Effect of therapeutic hypothermia against renal injury in a rat model of asphyxial cardiac arrest: A focus on the survival rate, pathophysiology and antioxidant enzymes

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Abstract. Although multi-organ dysfunction is associated with the survival rate following cardiac arrest (CA), the majority of studies to date have focused on hearts and brains, and few studies have considered renal failure. The objective of the present study, therefore, was to examine the effects of therapeutic hypothermia on the survival rate, pathophysiology and antioxidant enzymes in rat kidneys following asphyxial CA. Rats were sacrificed one day following CA. The survival rate, which was estimated using Kaplan-Meier analysis, was 42.9% one day following CA. However, hypothermia, which was induced following CA, significantly increased the survival rate (71.4%). In normothermia rats with CA, the serum blood urea nitrogen level was significantly increased one day post-CA. In addition, the serum creatinine level was significantly increased one day post-CA. However, in CA rats exposed to hypothermia, the levels of urea nitrogen and creatinine significantly decreased following CA. Histochemical staining revealed a significant temporal increase in renal injury after the normothermia group was subjected to CA. However, renal injury was significantly decreased in the hypothermia group. Immunohistochemical analysis of the kidney revealed a significant decrease in antioxidant enzymes (copper-zinc superoxide dismutase, manganese superoxide dismutase, glutathione peroxidase and catalase) with time in the normothermia group. However, in the hypothermia group, these enzymes were significantly elevated following CA. Collectively, the results revealed that renal dysfunction following asphyxial CA was strongly associated with the early survival rate and therapeutic hypothermia reduced renal injury via effective antioxidant mechanisms.

Introduction

Cardiac arrest (CA), also known as cardiopulmonary arrest or circulatory arrest, involves a sudden cessation of normal blood circulation due to the failure of the heart to pump blood adequately (1). CA induces whole-body ischemia, which causes damage to multiple organs, including the brain, heart, kidneys and liver. The majority of research studies involving CA over the past half-century have focused on improving the rate of successful return of spontaneous circulation (ROSC), with significant progress (2-4). Although immediate resuscitation may improve ROSC, the survival rate with a poor prognosis is a concern (5-7). Post-cardiac arrest syndrome (PCAS) refers to the pathophysiological consequences of ROSC following successful cardiopulmonary resuscitation (CPR) following CA (8). PCAS is the main cause of decreased survival following ROSC (9). The early-period PCAS survival rate in patients is only 30% (5). There is no doubt that the heart and brain are important organs in PCAS. Meanwhile, studies have rarely investigated renal failure following

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CA (1,10,11). Transient impaired renal function is common in patients surviving CA (12). The incidence and impact of kidney dysfunction following CA are not well described (13). In addition, our previous study suggested that the low early survival rate following ROSC in experimental studies (14) may be strongly related to renal failure such as acute kidney injury. One of the most common causes of acute kidney injury is CA (15). Acute kidney injury is a common PCAS developing in ~30% of in-hospital patients with CA (16).

Reactive oxygen species (ROS) are composed of a series of oxygen intermediates, including the free radical superoxide anion ($O_2^{\bullet-}$), the nonradical hydrogen peroxide (H_2O_2), the highly reactive hydroxyl free radical (OH•), peroxynitrite ($ONOO^-$) and singlet oxygen ($^1O_2$) (17). ROS have been revealed to play an essential role in several experimental renal conditions, such as acute ischemic renal failure, renal graft rejection, acute glomerulonephritis and toxic renal diseases (18). There is significant evidence supporting the synthesis of ROS immediately following acute ischemic stroke (19) and acute myocardial infarction (20). ROS are known to be important in ischemic diseases, such as stroke and myocardial infarction. For example, Hackenhaar *et al* (21) reported that ROS are generated in the blood of patients with PCAS; however, studies have rarely investigated ROS formation in the kidney following CA during the early post-PCAS period (22,23).

For this reason, it was hypothesized that ROS are important in kidney injury following CA and contribute to the low survival rate in the early stages of PCAS. To examine this hypothesis, asphyxial CA was induced in rats and the survival rate during the early stages of PCAS was observed. Additionally, immediate and delayed hypothermia were performed to increase the low survival rate associated with PCAS following ROSC. Furthermore, the renal dysfunction was analyzed histopathologically and the changes induced by ROS, such as copper-zinc superoxide dismutase (SOD-1), manganese superoxide dismutase (SOD-2), catalase (CAT) and glutathione peroxidase (GPX) were assessed via immuno-histochemical analysis following ROSC.

Materials and methods

Experimental animals and groups. A total of 62 male Sprague-Dawley (SD) rats (weight, 270-300 g; age, 10 weeks) were obtained from the Experimental Animal Center of Jeonbuk National University (Iksan, Republic of Korea). They were housed at a temperature of 23±2˚C and humidity of 60±10% under a 12-h light/dark cycle. They were supplied with free access to food and water. All experimental protocols were approved based on ethical procedures and scientific care by the Institutional Animal Care and Use Committee of Jeonbuk National University (approval no. JBNU 2020-084).

