

# Therapeutic hypothermia effect on asphyxial cardiac arrest-induced renal ischemia/reperfusion injury via change of Nrf2/HO-1 levels

ALI JAWAD<sup>1\*</sup>, YEO-JIN YOO<sup>1\*</sup>, JEONG-HWI CHO<sup>1</sup>, JAE CHOL YOON<sup>2</sup>, WEISHUN TIAN<sup>1</sup>,  
MOHAMMAD SADIKUL ISLAM<sup>1</sup>, EUI-YONG LEE<sup>1</sup>, HA-YOUNG SHIN<sup>1</sup>, SO EUN KIM<sup>2</sup>, KYUNGHWA KIM<sup>3</sup>,  
DONGCHOON AHN<sup>1</sup>, BYUNG-YONG PARK<sup>1</sup>, IN-SHIK KIM<sup>1</sup>, JUN HO LEE<sup>4</sup> and HYUN-JIN TAE<sup>1</sup>

<sup>1</sup>Department of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, Iksan, Jeollabuk-do 54696; <sup>2</sup>Department of Emergency Medicine, Research Institute of Clinical Medicine of Jeonbuk National University; Departments of <sup>3</sup>Thoracic and Cardiovascular Surgery and <sup>4</sup>Anesthesiology and Pain Medicine, Research Institute of Clinical Medicine of Jeonbuk National University-Biomedical Research Institute of Jeonbuk National University Hospital, Jeonju, Jeollabuk-do 54907, Republic of Korea

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**Abstract.** The present study aimed to investigate the renoprotective effect of therapeutic hypothermia (TH) on renal ischemia-reperfusion injury (RI/RI) induced by asphyxial cardiac arrest (CA) in rats. A total of 48 male rats were randomly divided into five groups: i) Sham (n=6); ii) Normothermia + CA (Normo.) (n=14); iii) Normo. and 2 h of TH after return of spontaneous circulation (ROSC) (n=12); iv) Normo. and 4 h of TH after ROSC (n=9); and v) Normo. and 6 h of TH after ROSC (n=7). All rats except the Sham group underwent asphyxia CA and were sacrificed 1 day after ROSC. The survival rate increased from 42.8% in the Normo. group to 50, 66.6 and 85.7% in the groups with 2, 4 and 6 h of TH after CA, respectively. TH attenuated the histopathological changes of the renal tissues following ROSC and the levels of blood urea nitrogen, serum creatinine and malondialdehyde in

renal tissues. On immunohistochemistry, the relative optical density of nuclear erythroid-related factor-2 (Nrf2) and heme oxygenase (HO-1) expression in renal tissues increased in the Normo. group compared with that in the Sham group and exhibited further significant increases at 6 h of TH after ROSC. In conclusion, TH attenuated renal injury and increased the expression of Nrf2 and HO-1 in a TH treatment time-dependent manner.

## Introduction

Cardiac arrest (CA) refers to loss of heart function that results in an abrupt halt of effective blood flow to the body; the morbidity and mortality due to CA have increased worldwide (1). The annual incidence of sudden CA (SCA) is ~3 million; however, the survival rate of SCA is <1% (2). Roberts *et al* (3) have reported that dysfunctions in various organs are common after CA following return of the spontaneous circulation (ROSC). In particular, kidney injury that results from CA following ROSC is a complex process; for heart disease patients admitted to hospitals, impairment of renal injury is common and is associated with a high mortality rate (4). Acute kidney injury (AKI) occurs frequently in patients with CA, occurring in ~50% of cases of CA (5). Geri *et al* (6) reported that the incidence of acute renal injury ranges from 12 to 40% in patients with CA. Most studies have focused on myocardial dysfunction and brain injury following ROSC after CA; however, renal injury has not been widely studied (7,8).

Oxidative stress contributes to the pathogenicity of renal ischemia/reperfusion injury (RI/RI) (9). Furthermore, RI/RI-induced oxidative stress generates high levels of reactive oxygen species (ROS). Subsequently, overproduction of ROS results in mutation of DNA, apoptosis, necrosis and lipid peroxidation, causing cellular death in numerous ways (10,11). A signaling pathway determined to have anti-oxidative stress properties and scavenge ROS production under oxidative stress conditions is nuclear erythroid-related factor-2 (Nrf2)/heme

**Correspondence to:** Professor Hyun-Jin Tae, Department of Veterinary Medicine and Bio-Safety Research Institute, Jeonbuk National University, 79 Gobong-ro, Iksan, Jeollabuk-do 54696, Republic of Korea  
E-mail: hjtae@jbnu.ac.kr

Dr Jun Ho Lee, Department of Anesthesiology and Pain Medicine, Research Institute of Clinical Medicine of Jeonbuk National University-Biomedical Research Institute of Jeonbuk National University Hospital, 20 Geonji-ro, Jeonju, Jeollabuk-do 54907, Republic of Korea  
E-mail: gojuno@jbnu.ac.kr

\*Contributed equally

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oxygenase (HO-1). Nrf2, an inducible transcription factor, binds with the antioxidant response element (ARE) located on the promoter regions of numerous antioxidant and detoxifying genes such as HO-1 (12). Previous studies (13,14) mainly focused on histopathological and pathophysiological ailments and the expression levels of Nrf2 and HO-1 in rats induced by RI/RI; however, this type of study in a rat model of asphyxial CA is rare.

The first successful TH application in humans following ROSC after CA was reported near the end of the 1950s (15). Since then, TH has been the most successful treatment for CA (16). Mild TH (MTH) increases the survival rate and attenuates neurological outcomes in patients with CA who achieve ROSC (17). Furthermore, MTH protects the heart, liver and kidney from damage (18). Previous studies demonstrated that MTH provides damage protection against oxidative stress (18-20). However, other studies reported that the neurological outcome and survival rate following ROSC after CA were not significantly different from those of patients who did not achieve ROSC (21-23). As TH has a controversial effect in patients with CA, an experiment was performed in the present study to confirm such an effect following ROSC.

Tujjar *et al* (24) demonstrated AKI in patients with CA following ROSC after CA, although the mechanism of kidney damage had remained elusive. Several studies reported increased expression of Nrf2 and HO-1 in the RI/RI experimental animal model (13,14). Thus, these parameters were selected for the present study to assess the antioxidative effects of TH in asphyxial CA-induced RI/RI following ROSC. The present study aimed to investigate the renoprotective effect of TH on asphyxial CA-induced RI/RI in rats.

