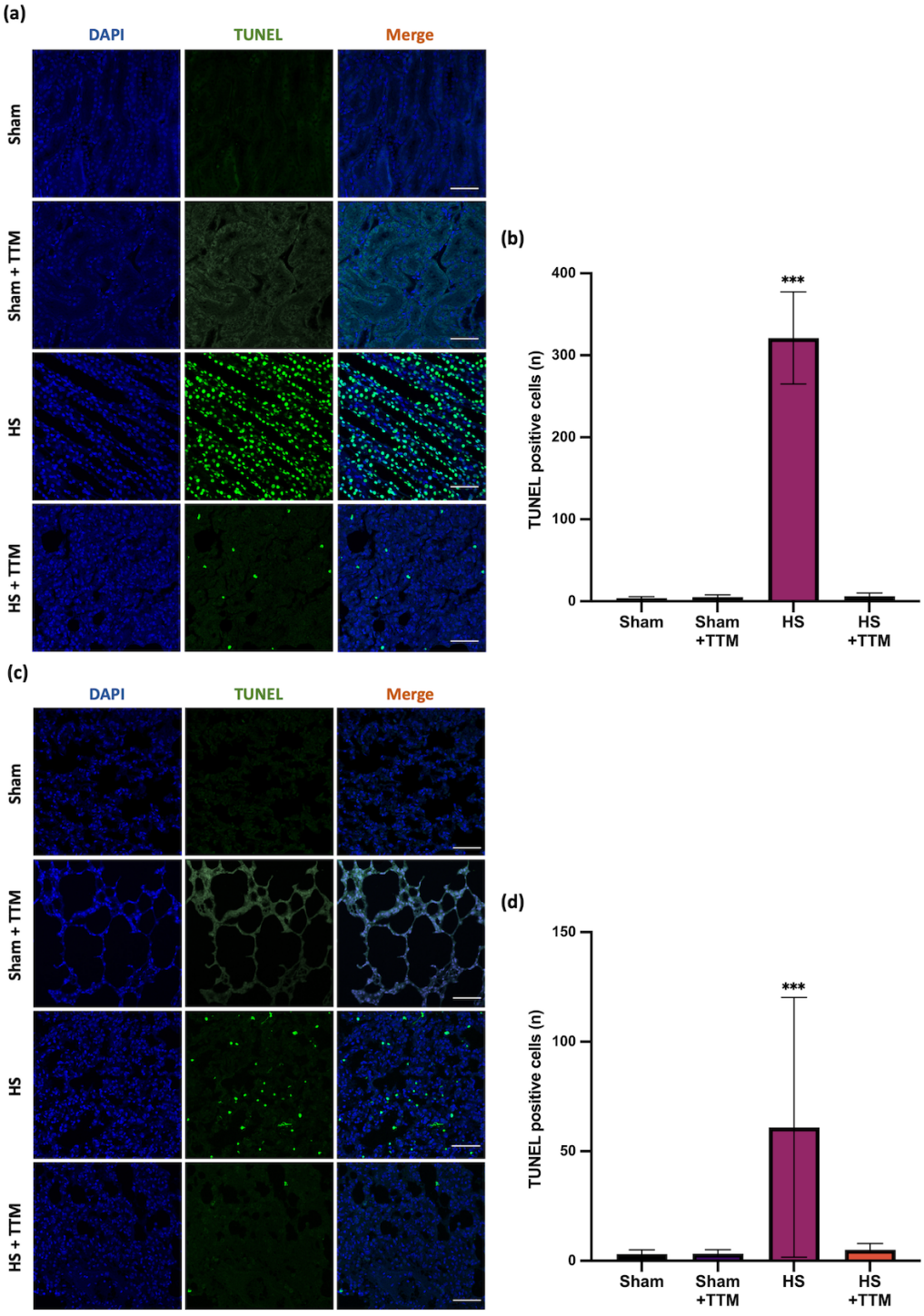

Fig. 2. Quantitative analysis of apoptotic cell death by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL).
a. Representative TUNEL assay results of kidney tissue from rats subjected to hemorrhagic shock (HS) and post-HS TTM at 36 °C. b. TUNEL assay results of kidney tissue (n = 5 in each group), ***$p < 0.001$, comparing HS with and without targeted temperature management (TTM) at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. c. Representative TUNEL assay results of lung tissue from rats subjected to HS and post-HS TTM at 36 °C. d. TUNEL assay results of lung tissue (n = 5 in each group), ***$p < 0.001$, comparing HS with and without TTM at 36 °C using ANOVA, followed by Bonferroni post hoc test. Scale bar, 50 μm. Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; DAPI, 4′,6-diamidino-2-phenylindole; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling.

3.3. TTM at 36 °C reduces intracellular levels of reactive oxygen species (ROS)

We evaluated intracellular ROS levels through DCFDA staining after a 4-hour period. Following HS, fluorescence intensities representing intracellular ROS levels showed a significant reduction in the kidneys (3.62 ± 1.43) and lungs (8.61 ± 1.34) of the HS-injured rats administered TTM at 36 °C compared with the kidneys (40.17 ± 2.86) and lungs (41.17 ± 1.30) of the HS-injured group without TTM (all $p < 0.001$) (Fig. 3). TTM at 36 °C was found to decrease intracellular levels of ROS.