Experimental animals were stratified in three categories [a sham operation group, CA under normothermia, and CA and hypothermia treatment (HT)] as follows: i) group I, a sham group (n=5) was maintained under normothermia conditions without CA; ii) group II, a normothermia group without hypothermia (33˚C) treatment following CA (n=17); and iii) group III (n=40), a group that underwent CA under normothermia and were treated with HT after CA for 2 h (n=17), 4 h (n=13) and 6 h (n=10) following ROSC, where all rats were reheated to normothermia.

CA induction and CPR. CA and CPR were performed as previously described (24,25) with minor modifications (Fig. 1). Briefly, the rats were anesthetized with 2-3% isoflurane and mechanically ventilated to maintain respiration using a rodent ventilator (Harvard Apparatus). To monitor peripheral oxygen saturation (SpO_2), a pulse oximetry oxygen saturation probe (Nonin Medical, Inc.) was attached to the left foot. Body temperature was maintained at 37±0.5˚C during and following the CA surgery. To monitor electrocardiogram (ECG) changes, electrocardiographic probes (Cytiva) were placed on the limbs to provide three-lead data, and which were monitored continuously. The left femoral artery and right femoral vein were separately cannulated to monitor the mean arterial pressure (MAP) (MLT 1050/D; ADInstruments, Ltd.) and intravenous injection.

Following a 5-min stabilization period, vecuronium bromide (2 mg/kg; Gensia Sicor Pharmaceuticals, Inc.) was intravenously administered, anesthesia was stopped and mechanical ventilation was withdrawn. A MAP below 25 mm-Hg and subsequent pulseless electric activity were used to define CA (25,26). CA was confirmed at 3-4 min following vecuronium bromide injection. At 5 min following CA, CPR was initiated by intravenously administering a bolus injection of epinephrine (0.005 mg/kg; Sigma-Aldrich; Merck KGaA) and sodium bicarbonate (1 mEq/kg; Sigma-Aldrich; Merck KGaA) followed by mechanical ventilation with 100% oxygen and manual chest compressions at a rate of 300/min until MAP reached 60 mm-Hg and electrocardiographic activity was observed. Once the animal was hemodynamically stable and spontaneously breathing (usually at 1 h following ROSC), the catheters were removed and the animal was extubated.

Temperature management among the groups. The body temperature of the normothermia group was maintained at 37±0.5˚C during and following the CA surgery and maintained until the rats were sacrificed according to the time schedule. In the hypothermia group, CA was established at normal temperature; then, the body temperature was maintained with ice packs and fans at 33±0.5˚C immediately following CPR for 2, 4 and 6 h, and they were rewarmed rapidly with the heating pad until the desired temperature (37±0.5˚C) was achieved. The rats were then returned to their cages until they were sacrificed one day following CPR/ROSC. The body temperature was monitored using a rectal temperature sensor (27).

Serum biochemical analysis. An intraperitoneal injection of 30 mg/kg pentobarbital sodium (JW Pharm Co., Ltd.) was used to anesthetize all animals. Blood was collected from the abdominal veins of each animal in each group. Serum was collected by blood centrifugation (2,774 x g, 15 min, 4˚C) and was preserved at -80˚C until analysis. The levels of blood urea nitrogen (BUN) and creatinine in the serum were determined according to methods outlined by the International Federation of Clinical Chemistry (28) using an automated chemical analyzer Hitachi 2070 (Hitachi, Ltd.). All assays were conducted in triplicate using fresh serum.
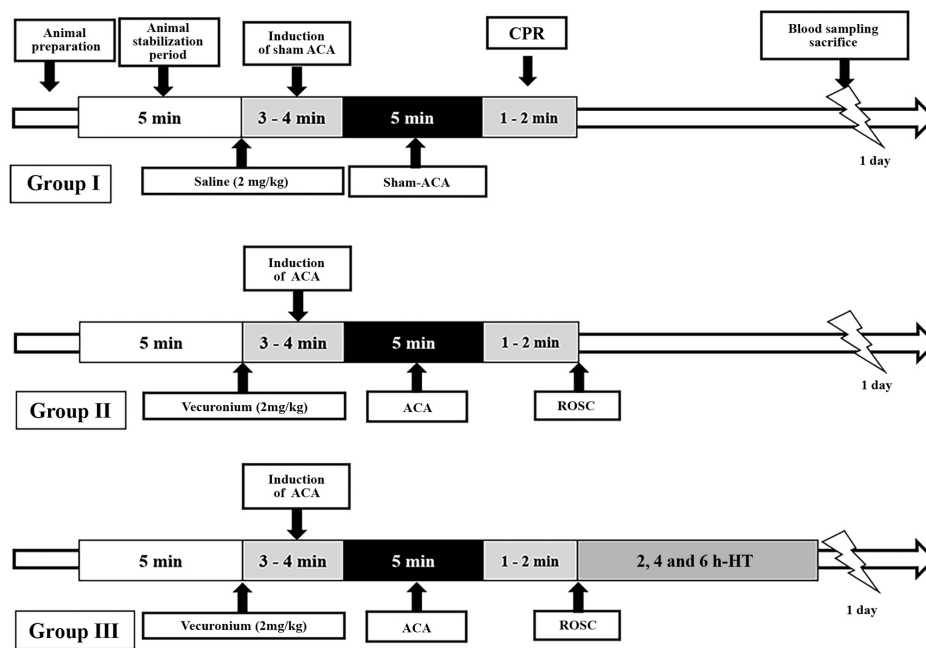
Figure 1. Experimental procedure showing animal stabilization, induction of ACA, CPR and ROSC, HT, blood sampling and sacrifice. HT, hypothermia treatment; ACA, asphyxial cardiac arrest; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation.

