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Original Article

Rapid rewarming after therapeutic hypothermia worsens outcome in sepsis

You Hwan Jo[1], Kyuseok Kim[1], Jae Hyuk Lee[1], Kwang Pil Rim[2], In Soo Cho[3]

[1]Department of Emergency Medicine, Seoul National University Bundang Hospital, Seongnam, Korea
[2]Department of Emergency Medicine, St. Carollo General Hospital, Suncheon, Korea
[3]Department of Emergency Medicine, Kepco Medical Center, Seoul, Korea

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Objective This study was performed to investigate the effect of the rewarming rate on survival and acute lung injury in sepsis.

Methods Male Sprague-Dawley rats underwent cecal ligation and incision. After 1 hour of sepsis induction, normothermia (37°C±0.5°C, NT group) or hypothermia (32°C±0.5°C) was induced. Hypothermia was maintained for 4 hours and rats were divided into two groups according to the rewarming rate: RW1 group, 1 hour of rewarming; and RW2 group, 2 hours of rewarming. In the survival study, rats were observed for 12 hours after sepsis induction (n=6 per group). In the second experiment, rats were sacrificed 7 hours after sepsis induction, and lung tissues and plasma were harvested (n=10 per group).

Results In the survival study, the RW2 group survived longer than the RW1 group ($P<0.05$), but the RW1 and NT groups showed no significant difference in survival duration ($P>0.05$). The histological lung injury score and malondialdehyde concentrations in the lung tissues were significantly higher in the RW1 group than in the RW2 group ($P<0.05$). Plasma interleukin (IL)-6 concentration and the ratio of IL-6 to IL-10 were higher in the RW1 group than in the RW2 group ($P<0.05$).

Conclusion Rapid rewarming after therapeutic hypothermia results in a shorter survival period and acute lung injury in sepsis, which could be associated with the inflammatory responses.

Keywords Hypothermia; Rewarming; Sepsis; Survival; Lung injury

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Correspondence to: Kyuseok Kim
Department of Emergency Medicine, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam 463-707, Korea
E-mail: dremkks@snubh.org

Capsule Summary

What is already known

The effect of therapeutic hypothermia on sepsis is under debate and it depends on the model of sepsis, time of application, rewarming and the severity of sepsis.

What is new in the current study

Rapid rewarming after therapeutic hypothermia shortens survival duration and worsens acute lung injury compared to slow rewarming.

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INTRODUCTION

Therapeutic hypothermia (TH) is currently recommended for comatose patients after cardiac arrest.[1] In addition to cardiac arrest, use of TH has been evaluated in various clinical and experimental conditions such as perinatal asphyxia, traumatic brain injury, stroke, myocardial infarction, hemorrhagic shock, and ischemia-reperfusion injury.[2-5]

Rewarming is an essential process following TH. The current recommendations are to rewarm slowly and avoid fever.[6] However, few studies have been performed to analyze the optimal rate and method of rewarming. Several studies have shown that rapid rewarming worsens outcomes including survival rate and tissue injury.[5,7,8] During the rewarming phase after TH, the inflammatory response may be altered in patients who experienced cardiac arrest,[9] and the detrimental effect of rapid rewarming has been reported to be associated with the inflammatory response and intracellular calcium concentration.[5,8,10,11]

The incidence of sepsis and resulting mortality rates are high despite improved therapeutic strategies. Studies on TH in sepsis have reported beneficial effects associated with the modulation of the inflammatory response.[12,13] In contrast, TH has also been reported to increase mortality and tissue injury in several sepsis models.[14,15] Therefore, the effect of TH on sepsis is debatable.

We previously reported that the effect of TH on sepsis varies according to the severity of sepsis and that TH had a protective effect only in a model of severe sepsis.[16] The inflammatory response is an important element in the pathophysiology of sepsis, and rapid rewarming could induce the inflammatory burst after TH. Therefore, we performed this study to investigate the effect of the rewarming rate following TH on survival rate and acute lung injury in a cecal ligation and incision model of sepsis. We hypothesized that rapid rewarming after TH would worsen outcomes in a severe sepsis model.

METHODS

Experimental animals

This study was approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital in accordance with National Institutes of Health Guidelines. Male Sprague-Dawley rats weighing 300–350 g were used for the experiments. Animals were housed in a controlled environment with free access to food and water for 1 week before the experiment.

