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ORIGINAL RESEARCH

Post-Cardiac Arrest Hydrocortisone Use Ameliorates Cardiac Mitochondrial Injury in a Male Rat Model of Ventricular Fibrillation Cardiac Arrest

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BACKGROUND: Steroid use after cardiac arrest has been reported to improve survival and neurological outcome in cardiac arrest survivors. The study aimed to evaluate the effect of post-arrest hydrocortisone use on myocardial damage and cardiac mitochondrial injury in a rat model of ventricular fibrillation cardiac arrest.

METHODS AND RESULTS: Ventricular fibrillation cardiac arrest was induced and left untreated for 5 minutes in adult male Wistar rats. Cardiopulmonary resuscitation and electric shocks were then applied to achieve return of spontaneous circulation (ROSC). Successfully resuscitated animals were randomized into 3 groups: control, low-dose hydrocortisone (2 mg/kg), and high-dose hydrocortisone (8 mg/kg). The low-dose hydrocortisone and high-dose hydrocortisone (treatment) groups received intravenous hydrocortisone immediately after ROSC and the control group received saline as placebo. Each group consisted of 15 animals. Within 4 hours of ROSC, both treatment groups showed a higher cardiac output than the control group. At the fourth hour following ROSC, histological examination and transmission electron microscopy demonstrated less myocardial damage and mitochondrial injury in the animals treated with hydrocortisone. In the treatment groups, hydrocortisone mitigated the acceleration of Ca^{2+}-induced mitochondrial swelling and suppression of complex activity observed in the control group. At the 72nd hour after ROSC, a significantly higher proportion of animals treated with hydrocortisone survived and had good neurological recovery compared with those given a placebo.

CONCLUSIONS: Hydrocortisone use after cardiac arrest may mitigate myocardial injury and cardiac mitochondrial damage and thus improve survival, neurological and histological outcomes in a rat model of ventricular fibrillation cardiac arrest.

Key Words: cardiac arrest ■ cytokine ■ hydrocortisone ■ mitochondria ■ myocardial damage

Sudden cardiac arrest is a challenge to clinical physicians. Extensive efforts have been devoted to improving the quality of cardiopulmonary resuscitation (CPR) and ameliorate post–cardiac arrest syndrome. Even in cases where initial return of spontaneous circulation (ROSC) is achieved under aggressive resuscitation and organized teamwork, the cerebral and myocardial damage along with the systemic ischemia/reperfusion (I/R) injury during CPR result in high in-hospital mortality and poor neurological recovery. Bundle care following cardiac arrest including ventilation, hemodynamic and targeted temperature management, and emergent coronary angiography as indicated has been proposed to improve the poor prognosis of cardiac arrest survivors.

For decades, the use of steroid during the resuscitation and post–cardiac arrest period has been investigated. Relative adrenal insufficiency is common in cardiac arrest survivors with vasopressor-dependent shock.[1] In both animal and human research, higher serum

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Nonstandard Abbreviations and Acronyms

HE	hematoxylin and eosin
HSC	high-dose hydrocortisone
I/R	ischemia/reperfusion
LSC	low-dose hydrocortisone
ROSC	return of spontaneous circulation

cortisol has been associated with a higher likelihood of survival and a lower likelihood of death from circulatory shock.[2–4] Several studies, including population-based analyses and clinical trials, have demonstrated the benefit of steroid administration after ROSC on survival and neurological outcomes.[5–8] Steroid use after cardiac arrest suppresses inflammatory responses, regulates catecholamine synthesis, and protects against ischemia-reperfusion injury.[9] The cytokines and immune response after cardiac arrest are similar to sepsis and were once described as sepsis-like syndrome[10,11] until post-cardiac arrest syndrome was established in 2011. Prolonged low-dose corticosteroid therapy was suggested to benefit survival in patients with septic shock,[12,13] although the role of corticosteroid for severe sepsis has been undetermined for many decades.[14]

Several studies have demonstrated the cardioprotective effects of hydrocortisone for myocardial ischemic injury. In a dog model of epinephrine-induced myocardial injury, hydrocortisone reduced microscopic damage and myocardial calcium concentration.[15] In rats with asphyxia-induced myocardial ischemia, pretreatment with hydrocortisone or dexamethasone ameliorated myocardial mitochondrial damage.[16] However, these 2 ischemic models did not address reperfusion injury, nor did they simulate most sudden cardiac arrest, which is of cardiac origin. Our previous study demonstrated myocardial mitochondrial injury after resuscitation from ventricular fibrillation (VF)-induced cardiac arrest and electric shock.[17,18] Whether hydrocortisone administration after ROSC can reduce myocardial I/R injury and cardiac mitochondrial damage, thereby improve outcomes in VF-induced cardiac arrest, has not been investigated yet. Using a rat model of VF cardiac arrest, this study evaluated the effect of hydrocortisone use after cardiac arrest on myocardial damage, survival, and neurological recovery.

METHODS

The study was approved by the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine and College of Public Health, in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the Laboratory Animal Center of National Taiwan University College of Medicine. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animal Preparation

The animals were prepared as described previously.[18] Male Wistar rats weighing 500±50 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The trachea was orally intubated using a PE 200 catheter (Becton Dickinson, Franklin Lakes, USA). A saline-filled PE-50 tube (Becton Dickinson, Franklin Lakes, USA) was inserted through the right femoral artery to monitor arterial blood pressure and was advanced into the abdominal aorta. Left ventricle (LV) pressure was measured with another saline-filled PE-50 tube that was inserted through the right carotid artery and advanced into the LV. The third saline-filled PE-50 tube was inserted into the right jugular vein for fluid and drug administration and central venous pressure monitoring. To monitor temperature change, a thermodilution-tipped catheter (ADInstruments, Sydney, Australia) was inserted through the left femoral artery and advanced 10 cm into the abdominal aorta. Blood pressure, LV pressure, central venous pressure, body temperature, and needle-probe electrocardiogram monitoring data were recorded using a computer-based data acquisition system (ADInstruments). After surgical preparation, the animals were observed for 30 minutes to ensure hemodynamic stability. Before the experiment, body temperature was maintained at 37°C ±0.5°C by using an incandescent heating lamp.