Tissue processing. The rats were deeply anesthetized by an intraperitoneal injection of 200 mg/kg pentobarbital sodium (JW Pharm Co., Ltd.), and they were perfused transcardially with 0.1 M of phosphate-buffered saline (PBS; pH 7.4), followed by 4% paraformaldehyde in 0.1 M of phosphate buffer (PB; pH 7.4). Kidneys were isolated from each animal and fixed with 4% paraformaldehyde in 0.1 M PB (pH 7.4) at room temperature during the 1 week, then sliced sagittally, embedded in paraffin and sectioned (6 μm).

Hematoxylin and eosin (H&E), Periodic Acid-Schiff (PAS) and Masson's trichrome staining. H&E staining was performed to examine pathological changes in the kidneys according to a previously described procedure (29). PAS staining was performed to examine changes in glomeruli according to previously described procedures (30-32). Masson's trichrome staining method was used for defining tubular injury, considering tubular dilatation, tubular atrophy, tubular cast formation, vacuolization, degeneration, interstitial fibrosis and sloughing of tubular epithelial cells, or thickening of the tubular basement membrane according to previously described procedures (33,34).

A total of 2 experienced pathologists evaluated histopathological changes in a double-blinded manner. Images of 10 stained sections/rat were captured at x400 magnifications using a Leica DM 2500 light microscope (Leica Microsystems GmbH). A total of 10 fields were analyzed in each section. Histopathological analysis of renal lesions was performed according to previously described procedures (35,36). Briefly, lesions were categorized as no significant microscopic lesions (NSML), minimal, mild, moderate, or marked lesions, respectively, graded using the following scale with the blind test: normal, 0 points; <25% damage, 1 point; 26-50% damage, 2 points; 51-75% damage, 3 points; and 76-100% damage, 4 points. Glomerular lesions were defined by loss of cellular elements, collapse of capillary lumen, amorphous hyaline material with or without adhesions to the Bowman's capsule (30-32) and scored by the following numeric scales: no damage, 0 points; very mild, 1 point; mild, 2 points; moderate, 3 points; and severe, 4 points. Tubular injury was scored by the following scoring system: no tubular injury, 0 points; 1-9% of tubules injured, 1 point; 10-25% of tubules injured, 2 points; 26-50% of tubules injured, 3 points; 51-75% of tubules injured, 4 points; and at least 76% of tubules injured, 5 points (33,34).

Malondialdehyde (MDA). MDA concentration in the renal cortex was evaluated according to a previously described protocol (23,37,38). In short, the homogenization and centrifugation of the renal tissues were performed at 8,832 x g for 10 min at 4°C, and the supernatant was collected and stored at -80°C for MDA analysis. MDA content was determined according to the instructions of TBARS assay kit (cat. no. 10009055; Cayman Chemical Company).

Immunohistochemistry (IHC) for antioxidant enzymes. IHC was performed with SOD-1, SOD-2, CAT and GPX to study changes in antioxidant immunoreactivities in the kidney. IHC was carried out according to our previously described method (22). In brief, the sections (6 μm) were incubated with primary goat anti-SOD1 (1:500; cat. no. SAB2500976; Sigma-Aldrich; Merck KGaA), goat anti-SOD2 (1:1,000; cat. no. SAB2501676; Sigma-Aldrich; Merck KGaA), rabbit anti-CAT (1:1,000; cat. no. ab16731; Abcam) and rabbit anti-GPX (1:1,000; cat. no. ab22604; Abcam) overnight at 4°C, followed by the biotinylated-conjugated anti-rabbit (1:250; cat. no. BA-1000-1.5; Vector Laboratories, Inc.) and the biotinylated-conjugated anti-goat (1:250; cat. no. BA-5000-1.5; Vector Laboratories, Inc.) secondary antibodies for 2 h at 24°C and developed using Vectastain ABC (Vector Laboratories, Inc.).

Table I. Physiological condition, asphyxia time and CPR time in Groups I, II and III before CA.

Parameters	Group I	Group II	Group III		
			2 h-HT	4 h-HT	6 h-HT
Body weight, g	355.44±17.33	354.13±17.04	284.14±9.89	278.14±19.41	283.71±12.08
Heart rate, beats/min	335.14±8.51	336.34±6.51	339.78±16.13	333.50±9.65	338.67±9.55
Room temperature, °C	36.60±0.53	23.90±0.87	25.07±0.53	25.06±0.68	24.83±0.72
Asphyxia time to CA, sec	-	162.12±16.36	145.44±28.79	148.89±19.65	150.56±24.55
CPR time, sec	-	71.12±12.49	72.03±7.97	69.78±10.57	73.89±9.93