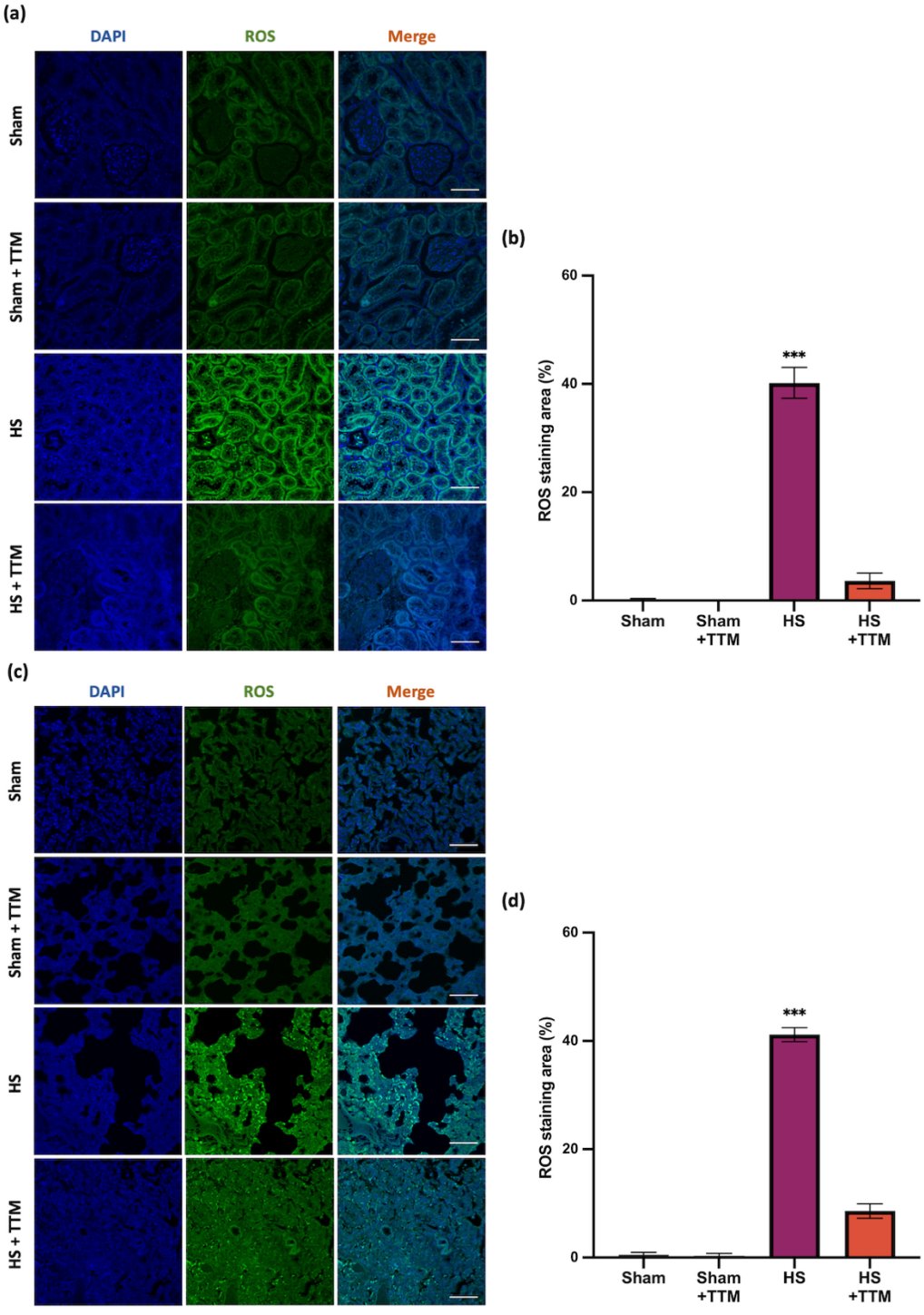

Fig. 3. Intracellular reactive oxygen species (ROS) level

a. Representative intracellular ROS results observed in the kidney following administration of targeted temperature management (TTM) after hemorrhagic shock (HS). b. ROS staining area of the lung (***$p < 0.001$), comparing HS with and without the administration of TTM at 36 °C. The comparison was conducted using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test (n = 4). c. Representative intracellular reactive oxygen ROS results observed in the lung following the administration of TTM after HS. d. ROS staining area of the lung (***$p < 0.001$), comparing HS with and without the administration of TTM at 36 °C. The comparison was conducted using ANOVA, followed by Bonferroni post hoc test (n = 4). Scale bar, 50 μm. Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; DAPI, 4′,6-diamidino-2-phenylindole; ROS, reactive oxygen species.

3.4. TTM at 36 °C inhibits extracellular release of HMGB1 and expression of proinflammatory cytokines associated with HMGB1 in the kidney and lung after HS

HMGB1 is released from kidney and lung cell nuclei during HS, resulting in a decreased number of HMGB1-positive cells after HS injury. Four hours after HS, we observed the disappearance of HMGB1 immunoreactivity in the kidney and lung. However, TTM at 36 °C significantly restored HMGB1-stained cells in both organs. In the uncontrolled body temperature group, we found that 9.92% ± 11.16% and 9.90% ± 5.36% of DAPI-positive cells in the kidney and lung, respectively, were HMGB1-positive. In contrast, TTM at 36 °C increased this number roughly three to five-fold to 49.83% ± 4.85% and 26.81% ± 3.81% in the kidney and lung, respectively. This suggests that TTM at 36 °C significantly reduced the extracellular release of HMGB1 (Fig. 4).