Current-Induced Cardiac Arrest Animal Model

VF was induced with a guidewire advanced from the right jugular vein into the right ventricle. An alternating current increasing progressively from 60 Hz to a maximum of 1 mA was delivered to the endocardium and sustained for 1.5 minutes to prevent spontaneous defibrillation. The animals were subsequently left untreated for 3.5 minutes. After 5 minutes of VF, a mechanical chest compressor was used to administer chest compressions at 200 beats per minute) and mechanical ventilation was started. Mechanical ventilation was initiated with a tidal volume of 0.65 mL/100 g body weight at a frequency of 100 breaths per minute and at an inspired oxygen fraction of 1.0. CPR was synchronized to provide a compression/ventilation ratio of 2:1 with equal compression–relaxation duration. After 1 minute of CPR, one 3-J monophasic electric shock was administered, and then 30 seconds of CPR was followed by one 5-J electric shock. Resuscitation was declared a failure

when ROSC could not be achieved after 8 shocks. All successfully resuscitated animals were closely monitored for another 4 hours following ROSC, wounds closed, extubated, and then put back in their cages. Dose of sodium pentothal at a dose of 10 mg/kg was given at 2nd hour after ROSC. Survival status was observed for 72 hours, and mortality was confirmed by loss of heartbeat and spontaneous respiratory movement over 2 minutes.[18]

Study Protocol

The resuscitated animals were block-randomized into 3 groups: control, low-dose hydrocortisone (LSC), and high-dose hydrocortisone (HSC). Immediately after ROSC, the LSC group received 2 mg/kg of intravenous hydrocortisone (Solu-cortef, Pfizer, Belgium), the HSC group received 8 mg/kg, and the control group received 0.9% saline at the same volume as placebo. The hydrocortisone dosage in the LSC group was based on bolus injection of intravenous hydrocortisone 100 mg in patients with suspicious adrenal crisis.[19] The hydrocortisone dosage in the HSC group was calculated by dosage conversion between rat and human on the base of body surface area.[20] All the animals received therapeutic hypothermia at targeted temperature of 32°C, which was maintained for 2 hours by ice water spray, electric fan, or heat lamp. During the rewarming process, the temperature was increased at the rate of 0.5°C/h with electric fan and heat lamp when intubated. After extubation, the animals were rewarmed naturally under room temperature and monitored hourly before they waked up. A sham group received preparation without induction of cardiac arrest, experimental drugs, or hypothermia (Figure 1A).

Hemodynamic Monitoring

The LV-positive dP/dt_{40} and maximal LV-negative dP/dt, which reflect systolic and diastolic function, respectively, were analyzed using a PC-based data acquisition system (ADInstruments, Sydney, Australia). For cardiac output measurement, 0.2 mL of isotonic saline indicator at room temperature was injected intravenously into the right atrium. As mentioned, temperature change was monitored using the thermodilution-tipped catheter (ADInstruments, Sydney, Australia) in the abdominal aorta, and cardiac output was calculated using a Cardio-Max II computer.

Evaluation of Neurological Function

The neurological function of the animals, including consciousness level, corneal reflex, respiration, righting reflex, coordination, and movement/activity, was evaluated with neurological function scoring on a scale of 0 to 12 at the 6th, 24th, 48th, and 72nd hours following ROSC (Table 1). Assessments were performed independently by 2 investigators who were blinded to group. A third investigator resolved any discrepancies in an independent assessment, and the score chosen by the majority was accepted. Good neurological recovery was defined as a score higher than 10.

Histological Examinations of Myocardial Damage

To further investigate the histological changes among the groups, the animals were prepared as described in the Methods section and euthanized at the end of the survival study (72-hour following ROSC) (Figure 1A) and 4 hours following ROSC (Figure 1B), respectively.

The apex, septum, and lateral wall of the LV were selected, embedded in paraffin, cut into sections, and observed under an optical microscope for hematoxylin and eosin (HE) and Gomori trichrome staining at 4 hours, and for HE and Masson Trichrome staining at 72 hours. In HE staining, myocytolysis was counted in 5 independent, randomly selected microscopic fields at ×200 magnification in each specimen. Each animal provided 6 specimens. In Gomori trichrome staining, aggregation of abnormal mitochondria was shown as red, ragged, thick, with irregular intracellular and intermyofibrillar deposits, and the damaged areas were counted as in HE staining. In Masson Trichrome staining, the collagen fibers were stained blue and the nuclei was stained black and the background was stained red.

In the transmission electron microscopy examination, the LV was fixed in glutaraldehyde, and 3 blocks were obtained in each myocardium. Thin LV sections (≈70 nm) were placed on uncoated 200-mesh copper grids, stained with 4% of uranyl acetate and 0.2% of lead citrate in 0.1 N NaOH solution, and examined under a Hitachi 7100 transmission electron microscope (Tokyo, Japan). An independent anatomist blinded to the grouping assessed the morphological and histological results.

Histologic Examination of Brain Injury at 72 Hours

The brains were removed, embedded in paraffin, and cut into coronal sections (5 μm) on a rotary microtome. The right half of the brain of each animal was selected and underwent H&E staining. The intact pyramidal cells of the hippocampus can be observed with complete cell structure with big and regular nuclei. When severely damaged, pyramidal cells were characterized by neuronal shrinkage and nuclear chromatin condensation. The morphological changes of neurons were examined in 3 independent, randomly selected microscopic fields at ×200 magnification in the Cornu amonis (CA1, CA2, CA3) of each hippocampal cut.