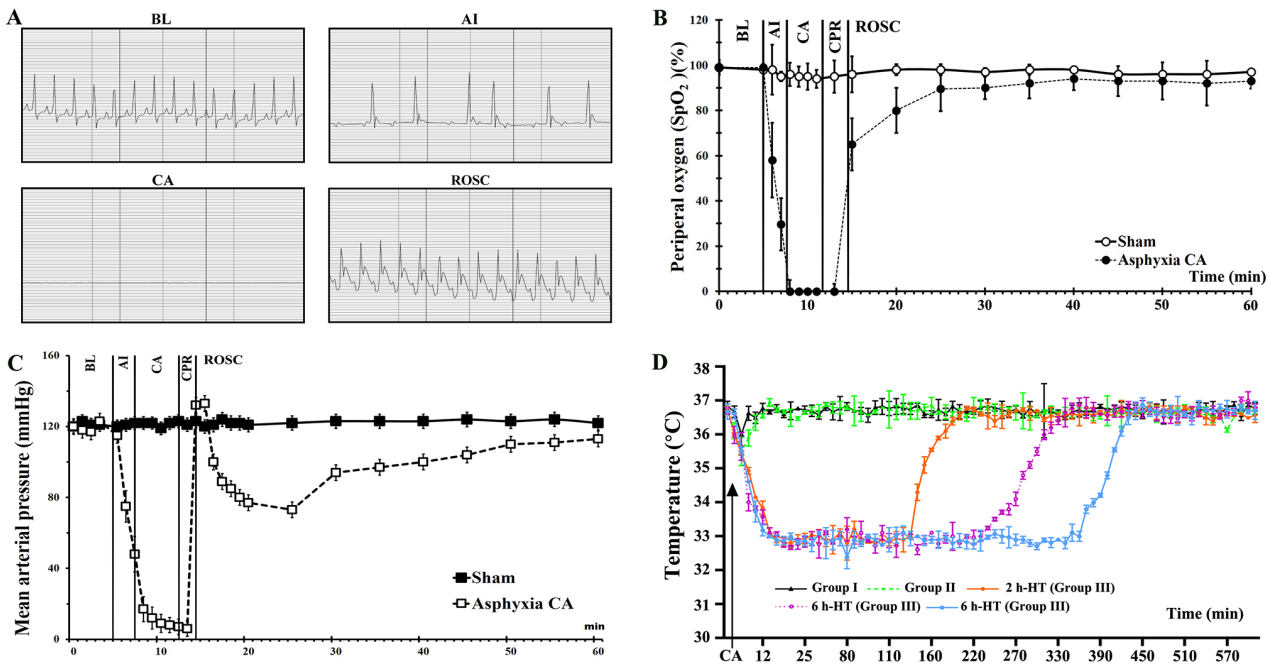CPR, cardiopulmonary resuscitation; CA, cardiac arrest, HT, hypothermia treatment.

Figure 2. Physiological variables in Groups I, II and III. (A) Electrocardiogram from a representative animal at BL, AI, CA and ROSC. Pulseless electrical activity is shown during CA, although it is often visible during CA. (B) SpO$_2$ levels were revealed during CA, CPR and ROSC. (C) Mean arterial pressure is shown during CA, CPR and ROSC. (D) Temperature management following ROSC. Data are expressed as the means ± SEM. HT, hypothermia treatment; CA, cardiac arrest; ROSC, return of spontaneous circulation; CPR, cardiopulmonary resuscitation; BL, baseline; AI, asphyxia induction.

Then, they were visualized with 3,3'-diaminobenzidine solution (in 0.1 M Tris-HCl buffer).

Leica DM 2500 microscope was used to image the sections at a magnification of x400. A total of 10 sections/rat were selected and 10 areas were captured. ImageJ threshold analysis software version 1.52a (National Institutes of Health) was used to measure the percent (%) of relative optical density (ROD).

Statistical analysis. All experiments were repeated in triplicate. Graph Pad Prism version 5.0 (GraphPad Software, Inc.) was used to analyze the data, which were expressed as the means ± standard error of the mean (SEM) values. Survival was analyzed using Kaplan-Meier statistics and the log-rank test. MAP and peripheral oxygen were compared using one- and two-way repeated-measures of analysis of variance to assess the effect of time. To determine the significance of differences, post hoc analyses were conducted using Tukey's

test for all pairwise multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Physiological changes, the survival rate and serum biochemical variables. There was no statistically significant difference among the groups regarding baseline characteristics, including body weight, MAP and SpO$_2$ (Table I and Fig. 2). The induction of CA occurred 3-4 min following the intravenous injection of vecuronium bromide (2 mg/kg). CA was confirmed with an isoelectric ECG, SpO$_2$ and MAP, and these changed as expected according to the experimental protocol (Fig. 2A-C). As revealed in Fig. 2D, the body temperature was different among all groups following ROSC.