Induction of sepsis

Animals were anesthetized with an intramuscular injection of Zoletil (50 mg/kg, Virbac, Carros, France) and xylazine (10 mg/kg, Bayer, Seoul, Korea). Sepsis was induced by cecal ligation and incision as previously described.[16,17] Briefly, a 2-cm midline incision of the abdomen was performed and the cecum was extruded. The distal one-third of the cecum was ligated, and a 0.5-cm incision was made on the anti-mesenteric surface of the ligated cecum. The cecum was gently squeezed to expel feces into the peritoneal cavity, and 20 mL/kg of 0.9% saline solution was then infused into the peritoneal cavity to distribute the feces evenly. The incised abdominal wall was then closed, and 30 mL/kg of 0.9% saline solution was administered subcutaneously for fluid resuscitation.

Experimental groups and protocols

Rats were randomly divided into one of the three groups: normothermia (NT) group; 1 hour of rewarming after 4 hours of hypothermia (RW1) group; and 2 hours of rewarming after 4 hours of hypothermia (RW2) group. We monitored the rectal temperature of the rats (Harvard Homeothermic Monitor, Boston, MA, USA) throughout the experiment. One hour after sepsis induction, NT (37°C±0.5°C) was maintained using a heating lamp and pad. Hypothermia was induced by surface cooling with ice packs, and the target temperature (32°C±0.5°C) was reached within 15 minutes and maintained using a cooling pad for 4 hours. The rats were then rewarmed to 37°C for 1 hour in the RW1 group and 2 hours in the RW2 group using a heating lamp and pad.

In the survival study, rats were observed for 12 hours after induction of sepsis (n=6 in each group). In the second experiment, rats were sacrificed 7 hours after the induction of sepsis (n=10 in each group). We performed laparotomy and withdrew blood samples from the abdominal aorta. The blood was then centrifuged for 15 minutes at 3,000 rpm at 4°C, and the separated plasma was stored at −70°C for subsequent analyses. We also harvested lung tissues, and the right lower lobe of the lung was fixed with 4% formaldehyde solution for histological examination. The remaining lung tissues were stored at −70°C for subsequent analyses.

Histological acute lung injury score

The right lower lobe of the lung was embedded in paraffin and the paraffin blocks were cut into 4-mm sections. The sections were deparaffinized and stained with hematoxylin and eosin. A board-certified pathologist blinded to the study groups scored the acute lung injury according to the following 4 criteria: alveolar congestion, hemorrhage, infiltration of neutrophils in the air spaces or vessel walls, and the thickness of alveolar wall/hyaline

membrane formation.[18] The score for each criterion ranged from 0 (minimal) to 4 (severe), and the sum of the scores was calculated ranging from 0 to 16.

Malondialdehyde concentration in lung tissue

Malondialdehyde (MDA) is a by-product of lipid peroxidation and an oxidative stress marker. MDA level was measured using Ohkawa's method with thiobarbituric acid and presented in nmol/g of tissue.[19]

Plasma interleukin-6 and interleukin-10 concentration

Plasma interleukin (IL)-6 and IL-10 concentrations were measured with enzyme-linked immunosorbent assay kits (R&D Systems Inc., Minneapolis, MN, USA). The concentrations are presented as pg/mL.

Statistical analyses

The survival rates were assessed with Kaplan-Meier curves and the log-rank test. Data are presented as mean ± standard error of the mean (SE) and Student t-test was performed to compare the variables. Statistical analyses were performed with SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA) and a P-value <0.05 was considered statistically significant.

RESULT

Survival rates

All rats died within 12 hours of sepsis induction (Fig. 1). The median survival duration of the NT, RW1, and RW2 groups was 6.8, 7.6, and 9.4 hours, respectively. The survival duration of the RW2 group was significantly longer than that of the NT and RW1 groups ($P<0.001$ and $P=0.002$, respectively). However, there was no significant difference in survival duration between the NT and RW1 groups ($P=0.213$).

In the survival study, 4 of 6 rats in the NT group died within 7 hours of sepsis induction. Therefore, the subsequent measurements of tissue injury and plasma cytokines were performed only in the RW1 and RW2 groups, not in the NT group.