## Materials and methods

**Experimental animals.** Male Sprague-Dawley (SD) rats (total n=48; bodyweight, 270-300 g; 10-weeks-old) were supplied by the Experimental Animal Center Jeonbuk National University (Jeonju, South Korea). They were housed in a conventional manner with adequate temperature ( $23\pm 2^{\circ}\text{C}$ ) and humidity ( $60\pm 10\%$ ) control under a 12-h light/dark cycle. They were provided with food and water *ad libitum*. All of the experimental procedures were approved by the Institutional Animal Care and Use Committee of Jeonbuk National University (approval no. JBNU 2019-005). Experimental animals were divided as into two major groups: Sham group (not subjected to CA surgery) (n=6) and rats subjected to CA surgery (total n=42). Rats that underwent CA surgery were further divided into the following groups: i) Normothermia + CA (Normo.) group (n=14); ii) Normo. with 2 h TH immediately after ROSC and gradual increase of the temperature to the normal temperature until sacrifice (Hypo 2 h) (n=12); iii) Normo. group with 4 h TH following cardiopulmonary resuscitation (CPR) and gradual rewarming to the normal temperature, which was maintained until the sacrifice of the rats (Hypo 4 h) (n=9); iv) Normo. group with 6 h TH immediately after ROSC and rewarming was performed to attain normal temperature, which was maintained until the rats were sacrificed (Hypo 6 h) (n=7). The number of rats in each group was selected according to the expected survival rate of each group with a target of having 6 animals per group at the end of the protocol;

in contrast to the estimated high survival rate at 2 and 4 h of TH (25).

**Induction of CA and CPR.** The induction of CA and CPR was performed according to the preferred protocol (26). In short, a rodent ventilator (Harvard Apparatus) was used to anesthetize the rats with 2-3% isoflurane and for mechanical ventilation. Peripheral oxygen saturation ( $\text{SpO}_2$ ) was checked by connecting the pulse oximetry with the right leg. For electrocardiographic (ECG) assessment, ECG probes were inserted in the limbs and data were monitored regularly. The cannulation of the left femoral artery was for monitoring the mean arterial pressure (MAP) and the right femoral vein was for intravenous administration. Vecuronium bromide (2 mg/kg; Gensia Sicor Pharmaceuticals) was injected intravenously after a stabilization period of 5 min (27,28). Furthermore, mechanical ventilation was also stopped for the induction of asphyxial CA. The MAP reaching below 20 mmHg resulting in pulseless electrical activity is defined as CA and it took 3-4 min for CA induction. After 5 min of CA with the administration of a bolus injection of epinephrine (0.005 mg/kg) and sodium bicarbonate (1 mg/kg), CPR was performed with an rodent CPR machine (Jeung Do Bio & Plant Co., Ltd.) on the chest of the rat at the depth of one-third of the anteroposterior region with mechanical chest compression at the rate of 300/min to provide 100% oxygen supply until the MAP became 60 mmHg (29). ECG was monitored continuously and the temperature was maintained according to the experimental protocol (Fig. 1A).

**Temperature management among the groups.** The body temperature of the Normo. group was maintained during and after surgery at  $37\pm 0.5^{\circ}\text{C}$  and this temperature was further maintained until the rats were sacrificed. In the 2 h of TH group, CA was performed at a normal temperature ( $37\pm 0.5^{\circ}\text{C}$ ) and then the body temperature was maintained at  $33\pm 0.5^{\circ}\text{C}$  immediately after CPR and the same temperature was maintained for 2 h, following which rapid rewarming was performed with the heating pad until the normal temperature ( $37\pm 0.5^{\circ}\text{C}$ ) was achieved and the rats were returned to their cages until they were sacrificed (Fig. S1). In the 4 and 6 h of TH groups, the duration of maintaining the body temperature at  $33\pm 0.5^{\circ}\text{C}$  CA after CPR was extended to 4 and 6 h, respectively. The body temperature was monitored by a rectal temperature sensor (30). After 24 h of CPR, all rats were sacrificed (13,31).

**Detection of blood urea nitrogen (BUN) and serum creatinine (Cr).** All of the rats were anesthetized with 30% urethane (1,400 mg/kg, intraperitoneal) at 24 h after ROSC and transcardially perfused with 4% paraformaldehyde and 5 ml of blood was collected from the inferior vena cava. The blood was centrifuged at  $1,413 \times g$  for 15 min at  $4^{\circ}\text{C}$  and serum was obtained for the determination of BUN and Cr with an Olympus AU 2700 Analyzer (Olympus Optical Co., Ltd.).

**Measurement of malondialdehyde (MDA) content of renal tissues.** The MDA concentration in renal tissues was measured according to a previously published protocol by our group (32). In short, homogenization and centrifugation of the renal tissues were performed at  $8,832 \times g$  for 10 min at  $4^{\circ}\text{C}$  and

