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Original Contribution

The difference in myocardial injuries and mitochondrial damages between asphyxial and ventricular fibrillation cardiac arrests

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Abstract

Introduction: Ventricular fibrillation (VF) and asphyxia account for most cardiac arrests but differ in cardiac arrest course, neurologic deficit, and myocardial damage. In VF resuscitation, cardiac mitochondria were known to be damaged via excess generation of reactive oxygen species. This study evaluated the difference of cardiac mitochondrial damages between VF and asphyxial cardiac arrests.

Methods: In the VF + electrical shock (ES) group, VF was induced and untreated for 5 minutes, followed by 1 minute of cardiopulmonary resuscitation (CPR) and 1 ES of 5 J. Animals were killed immediately after ES. In the asphyxia group, cardiac arrest was induced by airway obstruction, and then pulselessness was maintained for 5 minutes, followed by 1 minute of CPR. The animals were killed immediately after CPR. The histology and ultrastructural changes of myocardium and complex activities and respiration of mitochondria were evaluated. The mitochondrial permeability transition pore opening was measured based on mitochondrial swelling rate.

Results: The histopathologic examinations showed myocardial necrosis and mitochondrial damage in both cardiac arrests. Instead of regional damages of myocardium in the VF + ES group, the myocardial injury in the asphyxia group distributed diffusely. The asphyxia group demonstrated more severe mitochondrial damage than the VF + ES group, which had a faster mitochondrial swelling rate, more decreased cytochrome *c* oxidase activity, and more impaired respiration.

Conclusions: Both VF and asphyxial cardiac arrests caused myocardial injuries and mitochondrial damages. Asphyxial cardiac arrest presented more diffuse myocardial injuries and more severe mitochondrial damages than VF cardiac arrest.

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1. Introduction

Cardiac and respiratory etiologies account for most cardiac arrests [1]. Those resulting from cardiac etiology usually are accompanied by fatal arrhythmias requiring electrical shock (ES) [2-4]. This contrasts with cardiac arrest resulting from respiratory etiology presenting with pulseless electrical activity (PEA) and asystole [5,6]. Patients with initial PEA or asystolic rhythm usually have a higher mortality and less chance to gain return of spontaneous circulation (ROSC) [7-9].

Two prevalent cardiac arrest models exist in animal studies: ventricular fibrillation (VF) and asphyxia, representing clinical cardiac arrest from respective cardiac and respiratory etiologies. Asphyxial cardiac arrest has been reported to have worse morphologic brain damage than VF cardiac arrest, although without functional difference [10]. In contrast with brain damage, Kamohara et al [11] showed less postcardiac arrest myocardial impairment and longer survival duration in the asphyxial cardiac arrest than the VF cardiac arrest. In the VF cardiac arrest, both VF and ES contribute to cardiac mitochondrial damages via excess generation of reactive oxygen species (ROS) [12]. The cardiac mitochondrial injuries in the asphyxial cardiac arrest remain unclear, although hypoxia before cardiac arrest and ischemia-reperfusion are known to participate in myocardial damages. We hypothesized that asphyxial cardiac arrest also causes cardiac mitochondrial damages but may have different patterns from those of VF cardiac arrest. In the present study, we investigated the difference of damaged myocardium and mitochondrial functions between asphyxial and VF cardiac arrests with same pulselessness and cardiopulmonary resuscitation (CPR) durations.

2. Methods

The study was designed to evaluate myocardial injuries and mitochondrial damages between VF and asphyxial cardiac arrests and was approved by the institutional review board of the National Taiwan University College of Medicine and Public Health. The investigation complied with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health.

Male Wistar rats weighing 400 ± 50 g were anesthetized with pentobarbital sodium (50 mg/kg body weight) intraperitoneal injection. The animals were prepared as previously described [13].

The trachea was orally intubated with a PE 200 catheter (Angiocath; Becton Dickinson, Franklin Lakes, NJ). Mechanical ventilation was initiated with a tidal volume of 0.65 mL/100 g animal weight, a frequency of 100/min, and a fraction of inspired oxygen of 1.0. Arterial blood pressure was measured with a saline-filled PE-50 tube (Angiocath, Becton Dickinson, Franklin Lakes, NJ) inserted through the right femoral artery. Two other saline-filled PE-50 tubes were inserted through the right carotid artery and jugular veins, respectively. The first was advanced into the left ventricle (LV), confirmed by echocardiography and LV pressure waveforms. The other was inserted for fluid and drug administration, in addition to the pressure recording. Hemodynamics including cardiac output, LV positive dp/dt (40), and maximal negative dp/dt were recorded with a PC-based data acquisition system (Powerlab; ADInstruments, Milford, MA). Body temperature was maintained between 36.5°C and 37.5°C during surgical preparation by an incandescent heating lamp.