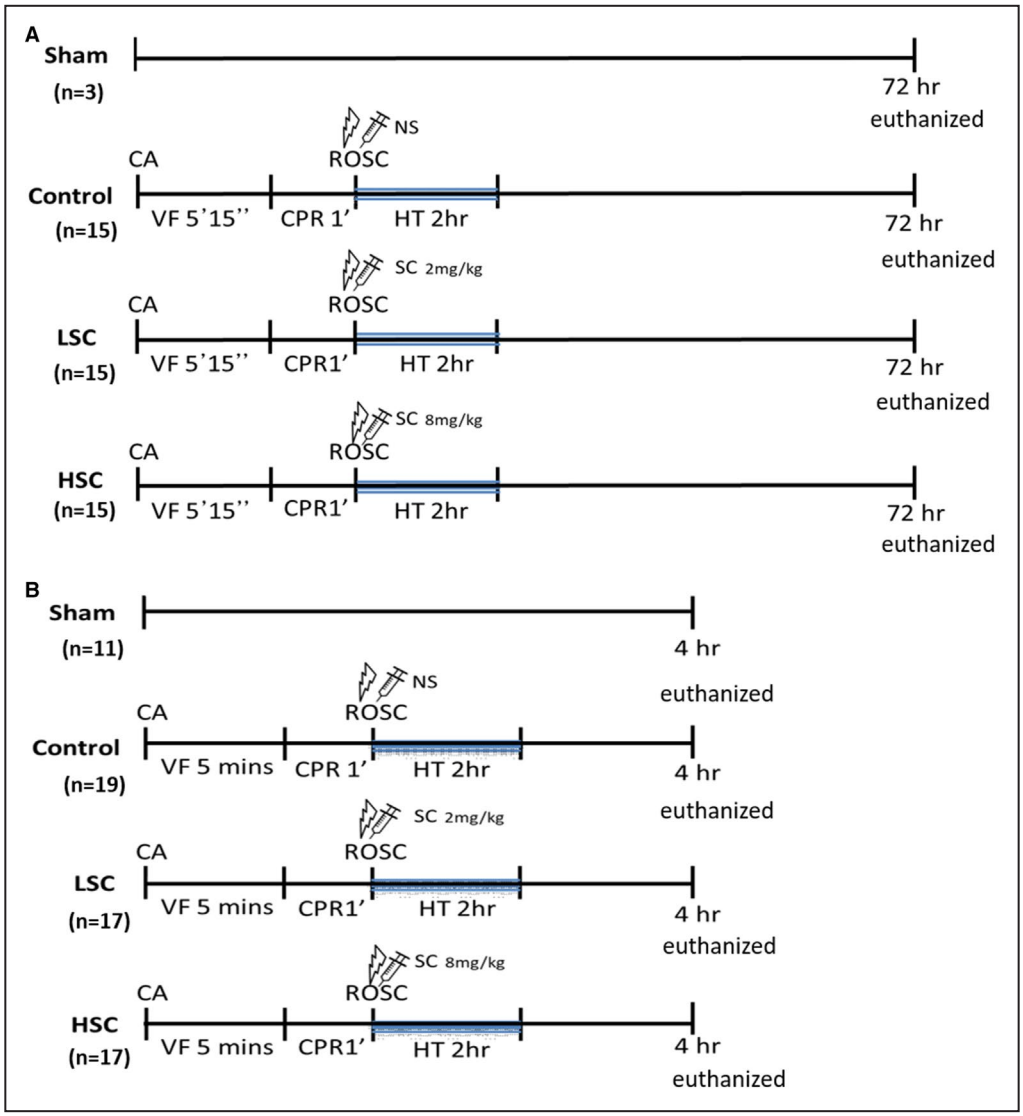

Figure 1. Study protocols.
A, Survival study (**B**) Histological and mitochondrial studies. CA indicates cardiac arrest; CPR, cardiopulmonary resuscitation; HSC, high-dose hydrocortisone; LSC, low-dose hydrocortisone; NS, normal saline; ROSC, return of spontaneous circulation; and SC, Solu-cortef.

The neuron death was presented as percentage of total neurons counted. Three hippocampal cuts were counted per animal.

Analysis of Cardiac Troponin I, Adrenal Hormones, and Cytokines

At the fourth hour following ROSC, blood samples were taken and centrifuged, and the plasma was separated and stored at –180°C. Commercially available ELISA kits for cardiac troponin I (Elabscience Biotechnology, Wuhan, China), adrenocorticotropic hormone and cortisol (USCN Life Science Inc., Wuhan, China), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) (R&D Systems, Minneapolis, USA) were used.

Evaluation of Myocardial Mitochondrial Injury

Mitochondria were isolated from the LV by differential centrifugation.[17] The final crude mitochondrial pellet was resuspended in I buffer (0.25 mol/L sucrose, 0.5 mmol/L EGTA, 3 mmol/L HEPES). Mitochondrial protein concentration was determined using the bicinchoninic acid method with bovine serum albumin as a standard. Fresh mitochondria were used for measurement of mitochondrial permeability transition pore opening and complex activity.

Isolated cardiac mitochondria were dissolved in a swelling buffer (200 mmol/L mannitol, 10 mmol/L HEPES, 5 mmol/L succinate, 70 mmol/L sucrose),

and mitochondrial concentrations were adjusted to achieved 1 of OD at 540 nm. Adding 400 μM/L $CaCl_2$ induced pore opening and caused mitochondrial swelling, which resulted in reduced absorbance at 540 nm. This reduction was measured using an ELISA reader for 30 minutes. The activities of NADH-cytochrome c reductase (NCCR) and succinate-cytochrome c reductase were evaluated by monitoring the reduction of oxidized cytochrome c. Cytochrome c oxidase activity was evaluated by measuring the oxidation of reduced cytochrome c. The change in absorbance was recorded using an ELISA reader.[17]

Statistical Analysis

We estimated that for HSC treatment to increase survival rate from 40% to 90%, the required sample size to achieve an 80% power at α=0.05 to correctly detect such a difference was 13. We chose 15 animals for each group and used block randomization for each group. Binomial variables were analyzed using a Chi-square test and Fisher exact test. Differences between the treatment groups and control group in continuous variables were evaluated using ANOVA with Dunnett post-hoc test. Repeated measurement was applied to compare mitochondrial swelling rates. Survival curves were determined by the Kaplan–Meier method and compared using the log-rank test. A *P* value of <0.05 was considered statistically significant. Multiple testing adjustment (*P*<0.025) was applied during the comparison of good neurological recovery between treatment and control groups. All analyses were performed using IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA).

Table 1. Neurological Scaling Score

	Score	Description
Consciousness level	0	No reaction to pinching of the tail
	1	Poor response to tail pinch
	2	Normal response to tail pinch
Corneal reflex	0	No blinking
	1	Sluggish blinking
	2	Normal blinking
Respiration	0	Irregular breathing pattern
	1	Decreased breathing frequency with normal pattern
	2	Normal breathing frequency and pattern
Righting reflex	0	No turning attempts
	1	Sluggish turning
	2	Urns over spontaneously and quickly
Coordination	0	No movement
	1	Moderate ataxia
	2	Normal coordination
Movement/ activity	0	No spontaneous movement
	1	Sluggish movement
	2	Normal movement

RESULTS

A total of 153 animals were used in the current study. Excluding 55 animals without ROSC (51 for mitochondrial experiments and histology examinations at 4 hours following ROSC, and 2 more in the control group because of insufficient blood sample), 98 successfully resuscitated animals were finally enrolled for further experiments. Another 14 were used as the sham group. Among them, 45 successfully resuscitated animals were equally randomized into the 3 groups. No significant between-group differences were observed in body weight, CPR events, body temperature, and hemodynamics (Table 2).