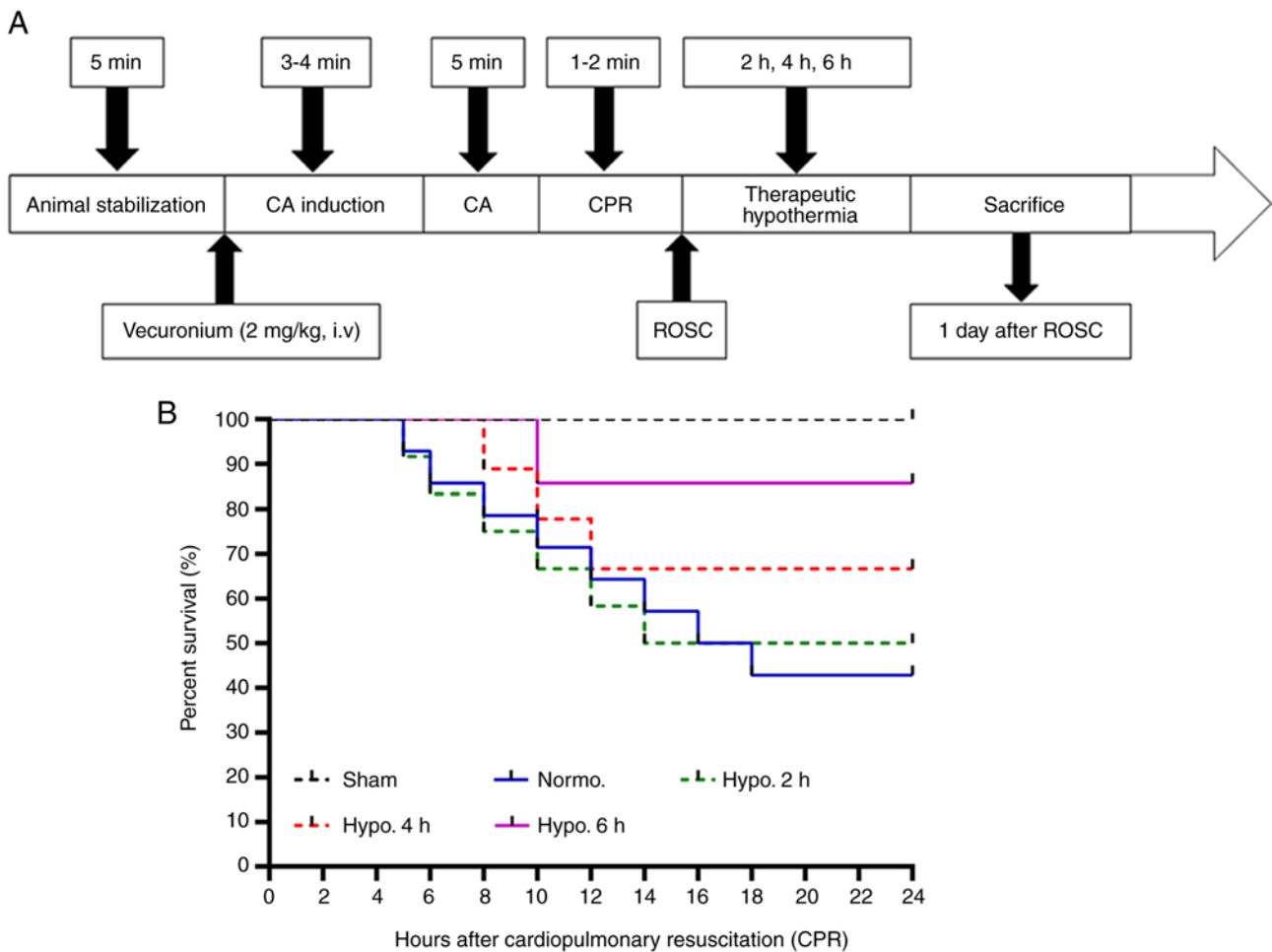


Figure 1. Schematic diagram and survival rate. (A) Schematic representation of the asphyxial CA model in rats and measurements obtained during the animal stabilization period (baseline), induction of CA, CA, CPR time, ROSC, TH duration and time-point of sacrifice. (B) The survival rate of the experimental rats was compared using Kaplan-Meier analysis ( $P < 0.05$ ). The Normo. group had a survival rate of 42.8% and it increased significantly at 2 h (50%), 4 h (66.6%) and 6 h (85.7%) of TH after CPR. Groups: Normo., Normothermia + CA; Hypo., TH after ROSC following CA. CPR, cardiopulmonary resuscitation; CA, cardiac arrest; TH, therapeutic hypothermia; ROSC, return of spontaneous circulation.

the supernatant was collected and stored at  $-80^{\circ}\text{C}$  for MDA analysis. Subsequently, the MDA content of the renal tissues was measured according to the instructions of a commercial kit (TBARS assay kit; Cayman Chemical).

**Tissue processing.** For transcardial perfusion, 0.1 M PBS (pH 7.4) was used following 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Kidneys were harvested from each rat and fixed with 10% neutral buffered formalin. Subsequently, the kidney was sagittally cut, embedded in paraffin and sectioned at  $5\ \mu\text{m}$ .

**Histopathological changes.** H&E staining was performed to observe the histopathological changes in the kidney according to the published protocol (33). The kidney sections were mounted on glass slides, dehydrated with ethanol after staining with H&E and mounted with Canada balsam (Kanto Chemical). Periodic acid Schiff (PAS) staining was performed according to the kit (cat. no. PAS-1-IFU; ScyTek Laboratories, Inc.) manufacturer's protocol. A Leica DM 2500 microscope (Leica Microsystems) was used to image the sections at fixed magnifications of  $\times 400$  (H&E) and  $\times 1,000$  (PAS), and in each group, 10 specific areas were captured. Analysis of glomerular

lesions was performed according to a previously published procedure (25). The scoring was as follows: Normal, 0;  $<25\%$  damage, 1; 26–50% damage, 2; 51–75% damage, 3; and 76–100% damage, 4 (25). Histopathological analysis of lesions was performed according to a published procedure (34). In short, histopathological changes were evaluated by quantitative measurement of interstitial tubular injury and through counting the number of apoptotic and necrotic cells, tubular brush border loss, dilatation of tubules, cast formation and infiltration of neutrophils. The injury scoring was as follows: 0, None; 1, 0–10; 2, 11–25; 3, 26–45; 4, 46–75; and 5, 76–100% (34).

**Immunohistochemistry (IHC).** IHC analysis of Nrf2/HO-1 was performed according to a previously published protocol by our group (32). In short, deparaffinization and dehydration of the paraffin sections were performed in xylene and ethanol. Antigen retrieval was performed with citrate buffer and 3% hydrogen peroxide was used for inactivation of endogenous peroxidase activity. Goat serum (cat. no. S-1000-20; Vector Laboratories, Inc.) was used for blocking of the tissue, followed by incubation with anti-rabbit polyvalent Nrf2 (cat. no. BP1-32822; Novusbio) and HO-1 (cat. no. ab13243; Abcam) antibody (dilution, 1:500).

Table I. Physiological variables of rats after asphyxial CA.

Physiological variable	Baseline	Normo.	Hypo. 2 h	Hypo. 4 h	Hypo. 6 h
Body weight, g	281±14.21	286±14.51	283±9.91	275±20.39	279±15.01
Asphyxial time to CA, sec	N. A.	139±34.68	145±23.71	156±31.41	149±24.84
CPR time, sec	N. A.	68±10.89	63±15.67	75±7.85	73±11.12
Heart rate, beats/min	331±10.91	339±19.54	335±18.74	338±10.31	333±8.79
Room temperature, °C	N. A.	24±0.70	25±0.49	25±0.57	24±0.81

Groups: Normo., Normothermia + CA; Hypo, TH after return of spontaneous circulation following CA. TH, therapeutic hypothermia; CA, cardiac arrest; CPR, cardiopulmonary resuscitation.