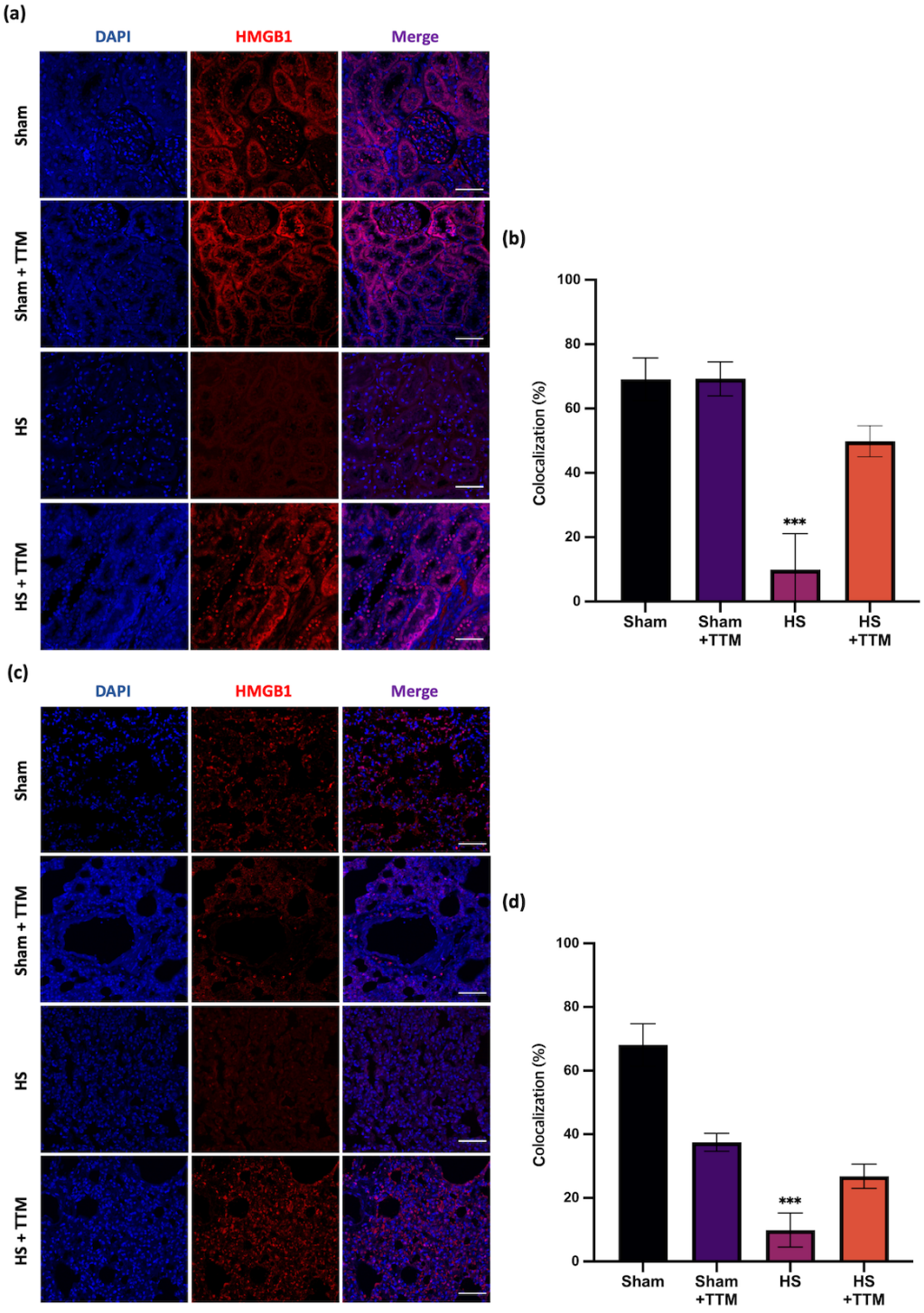

Fig. 4. Induction of targeted temperature management (TTM) suppresses the extracellular release of high mobility group box-1 (HMGB1) after hemorrhagic shock (HS).
a. Representative immunohistochemistry results of the kidney following TTM at 36 °C after HS. b. Immunohistochemistry results of the kidney (***$p < 0.001$), comparing HS with and without the administration of TTM at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test ($n = 5$ in each group). c. Representative immunohistochemistry results of the lung following TTM at 36 °C after HS. d. Immunohistochemistry results of the lung (***$p < 0.001$), comparing HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test ($n = 5$ in each group). Scale bar, 50 μm. Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; DAPI, 4′,6-diamidino-2-phenylindole; HMGB1, High mobility group box 1 protein.

To validate the expressions of proinflammatory cytokines associated with the extracellular release of HMGB1 based on the core temperature, we conducted RT-PCR at 4 hours after HS using isolated mRNA from whole kidney and lung tissue. The quantitative PCR findings revealed that the gene expression of TNF-α, IL-1β, and IL-6 decreased in both organs after TTM at 36 °C in comparison with uncontrolled body temperature after HS (Fig. 5).