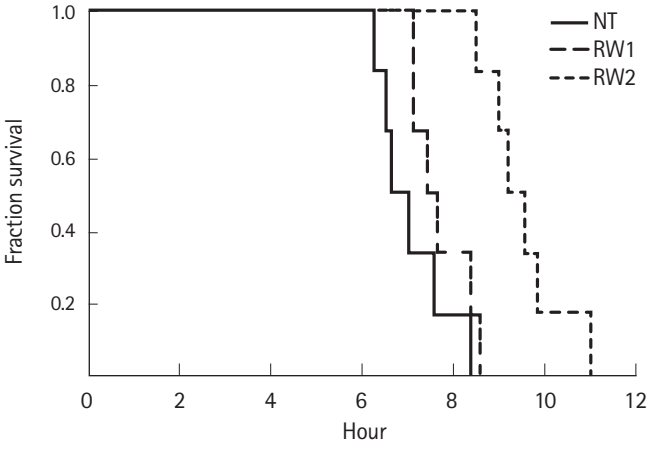

Fig. 1. Effect of the rewarming rate after therapeutic hypothermia on survival. The RW2 group survived significantly longer than the NT and RW1 groups ($P<0.05$). There is no difference in survival duration between the NT group and RW1 group ($P>0.05$). NT, normothermia; RW1, 1 hour of rewarming after 4 hours of therapeutic hypothermia; RW2, 2 hours of rewarming after 4 hours of therapeutic hypothermia. n = 6 per group.

Histological acute lung injury score

The acute lung injury scores of the RW1 and the RW2 groups were 8.4 ± 0.3 and 7.0 ± 0.4, respectively, and the acute lung injury score of the RW1 group was significantly higher than that of the RW2 group ($P=0.038$) (Fig. 2).

MDA concentration in the lung tissue

The MDA concentrations in the RW1 and RW2 groups were 83.8 ± 3.9 nmol/g and 73.4 ± 2.7 nmol/g, respectively (Fig. 3). The difference between the two groups was statistically significant ($P=0.039$).

Plasma IL-6 and IL-10 concentrations

The plasma concentration of IL-6 was significantly higher in the RW1 group than in the RW2 group (3,072 ± 281.4 pg/mL vs. 1,865 ± 410.5 pg/mL; $P=0.026$) (Fig. 4A). The IL-10 concentration did not differ significantly between the RW2 group and the RW1 group (352.6 ± 48.8 pg/mL vs. 301.0 ± 36.9 pg/mL; $P=0.443$) (Fig. 4B). The ratio of IL-6 to IL-10 was 8.8 ± 1.1 in the RW1 group and 3.9 ± 1.1 in the RW2 group; it was significantly higher in the RW1

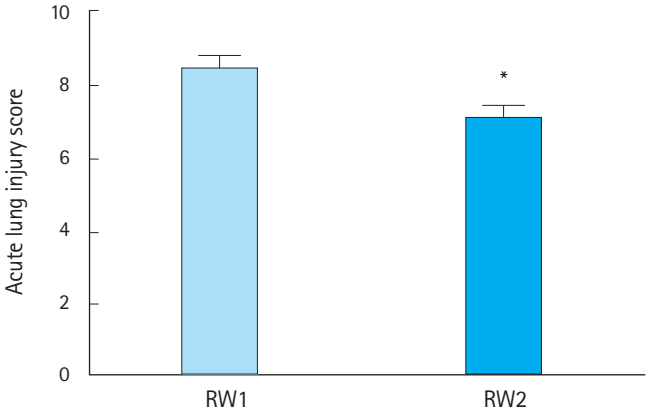

Fig. 2. Histologic lung injury. The acute lung injury score is significantly higher in the RW1 group than in the RW2 group. RW1, 1 hour of rewarming after 4 hours of therapeutic hypothermia; RW2, 2 hours of rewarming after 4 hours of therapeutic hypothermia. Values are presented as mean ± SE. n = 10 per group. *$P<0.05$.

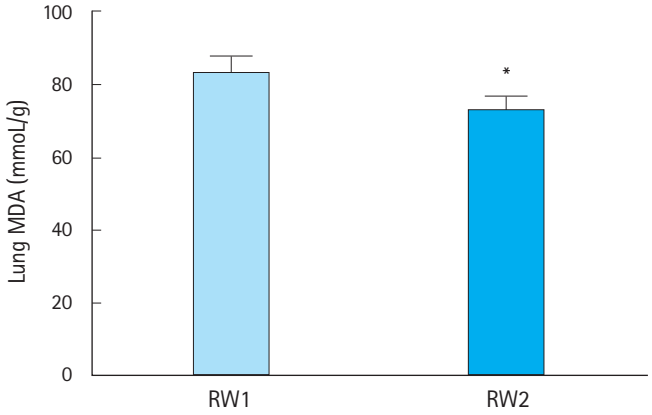

Fig. 3. Malondialdehyde (MDA) concentrations in the lung tissue. The lung MDA concentration is higher in the RW1 group than in the RW2 group. RW1, 1 hour of rewarming after 4 hours of therapeutic hypothermia; RW2, 2 hours of rewarming after 4 hours of therapeutic hypothermia. Values are presented as mean±SE. n=10 per group. *P<0.05.