Hemodynamics, Survival Outcomes, and Neurological Outcomes

The LV-positive dP/dt_{40} and maximal LV-negative dP/dt, which are representative of the systolic and diastolic functions of the heart, decreased following ROSC and recovered gradually in all groups. Compared with the control group, both treatment groups had significantly better LV-positive dP/dt_{40} in the second hour and better maximal LV-negative dP/dt in the fourth hour following ROSC (Figure 2A and 2B). In the first 4 hours after ROSC, the treatment groups had significantly

Table 2. Resuscitation Events and Post-Arrest Characteristics of Groups in the Survival Study

	Control (n=15)	LSC (n=15)	HSC (n=15)
Weight, g	501.8±35.4	487.8±39.9	491.3±35.6
Age, wk	14.7±0.4	14.8±0.3	14.9±0.4
Blood gas			
PH	7.41±0.04	7.41±0.03	7.39±0.04
HCO_3, meq/L	13.09±1.64	13.11±1.61	12.93±1.77
Lactate, mg/kg	0.77±0.44	0.80±0.23	0.79±0.45
Resuscitation events			
Electric shock no.	6.3±2.4	5.8±2.9	5.8±2.1
CPR duration, min	234.9±97.2	220.5±62.6	233.5±79.8
CPP (mm Hg) after 1 min of CPR	43.4±8.1	42.6±9.0	45.6±8.3
Post-arrest characteristics			
BT after ROSC, °	35.0±0.7	34.9±0.6	35.1±0.4
Heart rate, min	269.2±72.6	253.9±54.7	266.8±44.5
Systolic blood pressure, mm Hg	75.9±17.4	72.4±14.3	75.0±16.4

Data are reported as mean±SD. BT indicates body temperature; CPP, coronary perfusion pressure; CPR, cardiopulmonary resuscitation; LSC, low-dose hydrocortisone; HSC, high-dose hydrocortisone; and ROSC, return of spontaneous circulation.

improved cardiac output compared with the control group, except for a trend in the first hour for the LSC group (*P*=0.051; Figure 2C). No significant between-group differences were observed in mean arterial pressure between groups (Figure 2D).

Only 9 animals (60%) in the control group survived to 72 hours. By contrast, 14 animals (93.3%) in each of the treatment groups survived to 72 hours (*P*=0.000) (Figure 2E). Among the animals that survived, 3 (20%) in the control group, 10 in the LSC group (66.7%,

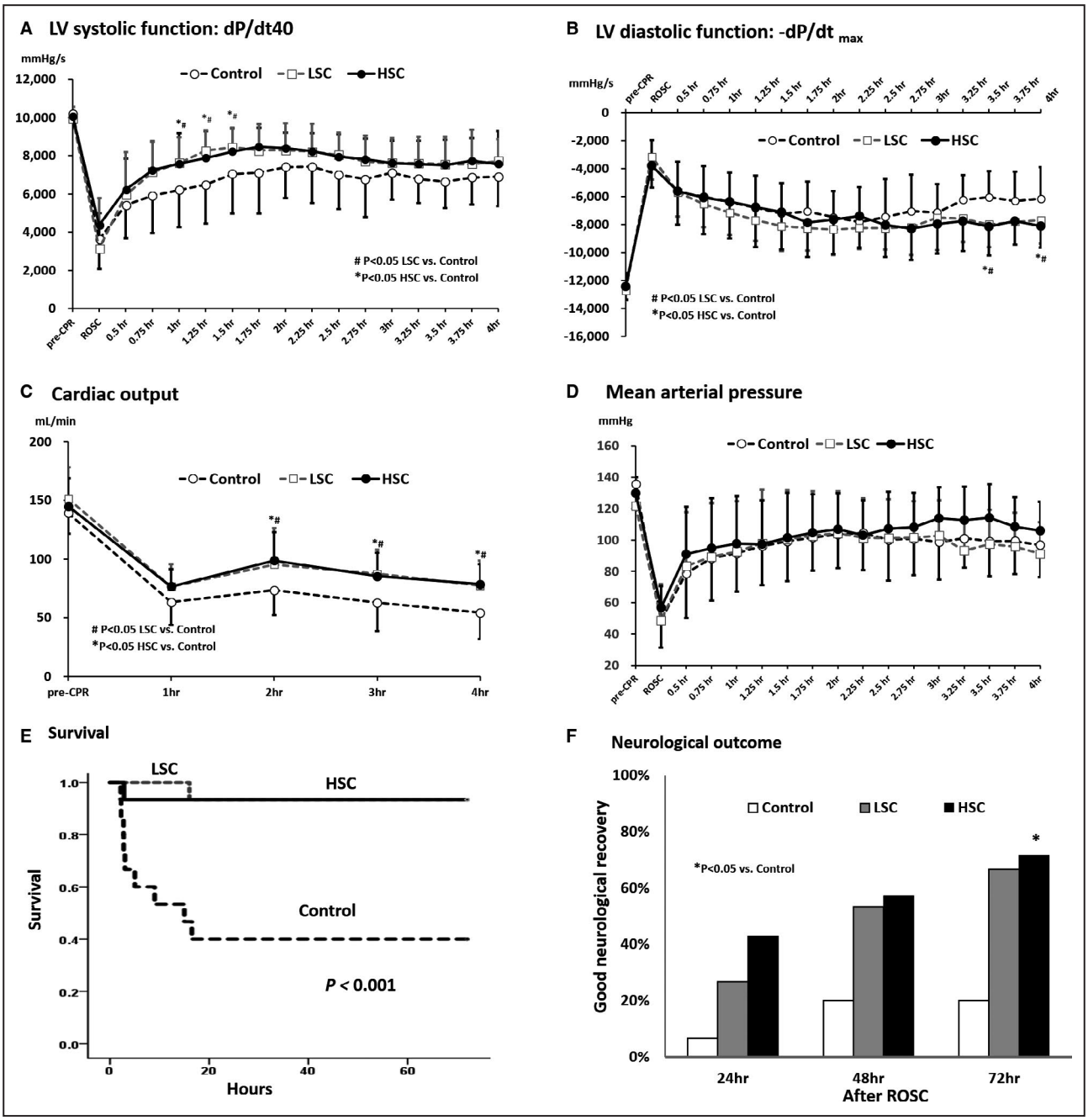

Figure 2. Hemodynamics, survival, and neurologic outcomes.
A and **B**, Both the low-dose hydrocortisone and high-dose hydrocortisone groups showed better but not sustained systolic and diastolic functions within the first 4 hours following return of spontaneous circulation (ANOVA with post-hoc correction; n=15). **C**, Animals receiving hydrocortisone had improved cardiac output compared with the control group during the first 4 hours after ROSC (ANOVA with post-hoc correction; n=15). **D**, No significant between-group difference in mean arterial pressure was noted (ANOVA with post-hoc correction; n=15). Animals receiving hydrocortisone had (**E**) better 72-hour survival rates (log-rank test; n=15) and (**F**) higher incidence of good neurological recovery (Fisher exact test with multiple testing adjustment; n=15). CO indicates cardiac output; CPR, cardiopulmonary resuscitation; HSC, high-dose hydrocortisone; LSC, low-dose hydrocortisone; LV, left ventricle; and ROSC, return of spontaneous circulation.

P=0.027 compared with control), and 11 in the HSC group (71.4%, *P*=0.020 compared with control) had good neurological recovery at the 72nd hour following cardiac arrest (Figure 2F).