2.1. Animal preparation for resuscitation and survival studies

The experimental animals were randomized into the VF + ES and the asphyxia groups. In the VF + ES group, VF was induced through a guide wire advanced from the right jugular vein into the right ventricle. A progressive increase in 60-Hz alternating current to a maximum of 1 mA was delivered to the right ventricular endocardium, and current flow continued for 2 minutes to prevent spontaneous defibrillation. The animals were subsequently left untreated for 3 minutes. Mechanical ventilation was stopped after VF onset. After 5 minutes of VF, 1 minute of CPR was applied, followed by 1 ES of 3 J. If ROSC could not be achieved postdefibrillation, then another 5-J shock was given after another 30 seconds of chest compressions. Resuscitation was declared a failure when ROSC could not be achieved after 4 shocks. In the asphyxia group, asphyxia was induced by endotracheal tube clamping. *Cardiac arrest* was defined as a mean arterial pressure less than 10 mm Hg with either asystolic or PEA rhythm, occurring approximately 4 minutes after asphyxia. Pulseness lasted for another 5 minutes, and then CPR was applied. Resuscitation was declared a failure when ROSC could not be achieved after CPR for 3 minutes (Fig. 1A). In both groups, precordial compression was delivered by a thumper at a rate of 200 beats/min. The animal was mechanically ventilated along with the start of precordial compression, and CPR was synchronized to provide a 2:1 compression/ventilation ratio with equal compression-relaxation durations. The depth of compressions was initially adjusted to secure a coronary perfusion pressure (CPP) of 23 ± 2 mm Hg. This typically yielded an end-tidal P_{CO_2} of 11 ± 2 mm Hg. The successfully resuscitated animals were closely monitored for another 4 hours, extubated, and then put back in their cages. Their survival status was recorded, and mortality was confirmed by a 2-minute loss of heart beat and spontaneous respiratory movements.

2.2. Animal preparation for malondialdehyde assay and histologic and mitochondrial experiments

The experimental animals were prepared as described above and randomized into 3 groups: the VF + ES group, asphyxia group, and sham group. In the VF + ES group, 1

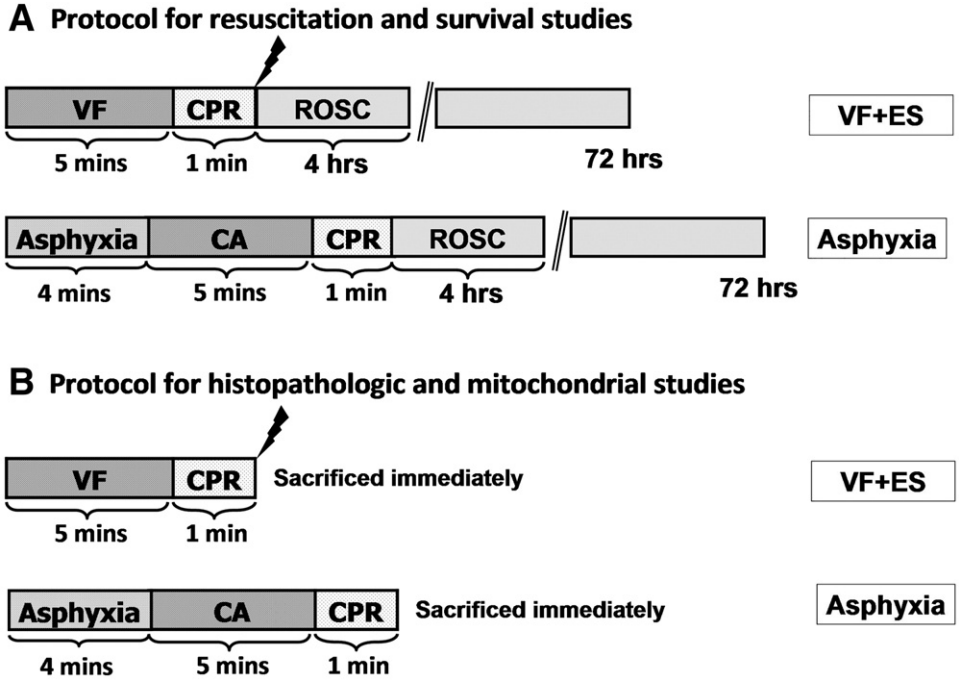

Fig. 1 Study designs. Resuscitation and survival studies (A) and histopathologic and mitochondrial studies (B). CA indicates cardiac arrest.

direct current ES of 5 J was applied after 5 minutes of VF and 1 minute of CPR. In the asphyxia group, cardiac arrest was induced by asphyxia and lasted for 5 minutes, and then CPR was applied for 1 minute. The sham group received same managements without cardiac arrest induction, precordial compression, and ES. Animals were killed immediately after the ES in the VF + ES group and precordial compression in the asphyxia group. The hearts were then removed for tissue malondialdehyde (MDA) assay, histopathology, and mitochondrial experiments (Fig. 1B). Except for the histopathologic evaluation, more than 6 animals were used in each group in every separate study.