Subsequently, sections were incubated with the biotinylated secondary antibody (dilution, 1:250; cat. no. BA-4000-1.5) and Vectastain ABC reagent (cat. no. PK-4000) (both Vector Laboratories, Inc.) at room temperature for 1 h. Diaminobenzidine was applied to the sections in the dark until the development of a brown color. After counterstaining with hematoxylin staining for 2-3 sec at room temperature, the sections were dehydrated and cleared in ethanol and xylene and then mounted on a glass slide. A Leica DM 2500 microscope (Leica Microsystems) was used to image the sections at a fixed magnification of x400. From each group, 10 specific areas were captured. ImageJ threshold analysis software version 1.52a (National Institutes of Health) was used to measure the relative optical density (ROD%).

**Statistical analysis.** GraphPad Prism 5.0 (GraphPad Software, Inc.) was used to analyze the data. Values are expressed as the mean ± standard error or the mean. The survival rate of the rats was analyzed using Kaplan-Meier curves and log-rank tests. Furthermore, values were expressed as the median with interquartile range and statistical comparisons were performed using the Kruskal Wallis test followed by Dunn's post-hoc test.  $P < 0.05$  was considered to indicate statistical significance.

## Results

**Physiological variables of the rats.** The physiological parameters were insignificant between the Sham and Normo. groups ( $P > 0.05$ ). There was also no significant difference between the Normo. group and the groups with 2, 4 and 6 h of TH ( $P > 0.05$ ; Table I). CA was confirmed with isoelectric ECG and  $\text{SpO}_2$  (Table I).

**Survival rate.** The survival rate of the rats was determined at 1 day post-CA. Kaplan-Meier analysis demonstrated a significant difference in the survival rate ( $P < 0.05$ ). In the sham group, the survival rate was 100%; however, the survival rate in the Normo. group was 42.8% at 1 day after ROSC. The survival rate of rats increased significantly in the groups with 2 h (50%), 4 h (66.6%) and 6 h (85.7%) of TH after ROSC ( $P = 0.0267$  treatment groups vs. Normo. group; Fig. 1B).

**Serum BUN and Cr.** Serum BUN and Cr levels, two major indexes of renal function, were significantly increased in the

Normo. group as compared with those in the sham group ( $P = 0.0018$  and  $0.0036$ , respectively). After 2 h ( $P > 0.05$ ) and 4 h ( $P > 0.05$ ), the levels of BUN and Cr decreased when compared with those in the Normo. group but the change was not significant. However, BUN and Cr significantly decreased at 6 h of TH as compared to the Normo. group ( $P = 0.0068$  and  $0.0189$ , respectively; Fig. 2A and B).

**MDA levels in renal tissues.** MDA, a final product of lipid peroxidation, which is induced by ROS production, was measured in renal tissues with an MDA kit (13). The MDA concentration significantly increased ( $P = 0.0338$ ) in the Normo. group compared with that in the sham group. However, its level was significantly decreased in the 6 h ( $P = 0.0450$ ) of TH groups. Its level also decreased in the 2 h ( $P > 0.05$ ) and 4 h ( $P > 0.05$ ) of TH group but not significantly compared with that in the Normo. group (Fig. 2C).

**Histopathological damage.** Renal histopathological changes were evaluated by H&E and PAS staining (Figs. 3 and 4). No histopathological changes were present in the kidneys of the Sham group (34). Kidney lesions were markedly increased in the Normo. group compared with those in the Sham group ( $P < 0.0001$ ). Histopathological damage was significantly decreased ( $P = 0.0046$ ) in the group with 6 h of TH; however, it was not significantly decreased in the groups with 2 h ( $P > 0.05$ ) and 4 h ( $P > 0.05$ ) of TH when compared with the Normo. group. The brush border of renal tubular epithelial cells was severely eroded. Tubular dilatation and acute renal tubular necrosis were more evident in the Normo. group when compared to the groups with 2, 4 and 6 h of TH. In addition, dilatation of glomerular capillaries was severe in the Normo. group ( $P = 0.0064$ ) as compared to the sham group; however, it was attenuated after 2 h ( $P > 0.05$ ), 4 h ( $P > 0.05$ ) and 6 h ( $P = 0.0460$ ) of TH (Figs. 3 and 4).

**Expression of Nrf2 and HO-1.** IHC was performed to investigate the mechanisms of the renoprotective effects of TH in each group. The expression of Nrf2 and HO-1 increased in the Normo. group compared to the sham group but without any statistical significance ( $P > 0.05$ ). The ROD% of Nrf2 expression increased insignificantly at 2 h ( $P > 0.05$ ) and 4 h ( $P > 0.05$ ), and was significantly increased at 6 h of TH ( $P = 0.0002$ ; Fig. 5). Furthermore, HO-1 expression was significantly increased after 6 h ( $P = 0.007$ ) of TH but not significantly after