Myocardial Damage and Mitochondrial Injury

Compared with the sham group, at 4 hours after ROSC, the HE-stained LV sections in all groups exhibited myocytolysis, waving, and transverse contraction bands. Both treatment groups had reduced myocytolysis compared with the control group (Figure 3A and 3B). With GT staining, significantly abnormal mitochondrial aggregation was noted in the control group but was less observed in both treatment groups (Figure 3A and 3C). Transmission electron microscopy examination of the control group showed mitochondrial swelling and edema, outer membrane rupture, and loss of inner-membrane cristae with amorphous densities. The treatment groups had less mitochondrial membrane damage, much lower swelling and edema, and more intact lamellar cristae (Figure 3A). Moreover, plasma troponin I levels were significantly lower in the treatment groups than in the control group (Figure 3D).

Compared with the sham group, the control group experienced an accelerated rate of Ca^{2+}-induced mitochondrial swelling. The HSC group mitigated the acceleration (Figure 4A). The control group demonstrated decreased activities of NCCR, succinate-cytochrome c reductase, and cytochrome c oxidase compared with the sham group. Administration of hydrocortisone, regardless of dosage, mitigated the inhibition of NCCR and cytochrome c oxidase but not succinate-cytochrome c reductase (Figure 4B through 4D).

Cytokines, Adrenocorticotropic Hormone, and Cortisol

Compared with the sham group, the control group demonstrated increased IL-6 and TNF-α levels.

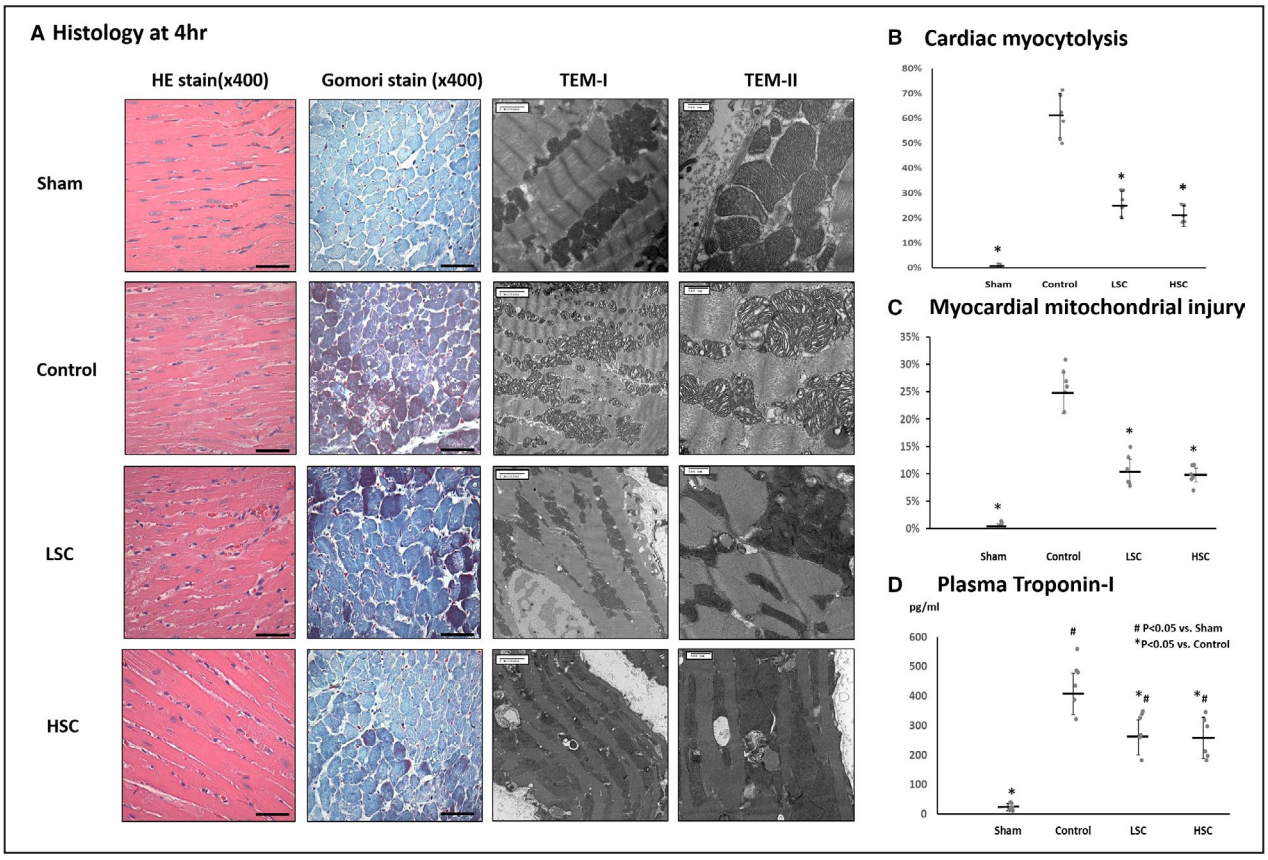

Figure 3. Histological examinations of heart and troponin-I at 4 hours.
A through **C**, Histological examinations showed less myocardial damage and cardiac mitochondrial injury in the treatment groups than in the control group (ANOVA with post-hoc correction; n=6 in the control and treatment groups, n=3 in the sham group). **D**, Hydrocortisone treatment also diminished troponin-I release (ANOVA with post-hoc correction; n=6). CPR indicates cardiopulmonary resuscitation; dP/dt_{40}, LV-positive dP/dt_{40}; dP/dt_{max}, maximal LV-negative dP/dt; HE, hematoxylin and eosin; HSC, high-dose hydrocortisone; LSC, low-dose hydrocortisone; LV, left ventricle; and TEM, transmission electron microscopy. HE and Gomori stains: ×400 magnification, scale bars=50 μm; TEM-I: ×12 800 magnification, scale bar=2 μm; TEM-II: ×38 400 magnification, scale bar=500 nm. *P<0.05 compared with the control group.