2.3. Malondialdehyde assay

To evaluate myocardial oxidative stress, the MDA-586 method (*Oxis*Research, Portland, OR) was used. Malondialdehyde forms a conjugate with *N*-methyl-2-phenylindole, resulting in blue chromogenic signal production (586-nm absorbance). The MDA concentration was corrected for protein concentration, and the blank sample values were subtracted to correct for any sample turbidity variance [14].

2.4. Histology and transmission electron microscopy examinations

The separate LV myocardium parts (apex, septum, and lateral wall) were selected and embedded in paraffin, cut into sections, and stained with both hematoxylin and eosin (HE) and Gomori trichrome. The pathologic changes were observed under an optical microscope. Myocardial damage was classified as minimal (score, 1), mild (score, 2), moderate (score, 3), and severe (score, 4), respectively, based on the numbers of myocytolysis and transverse contraction bands under 10 random microscopic fields at ×200 magnification. Six specimens were counted in each animal. In electron microscopic examination, the LV was fixed in glutaraldehyde and cut into ultrathin sections. Thin LV sections (approximately 70 nm) were placed on uncoated 200-mesh copper grids, stained with 4% uranyl acetate and 0.2% lead citrate in 0.1 N NaOH, and examined in a Hitachi 7100 transmission electron microscope (Tokyo, Japan). The morphologic and histologic results were examined by an independent anatomist blinded to the grouping.

2.5. Isolation of mitochondria

Mitochondria were isolated from LV by differential centrifugation as described elsewhere [15]. The final crude mitochondrial pellet was resuspended in SHE (sucrose-histidine-EDTA) buffer (0.25 mol/L sucrose, 0.5 mmol/L EGTA (ethylene glycol tetraacetic acid), 3 mmol/L HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulforic acid) buffer, pH 7.2). 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 (mPTP) opening, complex activity, and respiration.

2.6. Measurement of mitochondrial permeability in transition pore opening

The mPTP opening caused mitochondrial swelling, resulting in a reduction of absorbance at 540 nm. Isolated

cardiac mitochondria were dissolved in swelling buffer (200 mmol/L mannitol, 10 mmol/L HEPES, 5 mmol/L succinate, 70 mmol/L sucrose), and mitochondria concentrations were adjusted to achieve 1 of OD at 540 nm. The addition of 400 μmol/L $CaCl_2$ induced pore opening. The decreased absorbance was measured by an enzyme-linked immunosorbent assay ELISA reader for 30 minutes [16].

2.7. Measurement of complex activities

NADH cytochrome *c* reductase (NCCR) (complex I/III) and succinate cytochrome *c* reductase (SCCR) (complex II/III) activities were assayed by monitoring reduction of oxidized cytochrome *c*. The cytochrome *c* oxidase (CCO) activity (complex IV) was assayed by measuring the oxidation of reduced cytochrome *c* and recording the absorbance change at 550 nm with a spectrophotometer [17-19].

2.8. Measurement of mitochondrial respiration

Adenosine diphosphate (ADP)–independent (state 4) and ADP-dependent (state 3) respiration were measured by using a Clark-type oxygen electrode (Strathkelvin Instruments, North Lanarkshire, Scotland, UK). Mitochondria were diluted to a 0.5-mg/mL protein concentration in assay buffer (0.125 mol/L sucrose/150 mmol/L KCl/6 mmol/L $MgCl_2$/5 mmol/L HEPES/10 mmol/L $NaKPO_4$/20 μmol/L cytochrome *c*, pH 7.25). State 2 respiration was initiated by glutamate addition (15 mmol/L). After 2 minutes, state 3 respiration was initiated by ADP addition (0.5 mmol/L). Upon depletion, state 4 respiration was monitored. The substrate concentrations and assay buffers used were optimized to yield maximal respiration rates [20-22]. A high state 3 respiration indicates an intact respiratory chain and adenosine triphosphate (ATP) synthesis, and a low state 4 respiration indicates an intact mitochondrial inner membrane. The respiratory control ratio (RCR) is the ratio of state 3 to state 4 respiration, and a dramatically decreased RCR is diagnostic of extensive mitochondrial damage. The ADP/O ratio is the ratio between ATP synthesis and atomic oxygen consumption during state 3 respiration. The higher ADP/O ratio indicates better mitochondrial respiration.