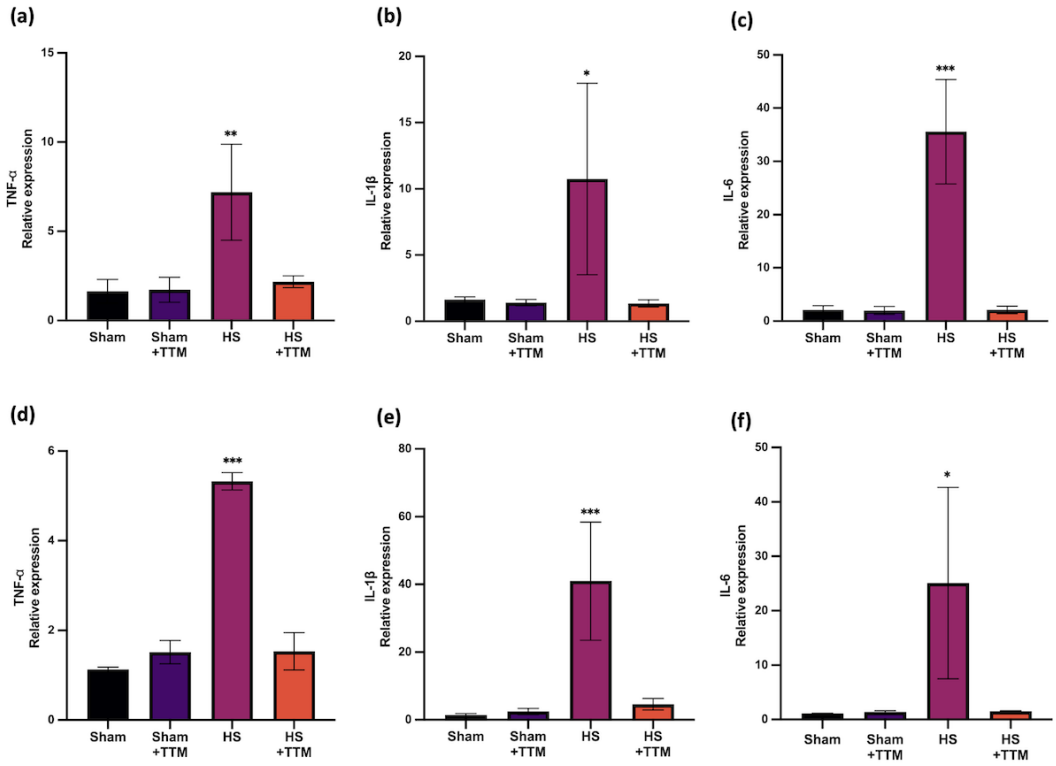

Fig. 5. mRNA expression of three major inflammatory cytokines in the kidney and lung.

a. Quantification of the expression of tumor necrosis factor-α (TNF-α) in the kidney by real-time polymerase chain reaction (RT-PCR) (**$p < 0.01$), comparing hemorrhagic shock (HS) with and without the administration of TTM at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. b. Quantification of interleukin-1β (IL-1β) expression in the kidney by RT-PCR (*$p < 0.05$). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test. c. Quantification of IL-6 expression in the kidney by RT-PCR (***$p < 0.001$). Comparison of HS with and without the administration of targeted temperature management (TTM) at 36 °C, using ANOVA followed by Bonferroni post hoc test ($n = 4$). d Quantification of the expression of TNF-α in the lung by RT-PCR (***$p < 0.001$), comparing HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test. e. Quantification of IL-1β expression in the lung by RT-PCR (***$p < 0.001$). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test. f. Quantification of IL-6 expression in the lung by RT-PCR (*$p < 0.05$).

Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test (n = 4). Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; TNF-α, Tumor necrosis factor-α; IL-1β, Interleukin-1β; IL-4, Interleukin-4.

3.5. TTM at 36 °C attenuates the activation of macrophages and modulates M1/M2 phenotype polarization of macrophages

Traditionally, CD68 is utilized as a valuable cytochemical marker for immunostaining monocytes/macrophages in the histochemical analysis of inflamed tissues. To characterize macrophages 24 hours after injury followed by TTM at 36 °C, we conducted CD68 detection to identify macrophages in both the kidney and lung. Additionally, we utilized iNOS and CD206 to label M1 and M2 macrophage phenotypes, respectively, estimating the numbers of labeled macrophages through immunohistochemistry (Fig. 6). The number of CD68-positive cells significantly decreased in groups treated with TTM at 36 °C. TTM at 36 °C also reduced the numbers of iNOS-positive cells by approximately 33.5% and 50.2% in the kidney and lung tissues, respectively, compared with uncontrolled body temperature group (Fig. 6c and 6h). This result indicates that TTM at 36 °C attenuated the activation and M1 polarization of macrophages in the kidney and lung tissues after HS. In groups treated with TTM at 36 °C, CD206-positive cells exhibited a similar percentage compared with uncontrolled body temperature group (Fig. 6d and 6i). However, TTM at 36 °C also demonstrated an increased ratio of CD206 to iNOS-positive cells by approximately 1.4- and 1.3-fold in the kidney and lung tissues, respectively, compared with uncontrolled body temperature group (Fig. 6e and 6j). This result indicates that TTM at 36 °C led to the induction of the M2 macrophage phenotype in the kidney and lung tissues after HS.