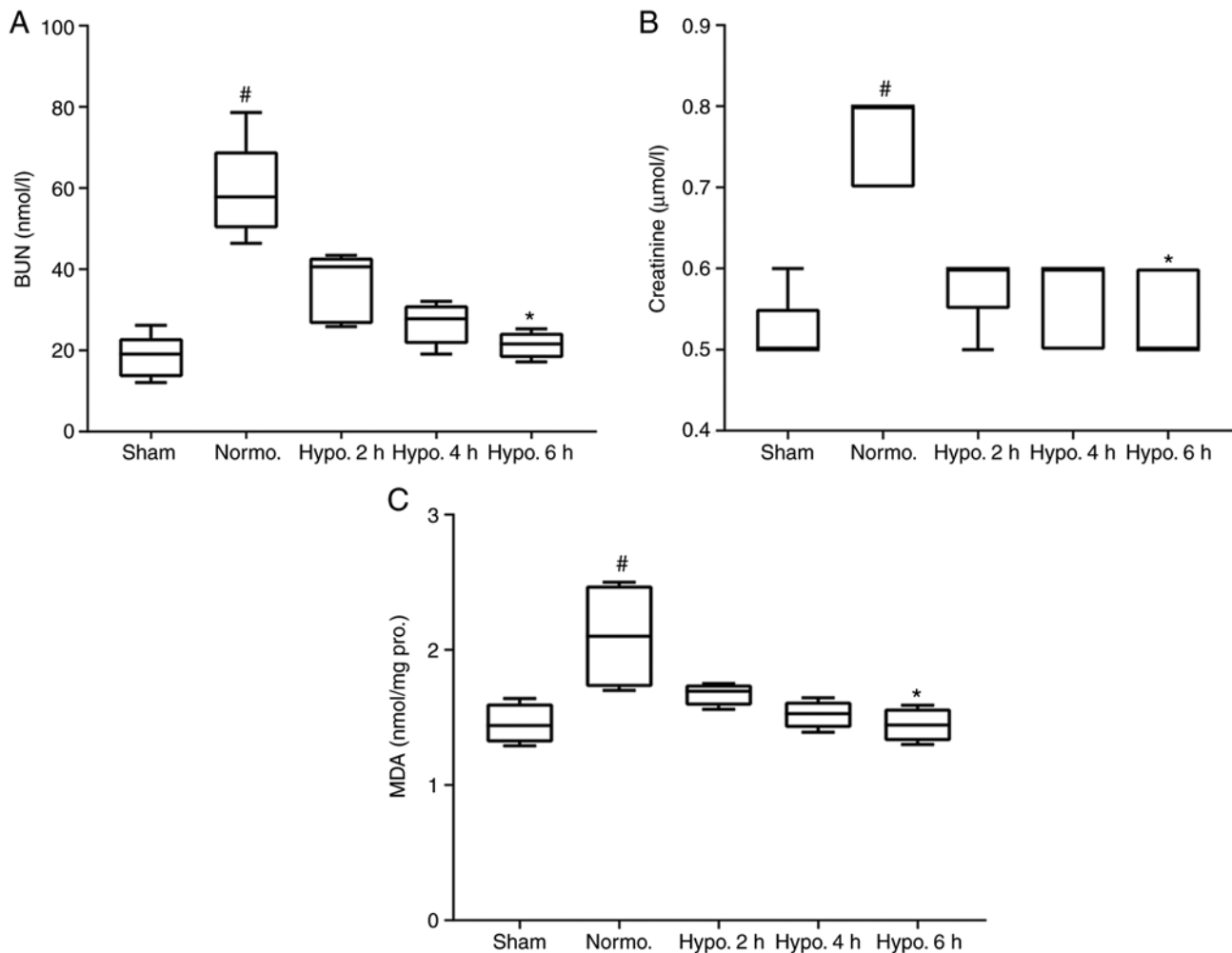


Figure 2. Serum BUN, Cr and MDA. Serum BUN, serum Cr and MDA content of the renal tissues increased significantly in the Normo. group compared to the sham group. The level of (A) BUN, (B) serum Cr and (C) MDA decreased insignificantly after 2 and 4 h of TH and increased significantly after 6 h of TH. The level of BUN, Cr and MDA data are presented as median and interquartile range. # $P<0.05$  compared with the Sham group; \* $P<0.05$  compared with the Normo. group. Groups: Normo., Normothermia + CA; Hypo., TH after return of spontaneous circulation following CA. BUN, blood urea nitrogen; Cr, creatinine; MDA, malondialdehyde; TH, therapeutic hypothermia; CA, cardiac arrest; pro., protein.

2 h ( $P=0.079$ ) and 4 h ( $P=0.1089$ ) of TH when compared with the Normo. group (Fig. 6).

## Discussion

The present study demonstrated that TH decreased the levels of BUN, serum Cr and MDA in renal tissues and increased the expression of Nrf2 and HO-1 in a TH time-dependent manner. The results suggested that TH ameliorated asphyxial CA-induced ischemia/reperfusion (I/R) renal dysfunction and oxidative stress. In the present asphyxial CA model, TH improved the survival rate and attenuated histopathological damage following ROSC after CA compared to those in the Normo. group. It is thus indicated that TH effectively mitigates oxidative stress markers in renal injury and increases the survival rate in a TH treatment time-dependent manner.

I/R injury is defined as paradoxical aggravation of cellular damage and death after the restoration of blood flow to previously viable ischemic tissue (35). CA is a case of whole-body I/R injury following ROSC characterized by multi-organ

dysfunction (3) and acute kidney failure is an outcome of whole-body I/R injury (24). In the present study, acute renal tubular necrosis, proximal convoluted tubule, brush border erosion and dilatation of renal glomerular capillaries were more severe in the Normo. group compared to those receiving 2, 4 and 6 h of TH after CA. Thus, it was indicated that TH treatment decreased renal injury and dysfunction in a TH treatment time-dependent manner.

The application of hypothermia treatment is a controversial topic. In one study, it achieved no beneficial effects on the survival of patients with CA following ROSC (23). Previous studies demonstrated that the survival rate of rats reached up to 7-8% at 2 days following ROSC after CPR in an asphyxial CA model (33,36). A study on ventricular fibrillation CA also revealed a low survival rate at 72 h following ROSC in rats (37). In the present study, the survival rate of the rats in the Normo. group also decreased, which was similar to previous results obtained with the asphyxial CA model (33,36,37). However, the present study reported that TH treatment increased the survival rate of the rats in a TH treatment time-dependent manner (23). Roberts *et al* (3)