Table 1 Baseline characteristics and resuscitation outcome

	VF + ES (n = 10)	Asphyxia (n = 10)
Baseline characteristics		
Weight (g)	368.5 ± 19.2	380.0.5 ± 32.4
Body temperature (°C)	36.92 ± 0.38	37.02 ± 0.43
Heart rate (beats/min)	452.7 ± 36.8	434.4 ± 35.6
Systolic blood pressure (mm Hg)	118.8 ± 12.6	118.8 ± 12.6
d*p*/d*t* (40) (×1000 mm Hg/s)	9.50 ± 1.58	9.46 ± 1.12
−d*p*/d*t* max (×1000 mm Hg/s)	−8.35 ± 1.78	−8.83 ± 1.80
Cardiac output (mL/min)	166.01 ± 33.29	149.32 ± 21.93
pH	7.42 ± 0.03	7.39 ± 0.04
Po_2 (mm Hg)	102.69 ± 19.22	104.21 ± 15.72
Hco_3 (meq/L)	13.23 ± 1.78	12.97 ± 1.57
Lactate (mg/kg)	0.79 ± 0.43	0.81 ± 0.51
Resuscitation events		
ROSC, n (%)	2 (20)	0 (0)
CPP, 0 min (mm Hg)	28.7 ± 8.9	24.2 ± 10.3
CPP, 1 min (mm Hg)	14.2 ± 3.6	16.2 ± 4.6

2.9. Data analysis

Binomial variables were analyzed with a χ^2 test. Continuous values were shown as mean ± SD. For continuous variables, group differences were evaluated with an analysis of variance test. Repeated general linear model measurements were applied for mitochondrial swelling rate comparisons. *P* values of less than .05 were considered statistically significant, and statistics were performed using SPSS 12.0 software (SPSS, Inc, Chicago, IL).

3. Results

There was no significant difference in baseline hemodynamics and blood gas between the VF + ES and asphyxia groups before inducing cardiac arrest. During cardiac arrest, the CPP immediately after the start and at the first minute of

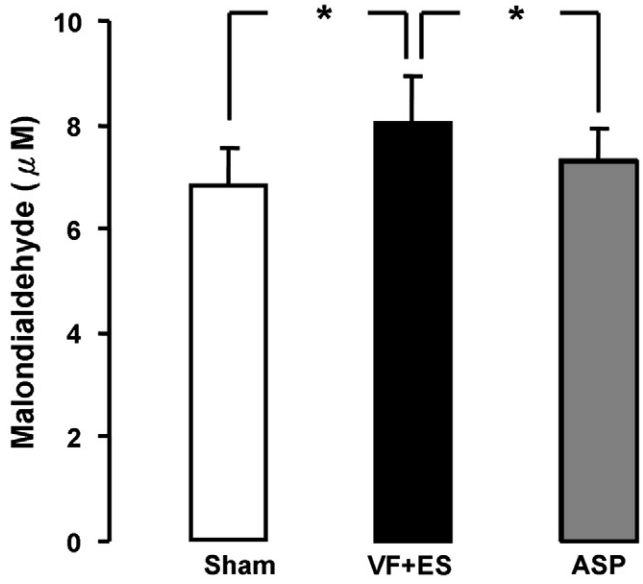

Fig. 2 The results of MDA assay. Compared with the sham and asphyxia groups, the MDA concentration significantly increased in the myocardium of the VF + ES group. There was no significant difference in MDA concentration between the sham and asphyxia groups. ASP indicates asphyxia. *$P < .05$.

CPR also showed no difference between the VF + ES and asphyxia groups. Compared with only 1 in the asphyxia group that achieved ROSC, 4 animals in the VF + ES group were successfully resuscitated ($P = .4$) (Table 1).

Compared with the sham and asphyxia groups, the MDA concentration significantly increased in the myocardium of the VF + ES group (VF + ES: 8.1 ± 0.9 μmol/L, asphyxia: 7.3 ± 0.6 μmol/L, sham: 6.8 ± 0.8 μmol/L). There was no significant difference in MDA concentration between the sham and asphyxia groups (Fig. 2). The results indicated less myocardial ROS generation and lipid oxidation in the asphyxia group than that in the VF + ES group.