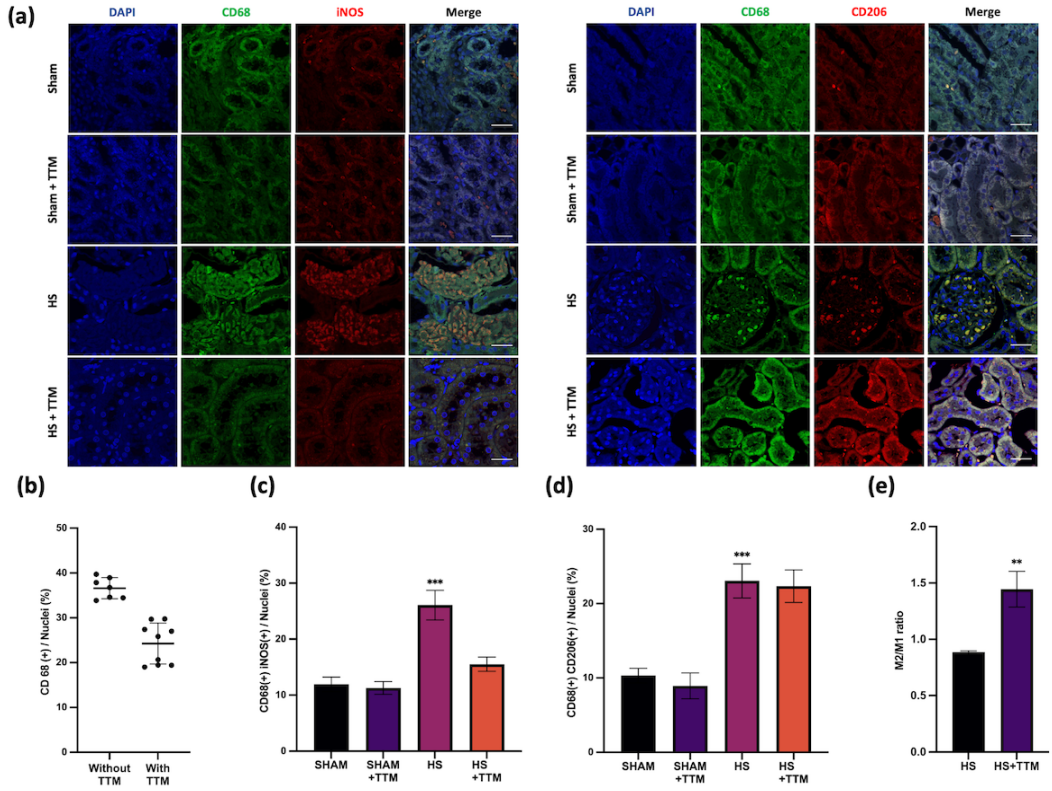

Fig. 6. Immunofluorescence analysis of macrophages in the kidney and lung.

a. Representative images of immunofluorescence staining in the kidney—M1 macrophages were detected using inducible nitric oxide synthase (iNOS); M2 macrophages, CD206; and all macrophage types, CD68. b. Quantitative analysis of cells positive for CD68 in the kidney. c. Quantitative analysis of cells positive for CD68 and iNOS in the kidney (***$p < 0.001$). Comparison of hemorrhagic shock (HS) with and without the administration of targeted temperature management (TTM) at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test ($n = 5$ in each group). d. Quantitative analysis of cells positive for CD68 and CD206 in the kidney (***$p < 0.001$). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test ($n = 5$ in each group). e. The ratio of M1 to M2 macrophages in the kidney (**$p < 0.01$) assessed using the Mann–Whitney U test.

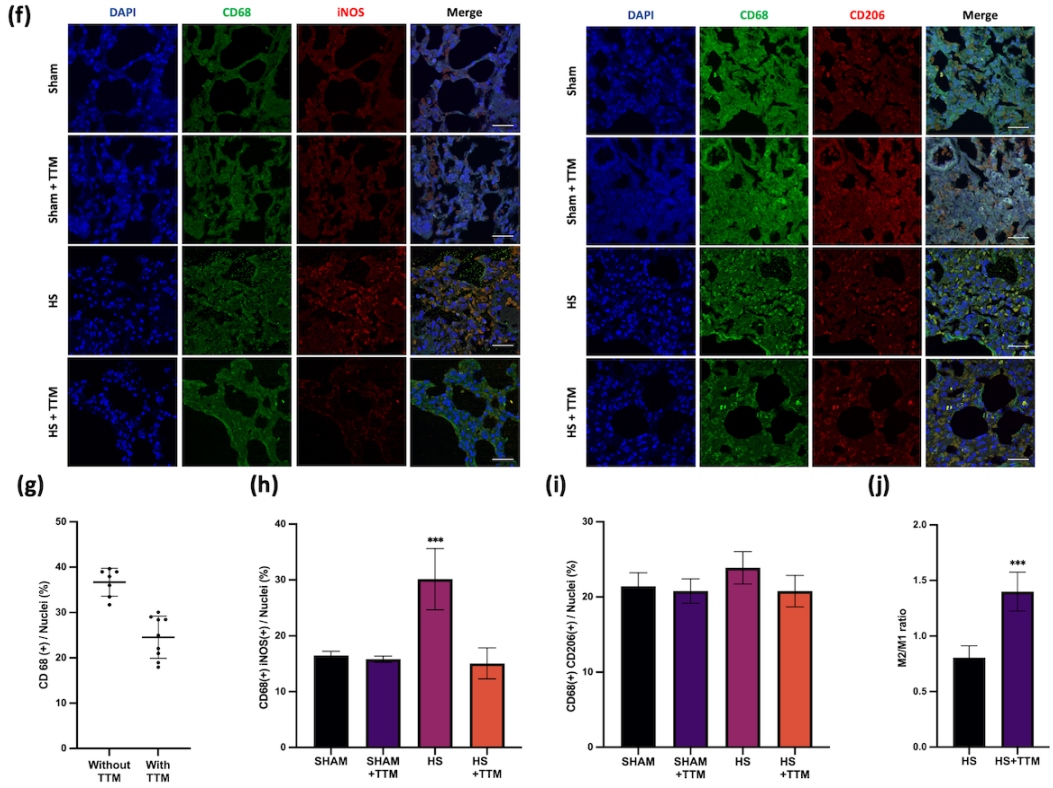

Fig. 6 (Cont.). Immunofluorescence analysis of macrophages in the kidney and lung.

f. Representative images of immunofluorescence staining in the lung—M1 macrophages were detected using iNOS; M2 macrophages, CD206; and all macrophage types, CD68. g. Quantitative analysis of cells positive for CD68 in the lung. h. Quantitative analysis of cells positive for CD68 and iNOS in the lung (***$p < 0.001$). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test ($n = 5$ in each group). i. Quantitative analysis of cells positive for CD68 and CD206 in the lung (p = NS). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test ($n = 5$ in each group). j. The ratio of M1 to M2 macrophages in the lung (***$p < 0.001$) assessed using the Mann–Whitney U test. Scale bar, 25 μm. Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; iNOS, inducible nitric oxide synthase.