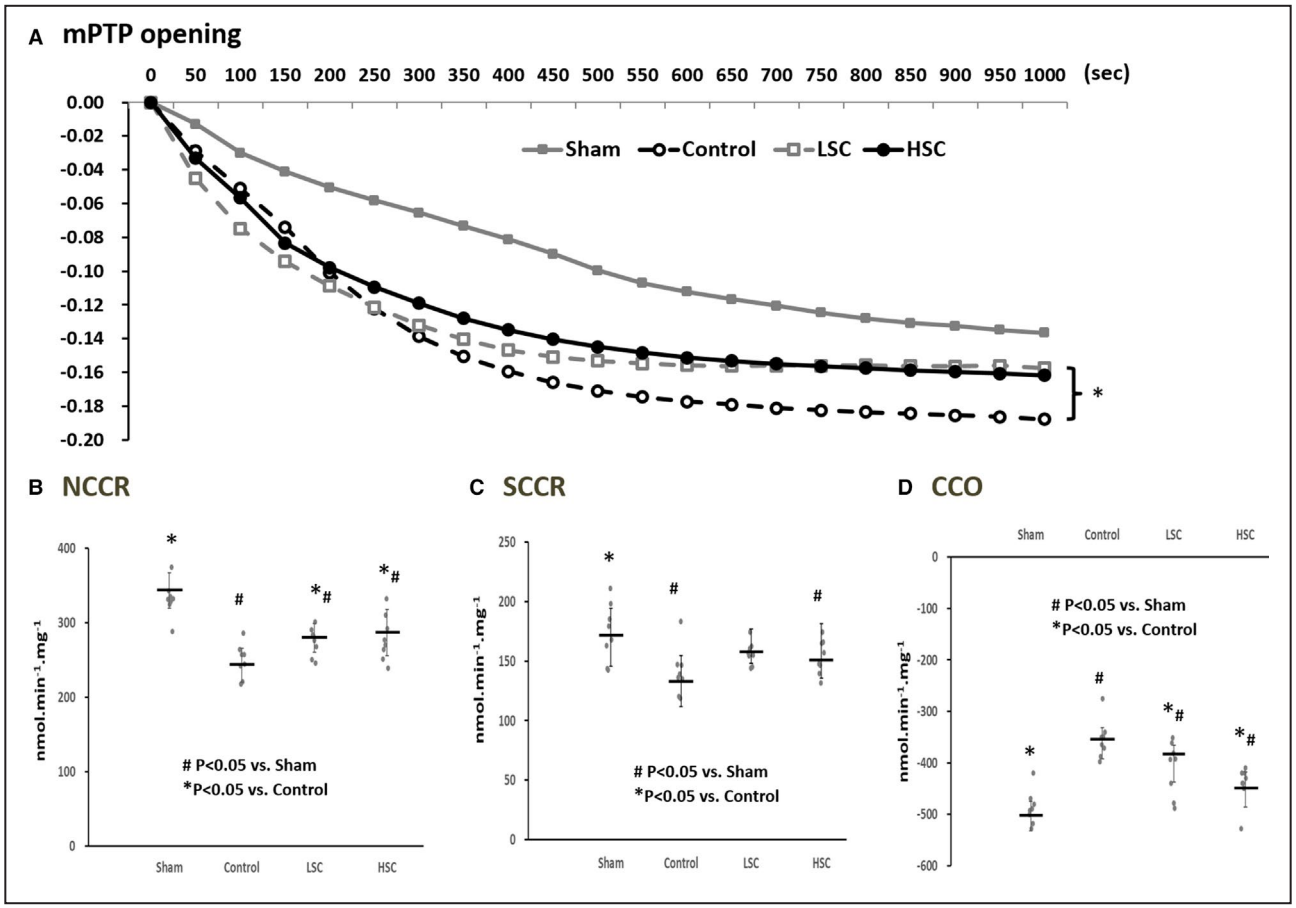

Figure 4. Mitochondrial function.
A, Acceleration of Ca^{2+}-induced mitochondrial swelling was significantly mitigated in the high-dose hydrocortisone group compared with the control group (repeated measurement; n=8). **B** through **D**, Hydrocortisone use after cardiac arrest, regardless of dosage, mitigated the inhibition of NADH-cytochrome c reductase and cytochrome c oxidase but not succinate-cytochrome c reductase (ANOVA with post-hoc correction; n=8). CCO indicates cytochrome c oxidase; HSC, high-dose hydrocortisone; LSC, low-dose hydrocortisone; mPTP, mitochondrial permeability transition pore; NCCR, NADH-cytochrome c reductase; and SCCR, succinate-cytochrome c reductase. *P<0.05 compared with the control group, #P<0.05 compared with the sham group.

Significantly reduced IL-6 and TNF-α levels were noted in the treatment groups (Figure 5A and 5B). No significant between-group differences were observed in adrenocorticotropic hormone or cortisol levels, except for a trend of higher cortisol level in the HSC group as compared with the control group (P=0.078; Figure 5C and 5D).

Cardiac and Brain Changes at 72 Hours Following ROSC

Compared with the sham group, at 72 hours following ROSC, the HE staining of LV in all groups showed increased fibroblast proliferation. Both LSC and HSC groups demonstrated improved histological outcomes as compared with the control group. Fibroblast proliferation and collagen fiber was less observed in the treatment groups than in the control group (Figure 6A through 6C). As to the cerebral injury, less neuron death was noted in the animals receiving post-arrest hydrocortisone than ones receiving saline as placebo (Figure 6A and 6D).

DISCUSSION

The current study demonstrated that hydrocortisone administration immediately after ROSC may mitigate post-arrest myocardial damage, ameliorate cardiac mitochondrial injury, and improve survival, neurological, and histological outcomes in a rat model of VF cardiac arrest. Besides, hydrocortisone decreased serum IL-6 and TNF-α level after I/R injury but did not affect serum ACTH and cortisol concentration.

As an extreme stressor, cardiac arrest causes devastating physiological changes. Compared with other stress states, cardiac arrest is associated with low cortisol levels during and after CPR.[3,21] Relative adrenal