As revealed in Fig. 3, the survival rate of each group was evaluated at one day post-CA. The rate of Group II was 42.9%.
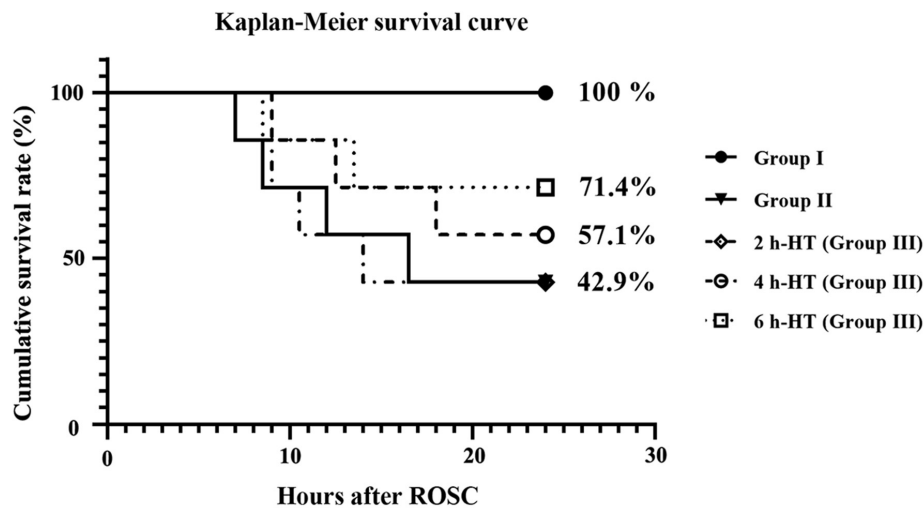
Figure 3. Cumulative survival rate using Kaplan-Meier analysis in Groups I, II and III by time point after ROSC. Group III had different survival rates from those revealed in Group II (log-rank test, P<0.05). ROSC, return of spontaneous circulation; HT, hypothermia treatment.
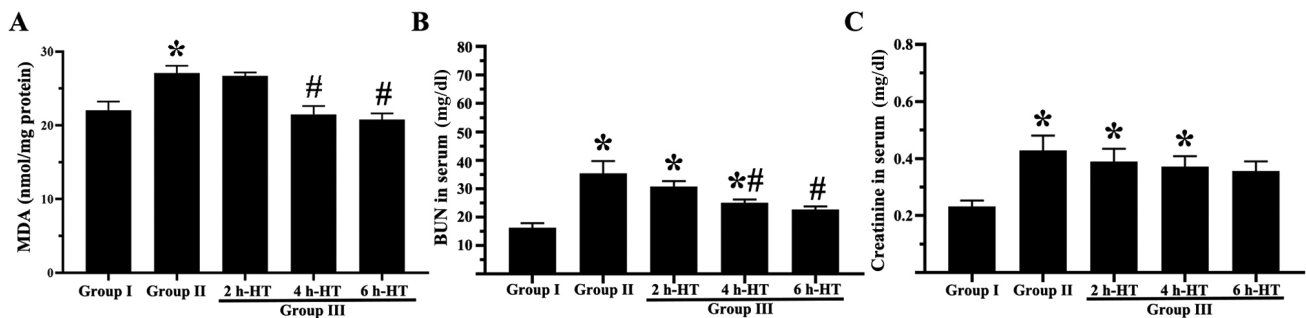

Figure 4. MDA, serum BUN and creatinine levels of the renal cortex tissue. (A) MDA levels. The levels were significantly increased in Group II 1 day following cardiac arrest; however, the levels in Group III were significantly decreased following 4 h- and 6 h-HT when compared with Group II. Serum (B) BUN and (C) creatinine levels. The levels were significantly increased in Group II; however, the levels in Group III were decreased following 6 h-HT when compared with Group II. *P<0.05 vs. Group I; #P<0.05 vs. Group II. MDA, malondialdehyde; HT, hypothermia treatment; BUN, blood urea nitrogen.

In group III, the rate following 2 h-HT was 42.9%, the rate following 4 h-HT was 57.1% and the rate following 6 h-HT was 71.4%. In this experiment, there was no difference between the rats in Group II and the rats with 2 h-HT in Group III.

As demonstrated in Fig. 4B, the serum BUN level in Group I was 13.8 mg/dl one day post-CA. In group II, the BUN level was significantly increased to 35.3 mg/dl. In Group III, the BUN level was 30.7 mg/dl following 2 h-HT, 25.0 mg/dl following 4 h-HT and 22.7 mg/dl following 6 h-HT. In addition, as revealed in Fig. 4C, the serum creatinine level in Group I was 0.23 mg/dl. In group II, the creatinine level was significantly increased to 0.43 mg/dl. In group III, the creatinine levels were lower than that observed in Group II as follows: 0.39 mg/dl following 2 h-HT, 0.37 mg/dl following 4 h-HT and 0.36 mg/dl following 6 h-HT.

Histopathological findings. In Group I (sham), intact histological structures were revealed by H&E, PAS and Masson's trichrome staining (Fig. 5A). The interstitial fibrosis was not detected in all groups, meanwhile in Group II, CA-induced renal histopathology was examined at one day following ROSC using H&E, PAS and Masson's trichrome staining. Severe CA-induced kidney injury was significantly increased in the proximal tubules and glomeruli; particularly, the brush borders of the renal tubular epithelial cells were seriously eroded (Fig. 5A and B). In addition, in this group, glomerular capillaries were dilated with inflammatory cells, and interstitial edema and acute renal tubular necrosis were serious as compared with those observed in Group I (Fig. 5A).

In Group III, kidney injury was attenuated at one day following ROSC (Fig. 5A and B). In particular, CA-induced injury in the proximal tubules was significantly decreased following 6 h-HT as compared with that observed in Group II. In addition, local expansion of the proximal tubules was decreased as compared with that revealed in Group II (Fig. 5A). For the glomeruli, 6 h-HT significantly attenuated glomerular injury as compared with that revealed in Group II (Fig. 5A and B).