3.6. TTM at 36 °C modulates cytokine production in both the kidney and lung after HS

To assess cytokine expression in uncontrolled body temperature and TTM at 36 °C, tissue samples from both organs were analyzed at 24 hours after HS using the cytokine array (Fig. 7). In uncontrolled body temperature group after HS, several cytokines, including soluble intercellular adhesion molecule-1 (sICAM-1), L-selectin, thymus chemokine, and vascular endothelial growth factor (VEGF), exhibited increased levels in the kidney tissue. Similarly, elevated levels of sICAM-1, interferon gamma-induced protein 10 (IP-10/CXCL10), lipopolysaccharide-induced CXC chemokine (LIX/CXCL5), L-selectin, and VEGF were observed in the lung tissue (Fig. 5b). Compared with uncontrolled body temperature group, the levels of these cytokines were significantly reduced in both organs following TTM at 36 °C.

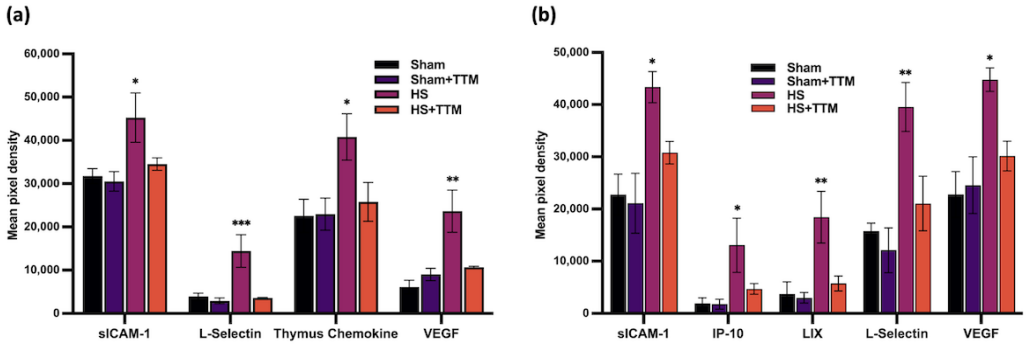

Fig. 7. Expression levels of several cytokines.

a. Expression levels of several cytokines in the kidney (*p < 0.05, **p < 0.01, ***p < 0.001). Comparison of hemorrhagic shock (HS) with and without the administration of targeted temperature management (TTM) at 36 °C, using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test (n = 5 in each group). b. Expression levels of several cytokines in the lung (*p < 0.05, **p < 0.01). Comparison of HS with and without the administration of TTM at 36 °C, using ANOVA followed by Bonferroni post hoc test (n = 5 in each group). Results are presented as mean ± SD. Abbreviations: TTM, targeted temperature management; HS, hemorrhagic shock; sICAM-1, soluble intercellular adhesion molecule-1; VEGF, vascular endothelial growth factor; IP-10/CXCL10, interferon gamma-induced protein 10; LIX/CXCL5, lipopolysaccharide-induced CXC chemokine.

4. DISCUSSION

In this study, we found that the application of TTM at 33 °C significantly increased short-term mortality after HS compared with HS without TTM treatment. Conversely, TTM at 36 °C significantly improved survival by attenuating cell death, lowering levels of ROS, and mitigating the deleterious inflammatory response. Inducing hypothermia in patients with severe hemorrhage is challenging due to potential excessive risks [24]. Jurkovich et al. reported no instances of survival when the core temperature fell below 32 °C after trauma, indicating that hypothermia in patients with trauma is generally considered an ominous sign [29]. While severity remains the most critical factor in predicting prognosis for patients with trauma, hypothermia is more prevalent in patients who experience shock. As the core temperature (Tc) decreases, the mortality rate among patients with shock rises rapidly (Tc < 34 °C, 52% mortality; Tc < 33 °C, 79% mortality; and Tc < 32 °C, 100% mortality) [29]. This is attributed to platelet dysfunction, worsening coagulopathy when the temperature drops below 35 °C. Additionally, the accumulation of lactic acid leads to metabolic acidosis, adversely affecting cardiovascular function, especially when the temperature falls below 32 °C [29]. Moreover, trauma patients often present with hypocalcemia in the setting of severe hemorrhage, which exacerbates acidosis and coagulopathy secondary to trauma, worsened by necessary transfusion and resuscitation [30]. Coagulopathy initially causes hypocoagulability and excessive bleeding, transitioning later to a hypercoagulable state associated with venous thromboembolism and MOF [7]. These processes involve synergistic activation of endothelial cells, the immune system, platelets, and the clotting system, aggravated by the “diamond of death” [7, 8]. Despite the variability stemming from the undetermined optimal core temperature for appropriate treatment after HS, the detrimental effects of prolonged hypothermia are recognized in HS [31]. However, Jiang et al. proposed that inducing hypothermia would not exacerbate the development of the “lethal triad” in trauma. Furthermore, even spontaneous hypothermia is not deemed entirely detrimental [32]. The 2010 American Heart Association (AHA) practice guidelines recommend inducing hypothermia with a goal temperature of 32–34 °C for 12–24 hours in patients successfully resuscitated after cardiac arrest [24]. In patients who have experienced cardiac arrest, maintaining a core temperature of 33 °C has demonstrated clinically favorable outcomes. However, the present study suggested that applying TTM at 33 °C significantly increases mortality rates after HS. This implies that employing TTM at 33 °C in HS poses a higher risk of harm than benefit, despite its

favorable outcomes in other clinical settings for patients with cardiac arrest. These findings highlight the potential danger associated with the application of TTM at 33 °C for the treatment of HS.