Nine animals (n = 3 in each group) underwent myocardial sampling for histopathology studies. The HE-stained LV sections in both the VF + ES and the asphyxia groups showed myocytolysis and transverse contraction bands. Waving was also observed in the VF + ES group. Compared with the VF + ES group, the asphyxia group had less severe myocytolysis but more diffusely distributed contraction band (Fig. 3A-C). The overall myocardial damage score between

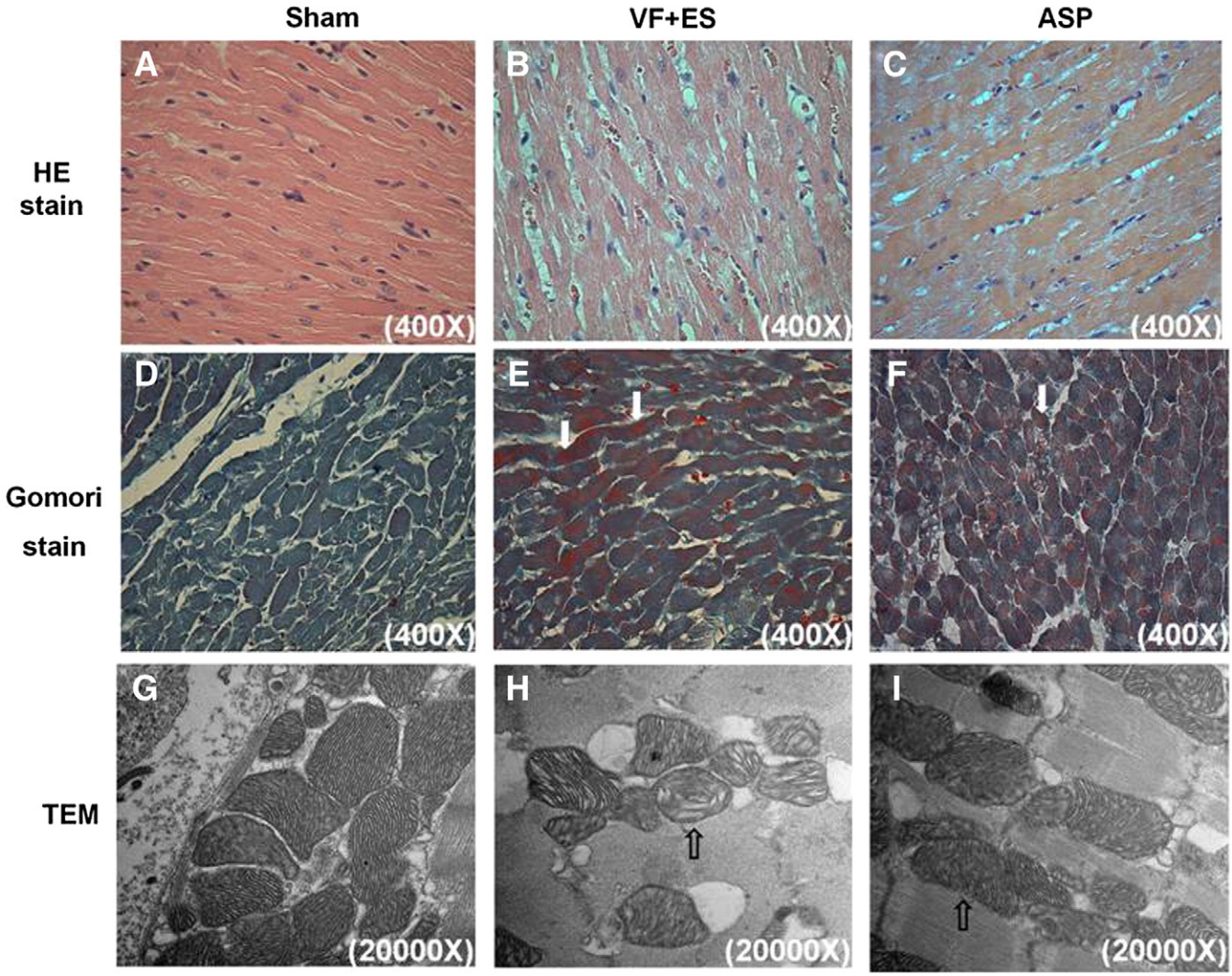

Fig. 3 Histologic results. A-C, HE staining disclosed myocytolysis and in the myocardium of the VF + ES and asphyxia groups. In the VF + ES group, waving was also observed. The distribution of contraction bands in the asphyxia group is more diffuse than that in the VF + ES group. D-F, Gomori staining of the VF + ES and asphyxia groups showed a ragged thick and irregular red subsarcolemmal layer and intermyofibrillar red deposits suggesting the aggregation of abnormal mitochondria (arrow). Rather than the mitochondrial aggregation distributed in some area of myocardium in the VF + ES group, the mitochondrial aggregation in the asphyxia group showed diffuse distribution in the myocardium. G-I, Electron microscopic analysis (×20 000) of the LV in the VF + ES and asphyxia groups showed mitochondrial damage characterized by mitochondrial swelling and edema, outer-membrane rupture, and loss of inner-membrane cristae with amorphous densities (hollow arrow). The mitochondrial damage in the VF + ES group was limited in some area of myocardium; in contrast, the asphyxia group showed generalized mitochondrial damage in the myocardium. Some unclear mitochondrial border in the sham group comes from the mosaic of the cutting edge. ASP indicates asphyxia; TEM, transmission electron microscopy.