MDA level. As revealed in Fig. 4A, the level of MDA in the renal cortex was significantly increased at one day following CA in Group II compared with Group I. However, in Group III, the level of MDA was significantly decreased following 4 h- and 6 h-HT. It was also decreased in the 2 h-HT, but there was no statistically significant difference compared with Group II.
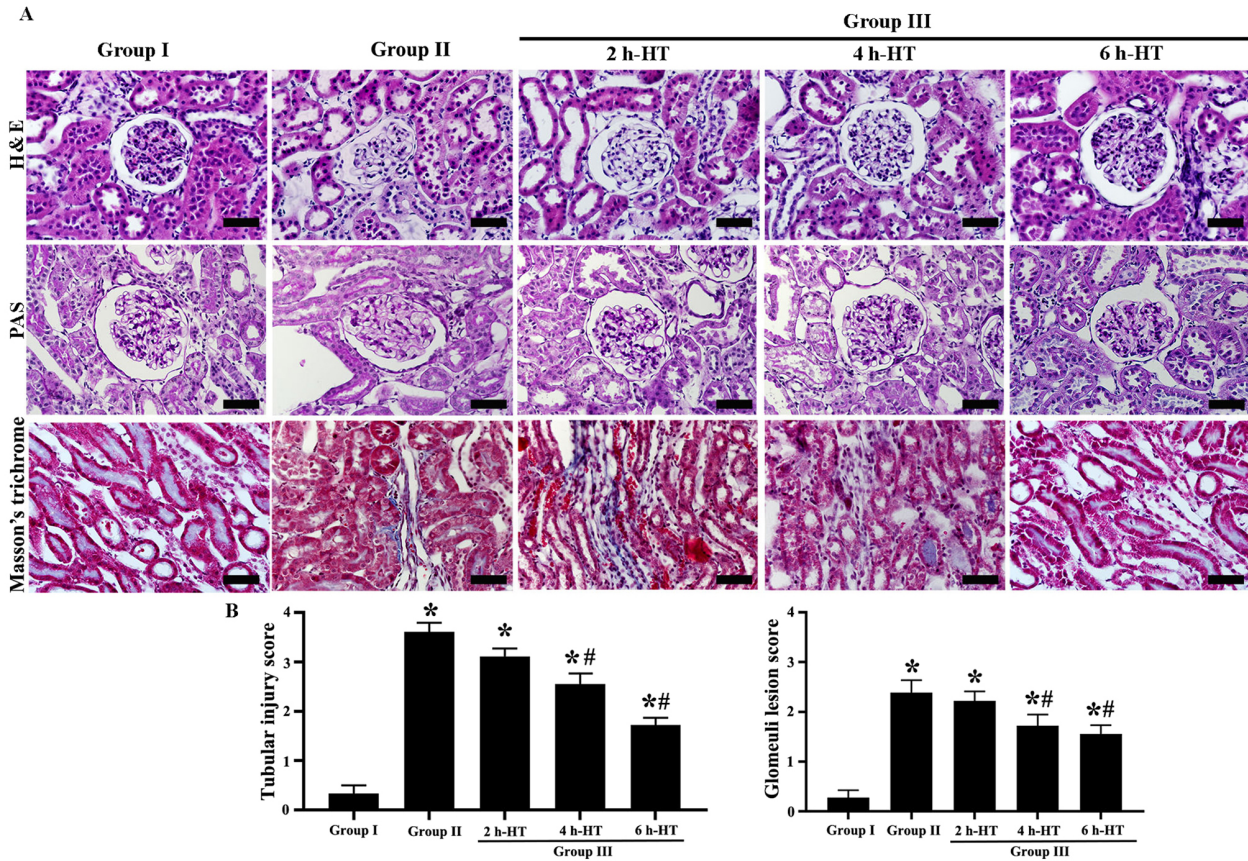
Figure 5. Histology of renal tissues in each group. (A) Histopathological findings of the kidneys of Groups I, II and III with 2 h-, 4 h- and 6 h-HT following CA. CA-induced renal injury was attenuated in Group III with 6 h-HT. Original magnification, x400. Scale bar, 50 μm. (B) Histograms of tubular injury score and glomerular lesion score. The scores in Group III with 6 h-HT were significantly decreased when compared with Group II. *P<0.05 vs. Group I; #P<0.05 vs. Group II. HT, hypothermia treatment; CA, cardiac arrest; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff.

Findings of antioxidant enzyme immunoreactivities. In Group I, normal SOD-1, SOD-2, GPX and CAT immunoreactivities were evaluated, revealing that they were principally located in the tubules (Fig. 6A). In Group II, SOD-1, SOD-2, GPX and CAT immunoreactivities were significantly reduced at one day following ROSC as compared with those revealed in Group I (Fig. 6A and B).