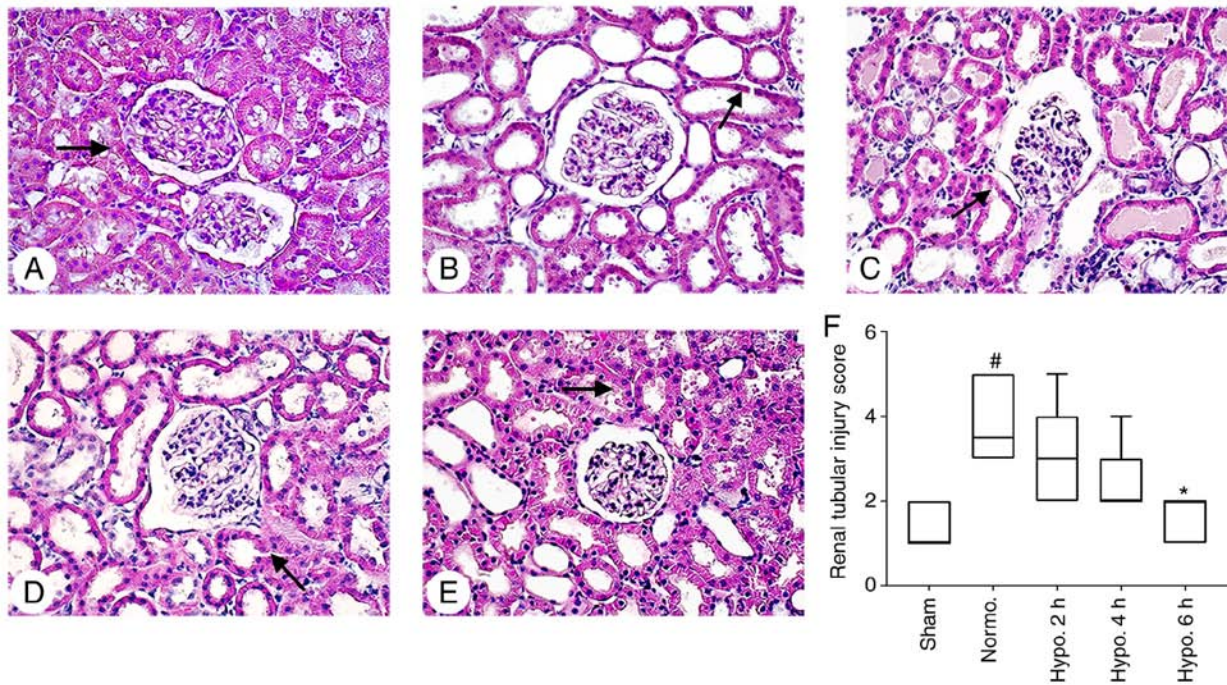


Figure 3. Histology of renal tissues in the different groups. H&E staining of (A) the sham group indicated no tubular injury. (B) In the Normo. group, obvious tubular injury was present (arrow). Renal tubules exhibited severe dilatation and loss of brush borders with necrosis. (C-E) TH ameliorated tubular injury (arrows); (C) 2 h, (D) 4 h and (E) 6 h of TH (magnification, x400). (F) Tubular injury score in the different groups. Glomerular tubular injury data are expressed as the median with interquartile range. \* $P < 0.05$  compared with the Sham group; # $P < 0.05$  compared with the Normo. group. Groups: Normo., Normothermia + CA; Hypo., TH after return of spontaneous circulation following CA. TH, therapeutic hypothermia; CA, cardiac arrest.

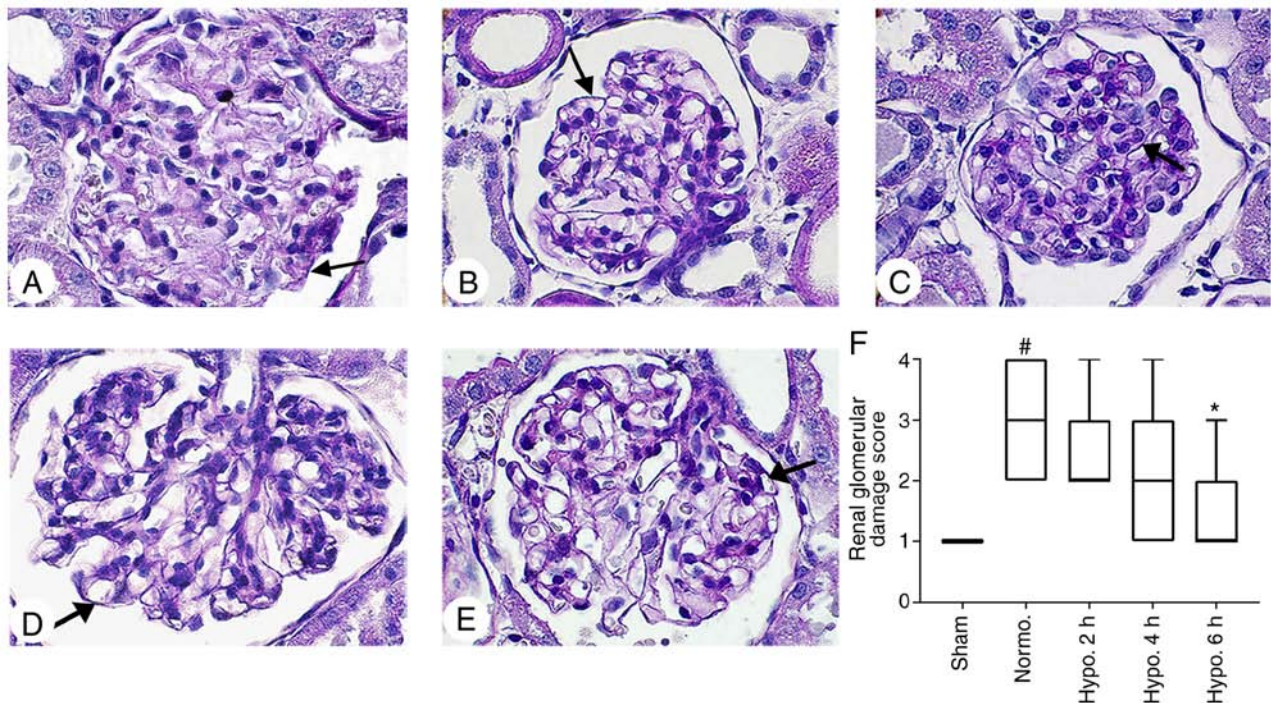


Figure 4. PAS staining. PAS staining of (A) the Sham group indicated no glomerular damage. (B) Glomerular capillary dilation (arrow) and the glomerular diameter increased significantly in the Normo. group. (C-E) Glomerular capillary dilation was ameliorated after TH (arrows); (C) 2 h, (D) 4 h and (E) 6 h of TH (magnification, x1,000). (F) Renal glomerular damage score in the different groups. Renal glomerular damage score data are expressed as the median with interquartile range. \* $P < 0.05$  compared with the Sham group; # $P < 0.05$  compared with the Normo. group. Groups: Normo., Normothermia + CA; Hypo., TH after return of spontaneous circulation following CA. TH, therapeutic hypothermia; CA, cardiac arrest; PAS, periodic acid Schiff.

demonstrated that TH ameliorated kidney damage following ROSC; however, the injury mechanism and attenuation via

TH remained elusive. In the present study, renal injury was attenuated with TH treatment in a time-dependent manner.