the VF + ES and the asphyxia groups did not show significant difference (2.8 ± 0.7 vs 2.8 ± 0.4; *P*, nonsignificant). The VF + ES and asphyxia groups showed ragged thick and irregular red subsarcolemmal layers and intermyofibrillar red deposits with Gomori stain, suggesting abnormal mitochondrial aggregation. In contrast to regional mitochondrial abnormality in the VF + ES group, the asphyxia group showed diffuse distribution (Fig. 3D-F). Ultrastructural analysis of LV in the VF + ES and asphyxia groups showed mitochondrial damage characterized by mitochondrial swelling and edema, outer-membrane ruptures, and loss of inner-membrane cristae with amorphous densities. The mitochondrial damage in the VF + ES group was limited in some myocardial areas; in contrast, the asphyxia group showed generalized mitochondrial damage (Fig. 3G-I).

After adding $CaCl_2$, both the VF + ES and the asphyxia groups had significantly accelerated mitochondrial swelling rates (a measure of mPTP opening). The mitochondrial swelling in the asphyxia group was faster than that in the VF + ES group (Fig. 4A).

The VF + ES group decreased the NCCR and CCO but not SCCR activities. However, in the asphyxia group, only the CCO activity decreased significantly. Cytochrome *c* oxidase activity decreased more in the asphyxia group than in the VF + ES group (0.28 ± 0.07 vs 0.33 ± 0.06 U/mg, $P < .05$). These results suggest that complexes I and IV are affected in VF cardiac arrest, and complex IV is affected in asphyxial cardiac arrest (Fig. 4B-D).

The VF + ES and asphyxia groups had a significantly decreased state 3 respiration. The state 3 respiration of the asphyxia group decreased more significantly than the VF + ES group (134.3 ± 34.5 vs 174.8 ± 54.7 nmol of O/mg mitochondrial protein, $P < .05$) (Fig. 5A). No significant difference was noted in the state 4 respiration between each group (Fig. 5B). The mitochondria from the VF + ES group showed a decreased RCR trend. The RCR of the asphyxia group was significantly lower than the VF + ES and sham groups (VF + ES: 3.5 ± 0.9, asphyxia: 2.9 ± 0.5, sham: 4.0 ± 0.7) (Fig. 5C). Both the VF + ES and the asphyxia groups had significantly decreased ADP/O ratios. The ADP/O ratio was lower in the asphyxia group than in the VF + ES group (1.5 ± 0.2 vs 1.9 ± 0.5, $P < .05$) (Fig. 5D).

4. Discussion

In the present study, we demonstrated dominant myocardial injury and mitochondrial damage in both VF and asphyxial cardiac arrests. Compared with the VF cardiac arrest, the asphyxial cardiac