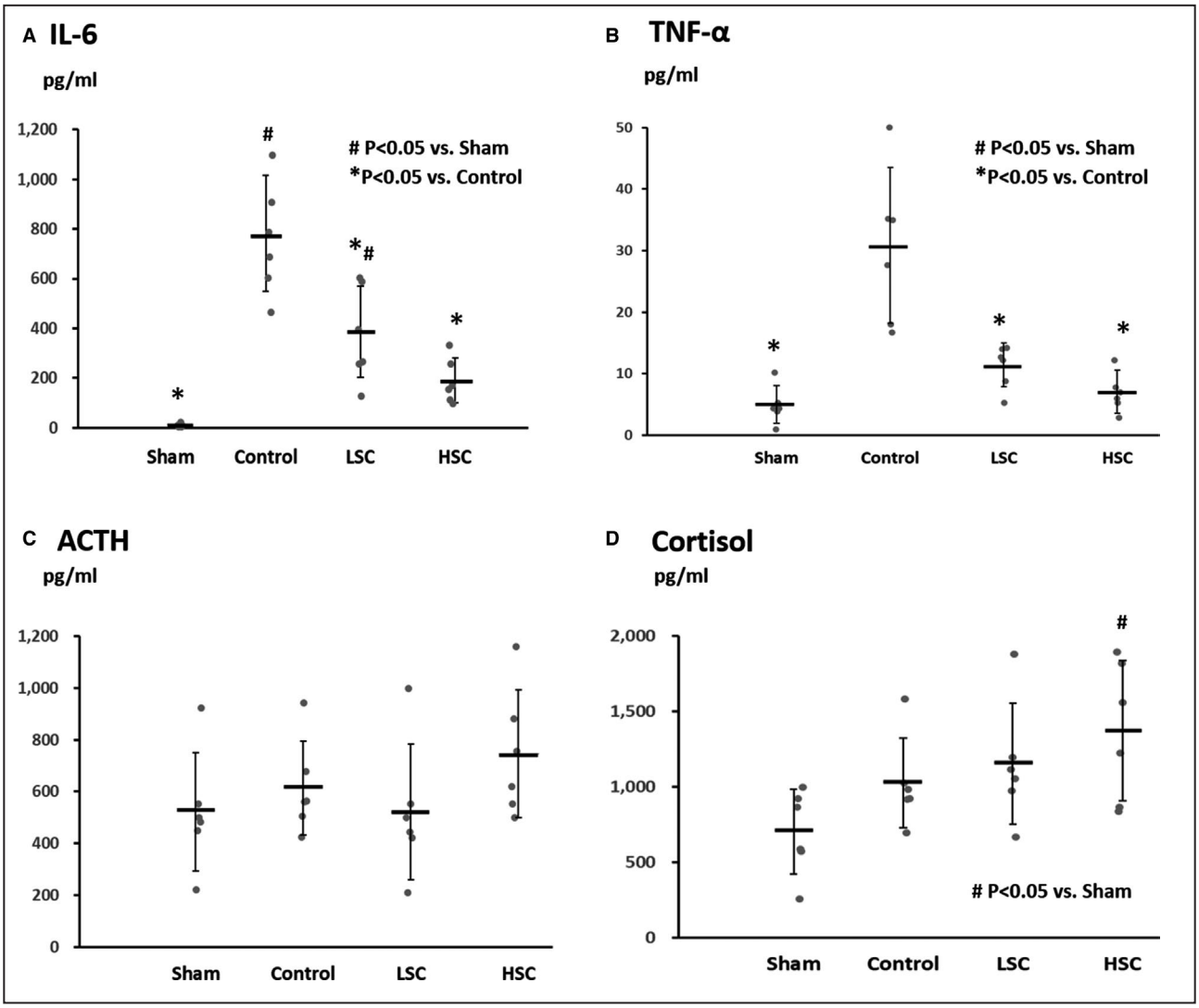

Figure 5. Cytokines, adrenocorticotropic hormone, and cortisol levels.
A and **B**, Low-dose hydrocortisone and high-dose hydrocortisone groups showed significantly reduced interleukin-6 and tumor necrosis factor-α levels compared with the control group (ANOVA with post-hoc correction; n=6). **C** and **D**, No significant differences in ACTH or cortisol levels between treatment groups and control group were noted (ANOVA with post-hoc correction; n=6). ACTH indicates adrenocorticotropic hormone; HSC, high-dose hydrocortisone; IL-6, interleukin-6; LSC, low-dose hydrocortisone; and TNF-α, tumor necrosis factor α. *P<0.05 compared with the control group, #P<0.05 compared with the sham group.

insufficiency was identified as a poor prognostic factor of shock-related mortality after cardiac arrest.[22] Several population-based studies[7,8] and clinical trials[5,6] have demonstrated the benefit of steroids on survival and neurological recovery in patients resuscitated from cardiac arrest, although some retrospective studies and one small clinical trial have not produced consistent findings.[23–25] Donnino et al showed no difference of shock reversal, survival to discharge, and neurological recovery between the cardiac arrest survivors receiving hydrocortisone and placebo.[26] A meta-analysis including 4 randomized controlled trials and 3 observational studies demonstrated that steroid use during CPR and after CA were significantly associated with an increased rate of ROSC and survival to discharge.[27] The significant improvement in both 72-hour survival and neurological outcomes in the treatment groups in the present study is consistent with the findings of these studies.

Post-resuscitation myocardial dysfunction has been reported, in both VF asphyxiaial cardiac arrest.[28–30] Two clinical trials have reported elevated systolic, diastolic, and mean blood pressure in patients receiving hydrocortisone for post-resuscitation shock. However, because the treatment groups also received vasopressin, epinephrine, and methylprednisolone during CPR, the individual effect of hydrocortisone is difficult to determine.[5,6] Hydrocortisone