A noteworthy randomized controlled trial conducted in 2013 compared mortality and neurological outcomes following TTM at core temperatures of 33 and 36 °C in patients with cardiac arrest. The study showed that the benefits of TTM at 36 °C were not inferior to those at 33 °C [33]. The 2015 AHA practice guidelines recommend TTM between 32 and 36 °C for at least 24 hours for all cardiac rhythms in both out-of-hospital and in-hospital cardiac arrests [34]. To improve clinical outcomes through TTM in patients with HS, considering essential knowledge to overcome clinical obstacles is crucial. Achieving faster induction, maintaining the core temperature effectively, implementing appropriate rewarming strategies, and minimizing systemic adverse effects have emerged as significant challenges for ensuring the efficacy and safety of TTM [35]. Compared with TTM at 33 °C, that at 36 °C may provide a more convenient approach in overcoming these challenges because 36 °C is close to the lower limit of the normal core temperature [36, 37]. In addition, shivering is a major adverse effect as it interferes with the cooling process by making it difficult to reach the target temperature. Furthermore, it causes detrimental effects through a massive and systemic increase in metabolic demand and energy consumption [38-40]. Shivering has proven to be one of the most challenging obstacles in awake patients, primarily due to the difficulty in employing sedative and anti-shivering regimens. TTM at 36 °C may offer an effective and safe approach to manage shivering and the rewarming processes [41].

In HS, TTM at 36 °C significantly improved survival in comparison with TTM at 33 °C and uncontrolled body temperature group. First, we may consider TTM at 36 °C as an optimal therapeutic core temperature in HS, with the goal of minimizing side effects while maximizing the therapeutic benefits of inducing hypothermia. Second, TTM at 36 °C could attenuate the progression of deleterious inflammatory damages after HS. HMGB1 emerges as a key initiator of sterile inflammation [15]. The interactive interplay between HMGB1 and macrophages establishes a detrimental, self-sustaining cycle—a vicious cycle—contributing significantly to the progression of inflammatory damage [15, 42, 43]. The present study suggested that TTM at 36 °C may reduce the initiation and progression of this vicious cycle in inflammatory damage after HS by blocking the extracellular release of HMGB1. Macrophage polarization is essential for tissue repair and maintenance of homeostasis, involving the production of distinct functional phenotypes in response to specific microenvironmental stimuli and signals [44]. M1 macrophages have robust microbicidal,

tumoricidal, and stimulatory/destructive activity by releasing proinflammatory cytokines. M2 macrophages orchestrate the remodeling of the tissue and tumor formation by releasing anti-inflammatory cytokines [44, 45]. We further identified that TTM at 36 °C could attenuate the activation of the M1 phenotype and increase the probability of macrophage transformation into the M2 phenotype after HS. We suggested that this could provide stimulatory/destructive (M1) or suppressive/protective (M2) therapeutic strategies.

Our study demonstrated that TTM at 36 °C, administered following HS, significantly reduced the RNA expression of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α in both kidney and lung tissues. Additionally, it led to a decrease in the production of several cytokines in both organs, such as intracellular adhesion molecule-1 (sICAM-1/CD54), L-selectin, and VEGF. Moreover, a notable reduction was observed in thymus chemokine (CCL17) in the kidneys, as well as in IP-10/CXCL10 and LIX/CXCL5 in the lungs.

The expression of ICAM-1 in vascular endothelial cells, macrophages, and lymphocytes facilitates leukocyte adhesion to endothelial cells through the ICAM-1/LFA-1 interaction, enabling their penetration into tissues [46]. LIX/CXCL5 serves as a potent chemoattractant for neutrophils and contributes to proangiogenic factors, participating in the innate immune response [47]. The expression of ICAM-1 and the production of LIX/CXCL5 are also stimulated by IL-1 or TNF, acting as inflammatory cytokines [47]. In acute and chronic inflammation, L-selectin also plays a crucial role in regulating leukocyte adhesion and recruitment to lymph nodes in both peripheral and inflammatory sites [48]. Inflammatory stimuli trigger abundant secretion of IP-10/CXCL10, which regulates the inflammatory potential of human monocytes and induces robust production of proinflammatory cytokines, including IL-12 and IL-23 [49]. The role CCL17 extends beyond T-cell chemotaxis to influence the migration of various cell types, including airway eosinophils, megakaryocytes, platelets, and cutaneous dendritic cells [50]. Increased expression of VEGF and its receptors enhances vascular permeability and angiogenesis, ultimately resulting in tissue edema [51].

Evidence indicates that TTM at 36 °C promotes macrophage polarization toward the beneficial M2 phenotype after HS. However, we propose that the polarization of the activated cells from the M1 to M2 phenotypes does not occur prominently, as TTM effectively blocks the activation of macrophages themselves. As a result, TTM at 36 °C mitigates macrophage activation induced by HS. Further studies are required to investigate adjunctive modalities that can promote notable

polarization toward the beneficial M2 phenotype in conjunction with the application of TTM at 36 °C.