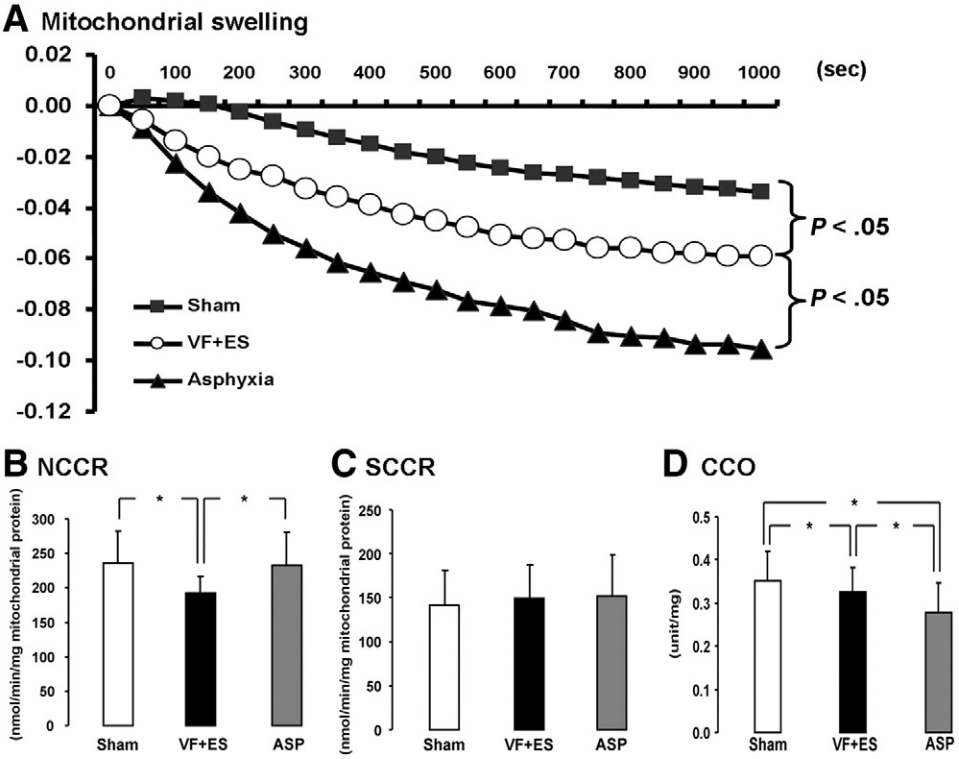

Fig. 4 The results of mPTP opening and complex activity studies. A, After adding $CaCl_2$, both the VF + ES and the asphyxia groups had significantly accelerated rates of Ca^{2+}-induced mitochondrial swelling, a measure of mPTP opening, compared with sham-operated rats. The mitochondrial swelling of the asphyxia group was faster than that of the VF + ES group. B-D, The VF + ES group decreased the activities of NCCR and CCO but not SCCR when compared with sham. However, in the asphyxia group, only the CCO activity significantly decreased when compared with the VF + ES and sham groups. There was no significant difference in NCCR between the asphyxia and the sham groups. ASP indicates asphyxia. *$P < .05$.

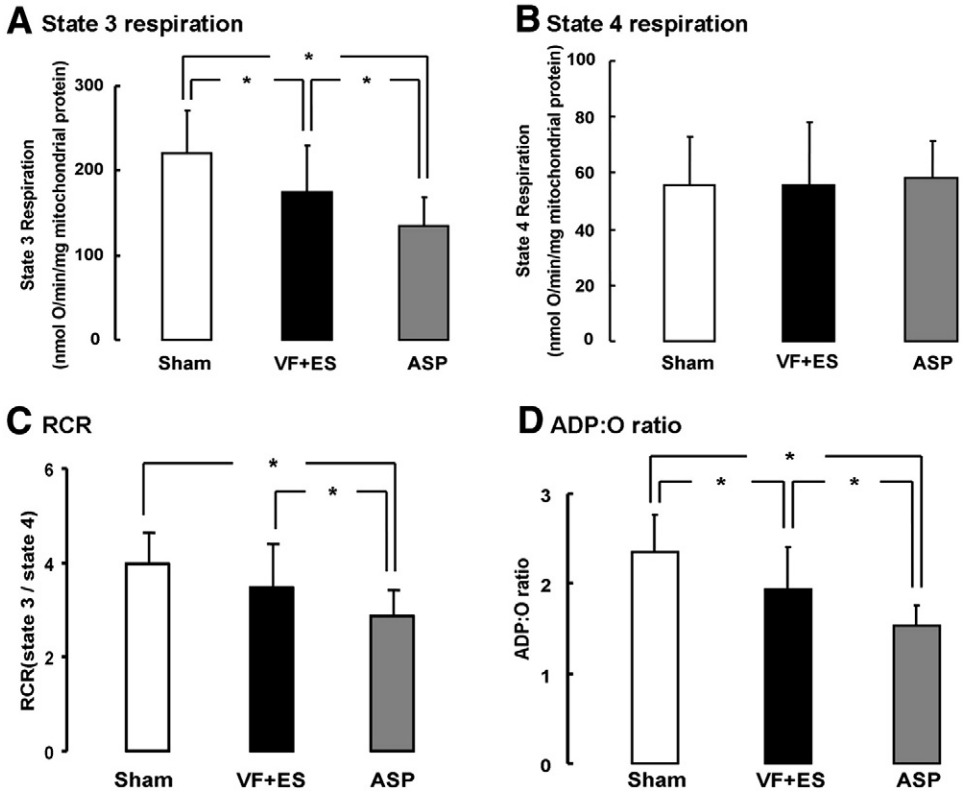

Fig. 5 Mitochondrial respiration. A, The state 3 respiration was significantly lower in the VF + ES and asphyxia groups than in the sham group. The state 3 respiration of the asphyxia group decreased more significantly than that of the VF + ES group. B, No significant difference in the state 4 rate between each group was noted. C, The mitochondria from the VF + ES group showed a trend of decreased RCR when compared with the sham group. The RCR of the asphyxia group was significantly lower than the VF + ES and sham groups. D, When compared with the sham group, the ADP/O ratios of the VF + ES and asphyxia groups were significantly decreased. Moreover, the ADP/O ratio was lower in the asphyxia group than in the VF + ES group. ASP indicates asphyxia. *$P < .05$.

arrest caused more diffuse myocardial injury and mitochondrial damage and, thus, less successful resuscitation.