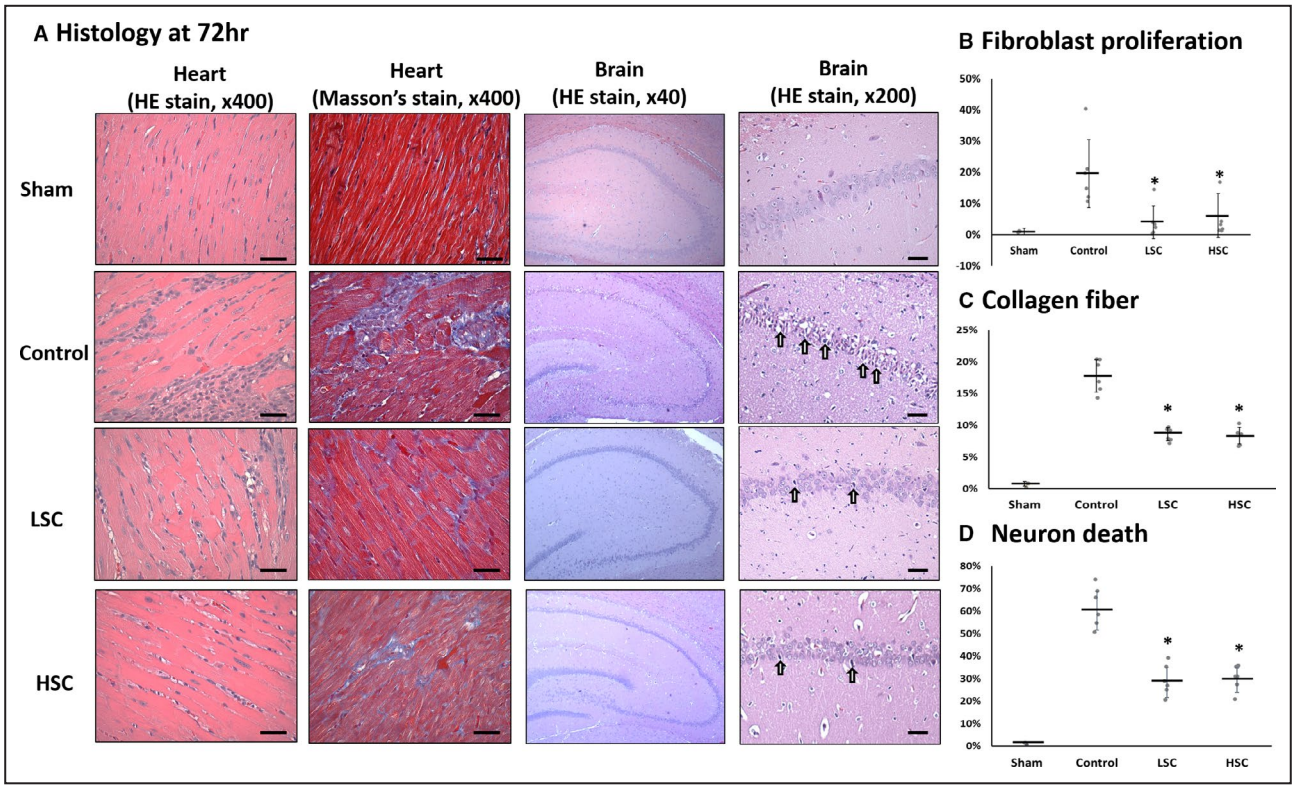

Figure 6. Histological examinations of heart and brain at 72 hours.
A through **C**, Histological examinations showed less fibroblast proliferation and collagen fibers in the treatment groups than in the control group (ANOVA with post-hoc correction; n=6 in the control and treatment groups, n=3 in the sham group). **A** and **D**, Less neuron death of hippocampus in the treatment groups as compared with the control group (ANOVA with post-hoc correction; n=6). HE indicates hematoxylin and eosin; and MT, Masson Trichrome. HE and MT stains of heart: ×400 magnification, scale bars=50 μm; HE stain of brain: ×200 magnification, scale bar=50 μm. *P<0.05 compared with the control group.

has been reported to ameliorate vasoplegic syndrome (vascular hyporesponsiveness to vasopressors)[31] and critical illness–related corticosteroid insufficiency in patients with septic shock.[32] In this study, although no significant differences in mean arterial pressure were observed, the animals treated with hydrocortisone showed improvement in cardiac output and systolic/diastolic function. The improved outcomes may be attributed from not only the alleviation of vasoplegia and critical illness–related corticosteroid insufficiency but also improved cardiac performance.

Steroids exert both positive and negative effects on the heart. The use of steroids after myocardial infarction may impair myocardial healing.[33] In an experimental myocardial infarction model of isolated perfused rat heart, dexamethasone aggravated cardiac damage.[34] Moreover, a preliminary study revealed an association between corticosteroid therapy and increased mortality in patients with shock following acute myocardial infarction.[35] However, steroids appear to be cardioprotective against I/R injury without definite vessel occlusion. In cardiac surgery, steroids strongly inhibit inflammatory responses and attenuate postreperfusion myocardial injury.[36,37] In epinephrine-induced myocardial ischemia, hydrocortisone pretreatment mitigated microscopic myocardial damage.[15] Similarly, in the present study, both microscopic and troponin I examinations revealed reduced myocardial injury in the animals that received hydrocortisone.

During cardiac arrest, cardiac mitochondria is progressively damaged and CPR is able to preserve their function and viability.[38] Upon full resumption of reperfusion, the cardiac mitochondrial damage aggravated by oxidative stress surges.[17,18,39] Our results revealed overt morphological damage to cardiac mitochondria after successful resuscitation. Adequate mitochondrial function is highly dependent on the complex ultrastructure of mitochondria, to which damage may play a vital role in the pathogenesis of cardiac mitochondrial dysfunction.[40] Therefore, in the present study, inner-membrane instability (mitochondrial permeability transition pore opening) and mitochondrial dysfunction (complex activity) following I/R injury may have resulted from ultrastructural change. Post-arrest hydrocortisone attenuated both cardiac mitochondrial injury and dysfunction.

The cardioprotective effects of steroids against I/R injury may also come from their modulation of

immunological and inflammatory responses. Post–cardiac arrest syndrome is characterized by high levels of circulating cytokines and adhesion molecules, presence of plasma endotoxin, and dysregulated leukocyte production of cytokines.[21] Cardiac arrest significantly increased the level of TNF-α in striatum and hippocampus,[41,42] IL-1α, IL-1β, IL-6, and TNF-α in heart,[43] and IL-6 and TNF-α in plasma.[43,44] Compared with healthy individuals, production of endotoxin-induced TNF-α, IL-6, and IL-10 were substantially increased in cardiac arrest survivors and associated with increased need of vasopressor support.[10,45,46] In patients with sepsis, TNF-α and IL-6 are the most frequently detected circulating cytokines[47]; along with several others. These cytokines are involved in sepsis-induced cardiac dysfunction[48,49] and are correlated with the prognosis and severity of sepsis.[50] Hydrocortisone reduces the levels of proinflammatory cytokines, including interleukin-1β, interferon-γ, TNF-α, and IL-6.[51] Our current study also showed reduced TNF-α and IL-6 levels in animals treated with hydrocortisone compared with those given a placebo. Recently, extracellular mitochondria and mitochondria-derived molecular patterns were recognized as potent inducers of inflammatory responses.[52] The relationship and interaction between cytokines and cardiac mitochondria under hydrocortisone treatment after cardiac arrest warrant further investigation.

Our study has several limitations. We chose 72 hours as the observation period for survival and neurological outcomes, which may be insufficient for observation of long-term outcomes. Second, we used male and healthy animals, whereas in clinical practice, VF cardiac arrest usually accompanies heart diseases. Because cardiac arrest in clinical practice is more complex than in the animal model we used, our results should be interpreted cautiously with regard to clinical application. Besides, the exclusion of female rats may also limit application of our study. Third, troponin, adrenal hormones and cytokines were not checked at 72 hours. Therefore, evolution or resolution of damage can only be known partly from the histological examinations of heart. Besides, the blood gas and lactate were not followed during the post-arrest period. Finally, hydrocortisone was administered as a single dose immediately after ROSC. Therefore, whether continuous infusion or repeated bolus injection of hydrocortisone would better improve outcomes requires further investigation.

CONCLUSIONS

Hydrocortisone use after cardiac arrest may mitigate myocardial injury and cardiac mitochondrial damage, and thus improve survival, neurological, and histological outcomes in a rat model of VF cardiac arrest.

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Author contributions: Tsai, Wang, Huang, Chang, and Chen contributed to the study concept and design; Tsai, Cheng, and Wu contributed to the acquisition of the data; Tsai, Wang, and Wu analyzed and interpreted the data; Tsai and Wu drafted the study; Huang, Chang, and Chen provided critical revision of the study for important intellectual content; Tsai, Wu, and Wang performed the statistical analysis; and Huang and Chen supervised the study.

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Disclosures

None.

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