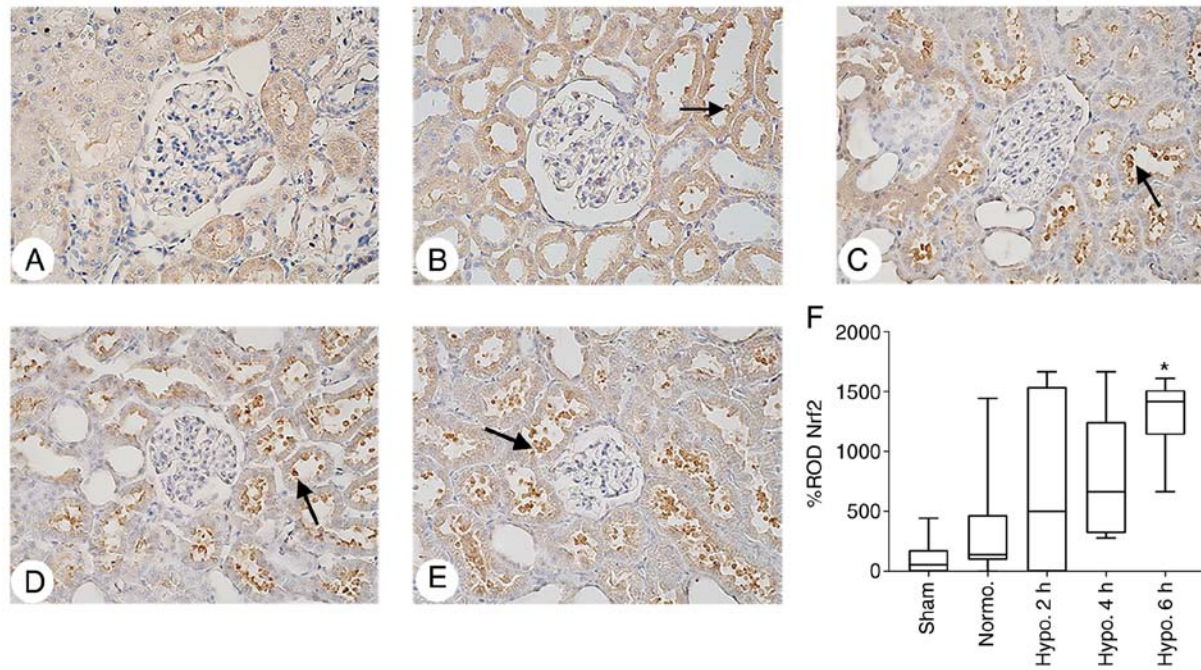


Figure 5. IHC analysis of Nrf2 expression in renal tissues. Representative IHC staining images for Nrf2 in the kidney tissues of (A) the Sham group, (B) Normo. group, (C) 2 h TH group, (D) 4 h TH group and (E) 6 h TH group (positive staining indicated by arrows; magnification, x400). It was revealed that TH increased the expression of Nrf2 in the renal cortex. (F) The ROD% of Nrf2 expression was significantly increased at 6 h of TH. ROD% of Nrf2 data are expressed as the median with interquartile range. \* $P < 0.05$  compared with the Normo. group. Groups: Normo., Normothermia + CA; Hypo., TH after return of spontaneous circulation following CA. TH, therapeutic hypothermia; CA, cardiac arrest; Nrf2, nuclear erythroid-related factor-2; IHC, immunohistochemical; ROD, relative optical density.