Ischemia-reperfusion injury during cardiac arrest and CPR has been known to cause oxidative stress and contribute to postcardiac arrest syndrome, including both neurologic and myocardial dysfunctions [23]. In the current study, the MDA concentration significantly increased in the myocardium of the VF + ES group. In addition to ROS generated by mitochondrial respiration during ischemia-reperfusion injury [24], cytosol electrolysis during ES is considered to be another ROS source because electrolysis of a physiologic buffer produces a milieu containing several free radicals [25]. However, the asphyxial cardiac arrest did not cause significant MDA generation in the current study. Low tissue O_2 availability during hypoxic period, short reperfusion duration, severe mitochondrial damage, and impaired mitochondrial respiration may account for less ROS formation in the asphyxia group.

In contrast to the myocardial lipid oxidation, there were more mitochondrial damages and dysfunction (accelerated mitochondrial swelling, impaired complex activities, and respiration) in the asphyxial cardiac arrest than in the VF cardiac arrest. Our previous study demonstrated that myocardial lipid oxidation and mitochondrial damages in VF cardiac arrest were ameliorated by administrating the antioxidant during CPR, suggesting that ROS mediates mitochondrial damages in VF cardiac arrest [12]. However, the mitochondrial damages in the asphyxial cardiac arrest may result from not only ROS generation but also hypoxemia, hypercarbia, and hypotension with incomplete ischemia for several minutes preceding the onset of pulselessness in the asphyxial cardiac arrest. In anoxia, mitochondria change from being ATP producers to potentially powerful ATP consumers, which hydrolyze glycolytically produced ATP to maintain mitochondrial proton motive forces [26]. If ATP is depleted during anoxia, necrosis occurs because of the mitochondria transmembrane potential (Ψ), followed by cell swelling and loss of plasma and mitochondrial membrane integrity [27,28].

The loss of mitochondria transmembrane potential (Ψ) and disruption of mitochondrial inner membrane result in the release of cytochrome *c* and proapoptotic factors and impaired mitochondria function, including oxidative phosphorylation and energy production, ultimately leading to apoptosis and necrosis [29-31]. Our current study showed myocardial injuries and mitochondrial damages in both cardiac arrests. Instead of regional myocardial injuries in VF cardiac arrest, the myocardial injury in asphyxial

cardiac arrest distributed diffusely. These results correspond to the finding in the canine study of Lerman et al [32], who reported that localized myocardial injury was noted after direct current ES with endocardial catheters, implying only tissue participating in the circuit would be damaged. Moreover, the less calcium-induced mitochondrial swelling, decreased complex activities, and respiratory dysfunction in the VF + ES group than in the asphyxia group implied less mitochondrial damage and better energy preservation during electron transport chain. The findings are compatible with more animals that were successfully resuscitated in the VF + ES group than in the asphyxia group.

5. Limitations

There are several study limitations. First, it is difficult to quantify and equalize the damages caused by VF and asphyxial cardiac arrests, respectively. The design of the current study is to evaluate the difference in histopathologic changes and mitochondrial damages between these 2 models with the same pulseless duration. Second, MDA is a byproduct produced when free radicals oxidize lipid and recognized as a standard measurement for determining the degree of cell oxidation. However, MDA cannot directly reflect the intracellular ROS concentration. Lastly, we used healthy animals as materials. However, in clinical practice, VF cardiac arrest usually accompanies coronary artery diseases, and asphyxial cardiac arrest usually accompanies infection, intoxication, and trauma. These clinical conditions are more complex than the animal models. Moreover, the clinical VF cardiac arrest mostly resulted from occlusion of coronary artery rather than electrical current in our present study without coronary artery lesion. Therefore, these results should be more carefully interpreted when applied into clinical practice.

6. Conclusion

We conclude, in the current study, that both VF and asphyxial cardiac arrests cause myocardial injuries and mitochondrial damages. With the same pulseless duration, there are more diffuse myocardial injuries, more severe mitochondrial damages, and, thus, less successful resuscitation in asphyxial cardiac arrest than VF cardiac arrest. Based on these findings, different cardiac arrest studies may have different investigated pathways and develop different research directions in the future.

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