There are a few limitations encountered in this study. Firstly, the administration of anesthesia, muscle relaxant (vecuronium), and mechanical ventilation in all experimental animals may significantly modify and restrict their innate physiological responses to hemorrhagic shock. Secondly, the use of only male rats constitutes a limitation. While it was necessary to select one sex due to differences in body weight and maintain consistency in body weight to monitor the effects on blood pressure and body temperature, this approach limits the generalizability of our findings. These factors may influence outcomes and therapeutic strategies in a sex-specific manner. Thirdly, in this study, autologous blood was obtained from the study subjects and processed using heparin-coated syringes for collection, storage, and subsequent transfusion. It is noteworthy that while the quantity of heparin utilized was minimal, typically less than 0.1cc, the potential for interaction with the circulating blood exists. This consideration arises due to the anticoagulant properties of heparin, which may influence coagulation dynamics and other blood parameters, potentially impacting experimental outcomes.

This study proposed that TTM at 36 °C provides protective and beneficial effects against inflammatory damage following HS, suggesting the potential clinical application of TTM at 36 °C. TTM in injured patients can be beneficial, though the extent of these benefits may vary based on when treatment begins. TTM initiation may be delayed in patients exhibiting unstable vital signs, such as those with hemorrhagic shock. Earlier initiation of TTM is generally associated with better outcomes; however, there is no universally agreed-upon critical time window where benefits sharply diminish. The effectiveness of TTM is influenced by factors including injury severity, patient health status, and the duration of delay in starting treatment. Further research is necessary to determine the optimal timing for initiating TTM to maximize its effectiveness.

5. CONCLUSION

The ultimate objective of this study was to identify an optimal core temperature that proves beneficial in the treatment and prognosis of patients with HS in clinical practice. This study showed that targeted temperature management (TTM) at 36°C confers protective effects on the kidneys and lungs by mitigating apoptosis, intracellular ROS production, inflammation, and cell death following heat shock (HS), leading to improved survival. Furthermore, TTM at 36 °C attenuates proinflammatory M1 macrophage activation while concurrently promoting the relative polarization of macrophages toward the beneficial M2 phenotype. These findings offer new mechanistic insights into TTM at 36 °C, laying the groundwork for its potential clinical application in critical care after HS. In the future, further studies are needed to validate the clinical effects of TTM itself. Furthermore, investigations into identifying the optimal core temperature that can maximize the positive effects of the treatment following HS should be undertaken.

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Abstract in Korean

출혈성 쇼크 모델의 급성 폐 및 신장 손상에서 목표 중심 체온 치료의 염증 반응 조절 기전 규명

연구의 배경: 출혈성 쇼크는 중대한 사망률 및 합병증과 연관된 중증의 상태로, 치료 접근법의 발전이 필요하다. 현재까지 시행되고 있는 치료 방침에도 불구하고, 상당한 출혈성 쇼크 관련 사망률이 지속적으로 보고되고 있다. 본 연구는 출혈성 쇼크 이후 염증 반응 완화 및 다발성 장기부전으로부터의 장기 보호를 위한 목표 중심 체온 치료의 잠재적인 치료 가능성을 연구하였다. 목표 중심 체온 치료가 염증반응의 핵심 매개체인 HMGB1 단백질과 대식세포 표현형 전환의 병태생리를 관여함으로써 염증 반응을 조절한다는 것이 연구의 가설로 설정되었다.

연구 방법: 본 연구에서는 대퇴동맥으로부터 혈액을 채혈하여 저혈압의 출혈성 쇼크 모델을 확립, 이후 자가수혈 및 수액주입을 통한 정상 혈압으로 회복하고 33도와 36도의 목표 중심 체온 치료를 시행하였다. 생존율, 조직병리학, 세포사멸 정도, 세포내 반응성 산소종 및 사이토카인 발현을 평가하였으며, 대식세포 활성화와 극성의 변화는 면역조직화학을 통해 분석되었다. 사이토카인 어레이는 신장과 폐 조직의 염증성 환경을 프로파일링하는 데 사용되었다.

연구 결과: 33도에서 목표 중심 체온 치료의 적용은 출혈성 쇼크와 관련된 사망률을 증가시켰다. 그러나 36도에서의 목표 중심 체온 치료는 전반적인 생존율을 크게 향상시켰으며, 출혈성 쇼크 이후 폐 및 신장 조직의 조직학적 손상을 완화시키고, 혈청 젖산 레벨을 감소시켰다. 또한, 세포사멸과 세포내 반응성 산소종 수준을 낮추는 보호 효과를 보여주었다. 36도에서 목표 중심 체온 치료는 세포 외로의 HMGB1 방출을 억제하고 폐와 신장, 두 조직 모두에서 염증성 사이토카인을 억제하였다. 추가적으로, 대식세포의 활성화를 조절하여 M1 표현형을 억제하고 M2 표현형을 촉진하였고, 사이토카인 어레이 분석 결과, 36도에서 목표 중심 체온 치료는 염증성 사이토카인을 상당한 수준으로 감소시켰다.

결론: 본 연구는 출혈성 쇼크 이후 유발되는 다발성 장기 손상에서 36도 목표 중심 체온 치료의 보호 효과를 지원하는 포괄적인 증거를 제시하였다. 다양한 긍정적인 효과는 사망률, 조직학적 손상, 세포사멸, 염증성 사이토카인의 감소 및 대식세포 표현형 조절의 변화로 나타났다. 이러한 결과는 출혈성 쇼크 이후 중환자 치료에 대한 목표 중심 체온 치료의 잠재적 치료 가능성을 제시한다.

핵심되는 말 : 출혈성 쇼크, 목표 중심 체온 치료, HMGB 1, 세포사멸, 염증, 대식세포