group than in the RW2 group (P=0.010) (Fig. 4C).

DISCUSSION

The effect of rewarming after TH has been studied in various situations. In a hemorrhagic shock model, rewarming was found to lower blood pressure and increase heart rate and the synthesis of reactive oxygen species (ROS).[10] Rewarming has also been reported to induce derangement of cerebrovascular activity in patients with traumatic brain injury.[20] In cardiac arrest patients, the inflammatory cytokines and complement and adhesion molecules changed during the TH and rewarming phases.[9] In addition, the effect of rewarming and its rate has been studied. Rapid rewarming has been reported to influence the survival rate in hemorrhagic shock and induce secondary axonal injury in traumatic brain injury.[7,21] During cardiopulmonary bypass, rapid rewarming has been reported to result in deterioration of neurocognitive function and brain injury.[8,22] In an intestinal ischemia-reperfusion model, gradual rewarming was found to improve the survival rate and attenuate injury to the lungs.[5] In the present study, our results showed that rapid rewarming shortens survival duration and aggravates acute lung injury in a severe sepsis model.

Since TH has been studied in various clinical and experimental models, several studies have evaluated the effect of TH on sepsis, but the results have been inconsistent. We previously reported that TH had a beneficial effect only in a severe sepsis model and that it might be associated with suppression of the exacerbated inflammatory responses.[16] However, in the present study, rapid rewarming worsened both survival rate and acute lung injury in a severe sepsis model. These results demonstrate that the duration of rewarming in addition to the severity of sepsis should be considered when applying TH to sepsis.