In Group III, SOD-1, SOD-2, GPX and CAT immunoreactivities following 2 h-HT were not significantly different from those observed in Group II (Fig. 6A and B). In the case of 4 h-HT, the four immunoreactivities were significantly higher than those revealed in Group II (Fig. 6A and B), demonstrating that, in particular, GPX immunoreactivity was significantly higher compared with the other immunoreactivities. In the case of 6 h-HT, all immunoreactivities were higher than those identified in the rats which received 4 h-HT, revealing that the ROD of each SOD-1, SOD-2, GPX and CAT immunoreactivity was 78.4, 67.4, 86.5 and 79.5%, respectively, as compared with Group I (Fig. 6A and B).

Discussion

In animal studies, the heart and brain are the most affected organs following ischemia/reperfusion (I/R) injury after CA (39,40). Nevertheless, certain studies have reported that acute kidney injury has an impact on neurological

recovery (41,42). Therefore, it is important to investigate acute kidney injury following CA and CPR. In the present study, adult male SD rats were used for asphyxial CA by injecting vecuronium bromide. CA was confirmed 3-4 min following induction of asphyxia and CPR was performed 5 min after CA. MAP, ECG and SpO$_2$ were altered as expected during CA and following ROSC. In our present study, the survival rate in Group III was 42.9% one day following ROSC in rats exposed to 2 h-HT, 57.1% in rats treated with 4 h-HT, and 71.4% in rats subjected to 6 h-HT. Che *et al* (26) reported that the survival rate was 40% two days following ROSC in a rat model of asphyxial CA. In addition, Wang *et al* (43) reported that in rats, the combination of hypothermia and levosimendan (a calcium sensitizer and potassium-channel opener) following ROSC significantly increased survival. Based on these findings, HT in rats with CA may increase the survival rate a few days following ROSC. However, in humans, HT after CA hardly increased the survival rate following ROSC (44).

Renal dysfunction was reported in 12-28% of patients with CA following successful resuscitation (13). In addition, acute kidney injury developed in 43% of patients resuscitated after CA, and more than 75% of these episodes occurred within three days following CA (45). In animal models, acute kidney injury induced by I/R (i.e., ROSC following CA) was significantly attenuated by HT (43,46,47). For example, Tissier *et al* (47) reported significant attenuation of kidney lesions by HT in
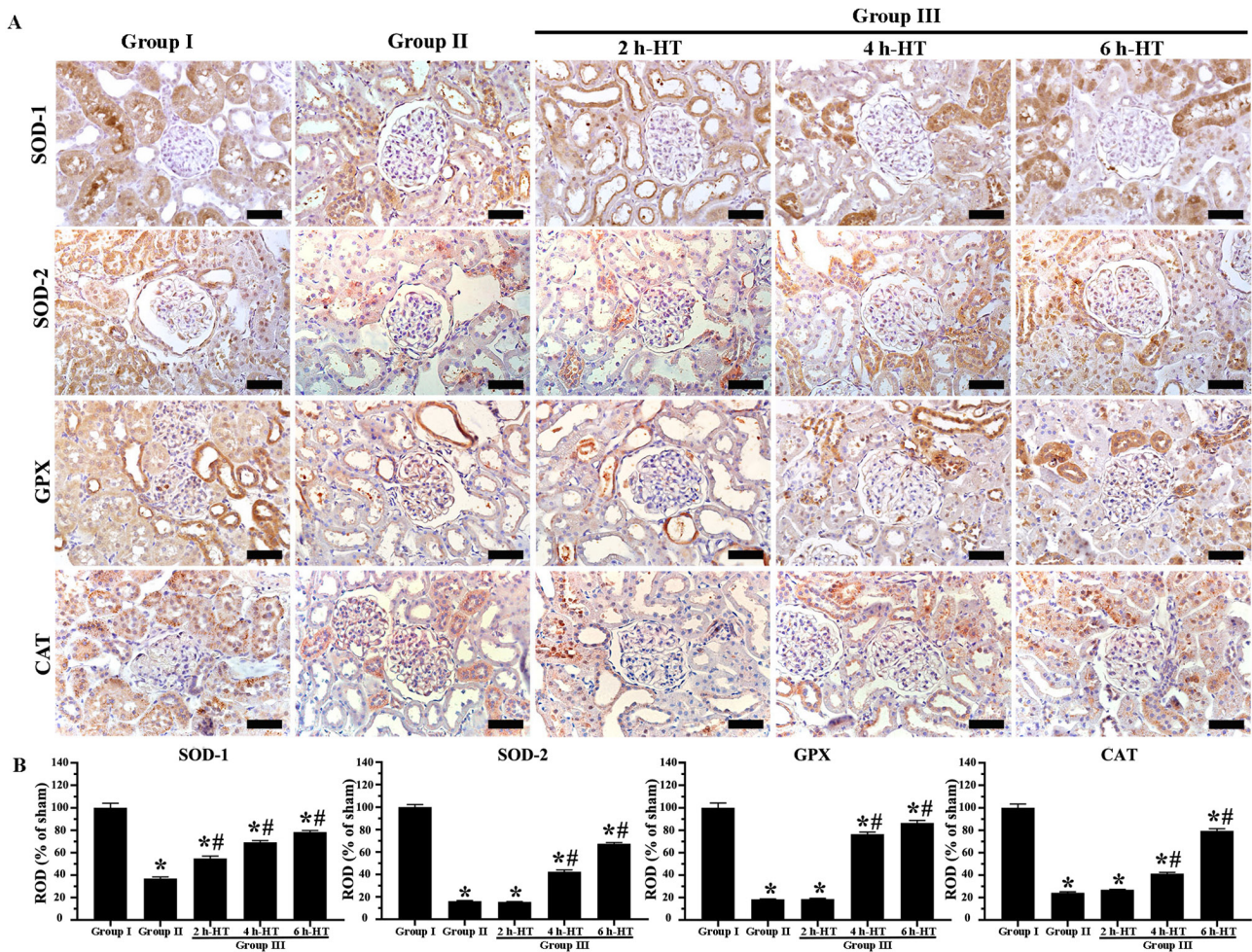
Figure 6. Immunohistochemistry analysis of antioxidant enzyme expression in renal cortex tissue. (A) Immunoreactivities of antioxidant enzymes (SOD-1, SOD-2, GPX and CAT) in the kidneys of Groups I, II and III were altered. All immunoreactivities in Group III with 6 h-HT were markedly increased when compared with Group II. Original magnification, x400. Scale bar, 50 μm. (B) ROD of SOD-1, SOD-2, GPX and CAT immunoreactivities. The ROD in Group III with 6 h-HT was significantly increased when compared with Group II. *P<0.05 vs. Group I; #P<0.05 vs. Group II. SOD, superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase; ROD, relative optical density; HT, hypothermia treatment.