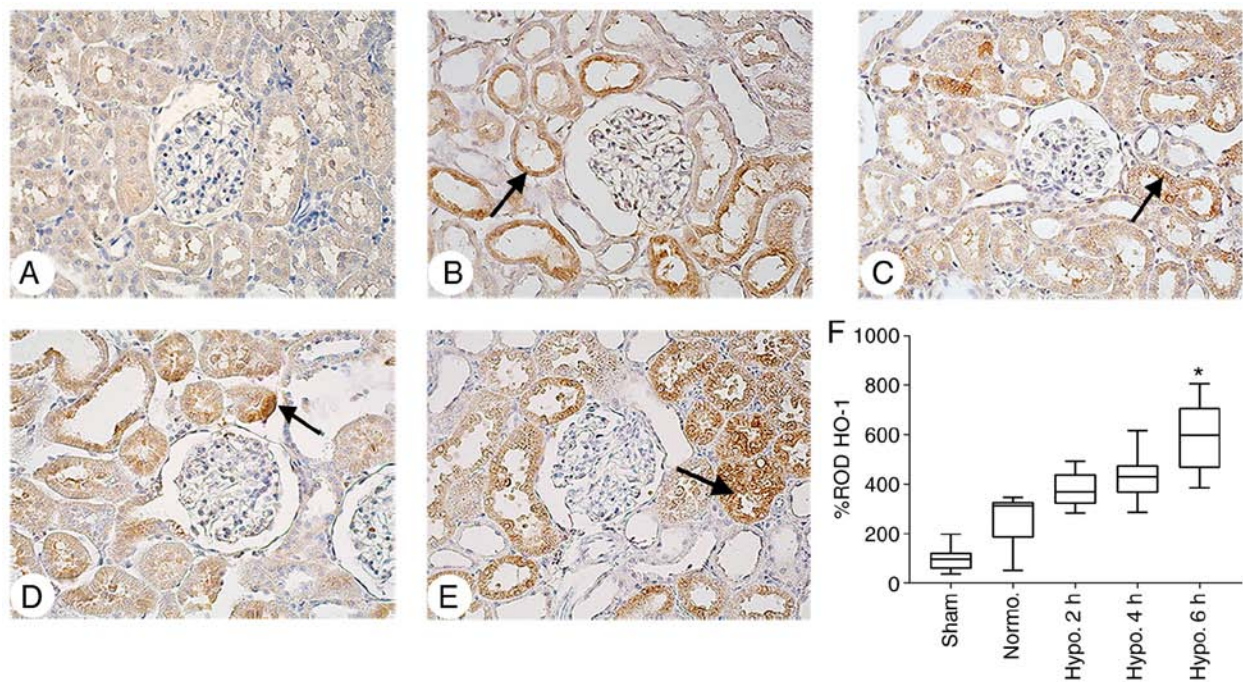


Figure 6. IHC analysis of HO-1 expression in renal cortical tissues. Representative IHC staining images for HO-1 in the kidney tissues of (A) the Sham group, (B) Normo. group, (C) 2 h TH group, (D) 4 h TH group and (E) 6 h TH group (positive staining indicated by arrows; magnification, x400). It was indicated that TH increased HO-1 expression in the renal cortical tissues. (F) The ROD% of HO-1 staining was elevated in the Normo. vs. Sham group and increased significantly at 6 h of TH in comparison with the Normo. group. ROD% of HO-1 data are expressed as the median with interquartile range. \* $P < 0.05$  compared with the Normo. group. Groups: Normo., Normothermia + CA; Hypo., TH after return of spontaneous circulation following CA. TH, therapeutic hypothermia; CA, cardiac arrest; IHC, immunohistochemical; ROD, relative optical density; HO-1, heme oxygenase.

The previous and present results suggested that TH treatment for various durations after ROSC is a favorable factor for

patients with CA and renal injury and may result in improved outcomes (3).

Atypical or unnecessary ROS is involved in the pathogenesis of tissue damage and injury (38). Guidet and Shah (39) reported that the capacity of the kidney to produce ROS is not accompanied by a related defense system against the resulting harm. A marked level of the pro-oxidation production MDA is generated due to reaction of ROS with components of cell membranes, and Grekas *et al* (40) suggested that I/R injury significantly elevated MDA production in renal tissues. Furthermore, Xia *et al* (41) reported that mild hypothermia decreased the MDA level in the kidneys of RI/RI mice. Hackenhaar *et al* (42) reported that TH reduces serum MDA concentration following ROSC after CA. In addition, Islam *et al* (25) reported that TH decreased the level of MDA in the renal tissues of their asphyxial CA rat model. In the present study, MDA levels in the renal cortical tissues following ROSC after asphyxial CA in rats were investigated, revealing that TH ameliorated MDA levels in the kidneys in a TH treatment time-dependent manner, which was in agreement with other studies. In the present study, TH ameliorated renal injury time-dependently and may be associated with an increased survival rate.

Previous studies demonstrated that the levels of kidney injury markers, MDA levels, renal histopathological ailments and Nrf2/HO-1 expression levels increased at 24 h following 45 min of RI/RI; however, in the present study, asphyxial CA induced 5 min of whole-body I/R injury (13,31). Despite the difference in ischemia duration and experimental models, the present study demonstrated consistency with the previous RI/RI rat models (13,31). Thus, it is suggested that renal injury markers, MDA and renal histopathological changes were attenuated and high expression level of Nrf2/HO-1 was achieved at 24 h after return of spontaneous circulation. Signaling pathways associated with asphyxial CA-induced RI/RI remain to be fully elucidated. In the Nrf2 signaling pathway, under normal physiological conditions, Nrf2 binds with Kelch-like ECH-associated protein in the cytoplasm (43). However, the oxidative stress in pathological conditions may lead to dissociation of the Nrf2-Keap1 complex (12,44), allowing Nrf2 to translocate into the nucleus, where it binds with ARE and HO-1 and results in an offset of cellular oxidative stress (12,44). The Nrf2/HO-1 expression level was correlated with scavenging and amelioration of ROS during the oxidative stress process (45). Therefore, Nrf2/HO-1 expression was considered beneficial in the case of RI/RI (45). Xia and Zhang (46) demonstrated that mild hypothermia significantly upregulated the expression of Nrf2/HO-1 in the cerebral cortex and hippocampus following asphyxial CA of rats; however, the role of mild TH on Nrf2 and HO-1 expression in asphyxial CA-induced-renal ischemia has remained elusive. In the present study, Nrf2 and HO-1 expression increased in the Normo. group compared with that in the sham group; however, application of TH for 2, 4 and 6 h increased the expression of Nrf2/HO-1 in the renal cortical tissues in a TH treatment time-dependent manner. Thus, it was indicated that immediate TH after CPR decreased the renal oxidative stress effects of post cardiac arrest syndrome, which is associated with post cardiac arrest myocardial dysfunction, brain injury and systemic ischemia/reperfusion response.

However, the present study still has certain limitations. TH treatment was followed by gradual rewarming in

previous studies on the asphyxial CA and CPR model, in which the focus was on the brain and myocardium and the results demonstrated improved neurological outcome and myocardial function (29,47). By contrast, rapid rewarming after TH treatment was reported to improve the survival rate, histopathological ailments and renal injury markers in the asphyxial CA rat model (29,47). In the present study rapid rewarming was performed after therapeutic hypothermia (2, 4 and 6 h) which was one of the potential limitations and requires further investigation. Furthermore, western blot analysis of Nrf2 and HO-1 expression, use of Nrf2 and HO-1 inhibitors/agonists, the lack of an alternative method for oxidative stress measurement and the lack of a variety of different experiments to verify the results are potential limitations of the present study and an objective of future studies by our group.

In conclusion, in the present study, immediate TH exhibited renoprotective effects against asphyxial CA-induced RI/RI by inhibiting oxidative stress and increased the survival rate of rats in a TH treatment time-dependent manner. It was indicated that TH confers protection by decreasing ROS levels, increasing the expression levels of Nrf2 and HO-1 in renal tissues and improving the survival rate in a TH treatment time-dependent manner. In short, immediate TH may be a favorable protective method for asphyxial CA-induced RI/RI; however, its role in the relationship among the renal system, heart and brain in CA remains elusive. Further study is required to evaluate the underlying protective mechanisms of TH in the kidneys via asphyxial CA-induced RI/RI in rats.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

AJ, YJY, JHL and HJT were responsible for the experimental design, data acquisition, data analysis and manuscript writing. JHC, WST, HYS, SEK, MSI, EYL and KHK performed the experiments and data analysis. DCA, BYP, JCY and ISK performed data analyses and made critical comments on the entire process of the study. All of the authors read and approved the final version of manuscript. JHL and HJT confirm the authenticity of the raw data.



## Ethics approval and consent to participate

All the procedures were performed in full compliance with the recommendations in the guidelines of the Institutional Animal Care and Use Committee of Jeonbuk National University (approval no. JBNU 2019-005).

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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