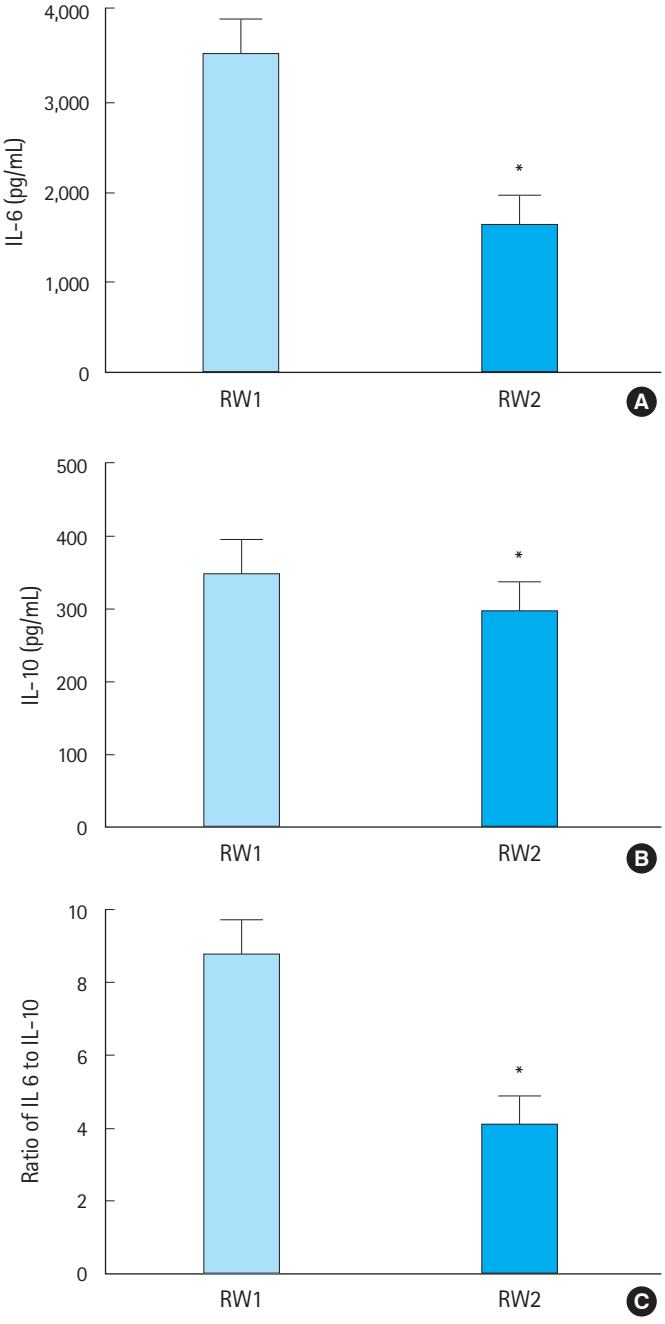

Fig. 4. Plasma interleukin (IL) concentrations. (A) Plasma IL-6 concentration is higher in the RW1 group than in the RW2 group. (B) Plasma IL-10 concentration is not significantly different between the RW1 and RW2 groups. (C) The ratio of IL-6 to IL-10 is significantly higher in the RW1 group than in the RW2 group. RW1, 1 hour of rewarming after 4 hours of therapeutic hypothermia; RW2, 2 hours of rewarming after 4 hours of therapeutic hypothermia. Values are presented as mean±SE. n=10 per group. *P<0.05.

It has been suggested that the harmful effect of rapid rewarming is associated with ROS production and inflammatory respons-

es. ROS have been known to play an important role in various tissue injuries. It has been reported that ROS production increased during rewarming following TH in hemorrhagic shock, and rapid rewarming resulted in a higher concentration of ROS in an intestinal ischemia-reperfusion model.[5,10] In the present study, the MDA concentration of the RW1 group was higher than that of the RW2 group, which indicated that rapid rewarming aggravated acute lung injury by ROS-induced lipid peroxidation.

IL-6 is a proinflammatory cytokine reported to be associated with poor outcome in sepsis.[23] TH has reportedly decreased the concentration of IL-6, and this phenomenon has been associated with the anti-inflammatory effect of TH.[4,9,16] In the present study, the RW1 group demonstrated a higher concentration of IL-6 than the RW2 group, which indicated that rapid rewarming induced the inflammatory response and was related to poor outcome in sepsis.

IL-10 is an anti-inflammatory cytokine, and few studies have analyzed the effect of rewarming after TH on IL-10. In patients with cardiac arrest and in an animal model of trauma, IL-10 concentrations did not differ between the TH and rewarming phases.[9,24] It has also been reported that surviving animals showed a tendency toward decreasing IL-10 concentrations after TH and rewarming compared to non-surviving animals in an emergency preservation and resuscitation model.[25] In the present study, IL-10 concentration did not differ between the RW1 and RW2 groups. The ratio of IL-6 to IL-10 represents the equilibrium of the pro- and anti-inflammatory responses, and a higher ratio of IL-6 to IL-10 was found to be correlated with poor outcome in sepsis patients.[26] In the present study, the ratio of IL-6 to IL-10 was significantly higher in the RW1 group than in the RW2 group, which suggests that rapid rewarming could induce the inflammatory response.

This study has several limitations. First, we did not measure the tissue injury and plasma cytokine levels in the NT group. However, 4 of 6 rats in the NT group died within 7 hours of induction of sepsis, so measurement of tissue injury only in the surviving animals would result in a survival effect in the NT group and bias results. Second, the rewarming rates in the present study were approximately 5°C/hour in the RW1 group and 2.5°C/hour in the RW2 group, which are much faster than those currently recommended.[6] As mentioned above, we previously reported that TH has a beneficial effect only in a severe sepsis model, and the median survival durations in the present study were 7.6 hours in the RW1 group and 9.4 hours in the RW2 group, respectively. Therefore, we could not prolong the durations of TH and rewarming to investigate the effect of the rewarming rate. Third, we did not use the approaches used in the clinical management of sepsis patients, such as antibiotics, vasopressors, or drainage. Fourth, TH has been reported to have a beneficial effect through various mechanisms including apoptosis, immune responses, ROS, and mitochondrial function.[27] Although these factors might be altered during rewarming following hypothermia, we did not assess them, with the exception of several inflammatory cytokines and MDA. Further studies are warranted to evaluate the mechanisms of the harmful effects of rapid rewarming.

In conclusion, rapid rewarming after TH shortens survival duration and worsens acute lung injury in sepsis, which could be associated with the inflammatory response and production of ROS.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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