a rabbit model of CA, based on histopathology and electron microscopy, compared with the control group. In our present study, the histopathological glomerular and tubular lesion scores of kidneys in Group II were apparently enhanced one day following ROSC. In this group, serum BUN and creatinine levels were significantly increased following ROSC when compared with the sham group (Group I). These results were similar to those of previous studies involving canine, rabbit and piglet CA models (47-49). Thus, kidney injury was severely increased in the early phase following CA in experimental animals. In our present study, renal glomerular and tubular lesions and histopathological scores in Group III were significantly reduced following 4 h- and 6 h-HT one day following ROSC compared with Group II. Ribeiro *et al* (46) and Souza *et al* (50) reported that HT was effective in animal models of renal I/R injury. Islam *et al* (23) and Jawad *et al* (22) determined that HT reduced the severity of renal injury and increased the survival rate in an asphyxial CA model. The findings suggested that HT has a significant renal-protective effect, which was associated with an increased survival rate.

Endogenous antioxidant enzymes mainly include SODs, CAT and GPX. These enzymes provide a first line of defense against $O_2^{\bullet-}$ and OH•. SOD-1 and SOD-2 provide a defense against oxidative stress by catalyzing the dismutation of $O_2^{\bullet-}$ into O_2 and H_2O_2 (51). Oxidative stress is a crucial factor in organ injury and hemodynamic dysfunction during PCAS and the generation of ROS during I/R injury. The activity of antioxidant enzymes is altered by I/R injury following CA (21). Our study revealed that SOD-1, SOD-2, GPX and CAT levels were decreased following ROSC in Group II compared with Group I. The levels of these antioxidant enzymes are reduced following I/R, which causes cell damage and death due to the consumption of endogenous antioxidants as a result of ROS release (52).

Xia *et al* (53) reported increased antioxidant activity in kidney tissues of mice exposed to HT in renal I/R injury. Hackenhaar *et al* (21) observed a significant increase in the activity of SOD-1, SOD-2, GPX and CAT after 6, 12, 36 and 72-h HT in humans following ROSC. In previous studies using a rat model of asphyxial CA, Islam *et al* (23) reported that HT following CA reduced oxidative stress in the kidney and Jawad *et al* (22) reported that HT following CA protected the kidney against injury induced by CA, demonstrating that Nrf2/HO-1 was increased in the kidney. In our present study,

the immunoreactivities of SOD-1, SOD-2, GPX and CAT were significantly increased following 4 h-HT and 6 h-HT in Group III when compared with Group II, suggesting that HT activated antioxidant enzymes and reduced the oxidative stress.

Based on the survival, histopathology, biochemical and immunohistochemical results of this study, it was determined that renal dysfunction is common and associated with mortality in the early stages of PCAS following ROSC, in our rat model of asphyxial CA. However, 4 h- or 6 h-HT following ROSC significantly reduced renal injury, suggesting that HT induces activation of antioxidant enzymes, such as SOD-1, SOD-2, GPX and CAT, resulting in reduced oxidative stress in the kidneys. As a result, it was hypothesized that HT reduces renal injury by an antioxidant mechanism and increases the early survival rate. However, western blot analysis is required to elucidate the mechanism of renal injury and HT in CA following ROSC. This is a potential limitation of the present study and underscores the need for further studies.

Acknowledgements

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

SEK, HYS, MHW and HJT were responsible for the experimental design, data acquisition, data analysis and manuscript writing. EYL, YJY, RHK, JHC and TKL performed the experiments and data analysis. DCA, BYP, JCY, SKH and ISK performed the data analysis and made critical comments on the entire process of the study. All authors have read and approved the final manuscript. HYS and HJT confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All experimental protocols were approved based on ethical procedures and scientific care by the Institutional Animal Care and Use Committee of Jeonbuk National University (approval no. JBNU 2020-084; Jeonju, South Korea).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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