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# The differences in brain damage between asphyxial and ventricular fibrillation cardiac arrests

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## Abstract

*Objective:* Asphyxia and ventricular fibrillation are the two most prevalent causes of cardiac arrest. The study investigated the differences in brain damage after cardiac arrest between asphyxial and ventricular fibrillation cardiac arrests in rats. *Methods:* Male healthy Sprague-Dawley rats were randomly assigned to the asphyxial group (cardiac arrest of 6 min, n=15), ventricular fibrillation group (cardiac arrest of 6 min, n=15) and sham group (n=5). Neurologic deficit scores and tape removal test were evaluated at 1, 3 and 7 days after cardiopulmonary resuscitation from three groups. Serum S-100B and brain histopathologic damage scores were also examined. *Results:* There were no differences in neurologic performance at 1, 3 and 7 days after cardiopulmonary resuscitation between the asphyxial group and ventricular fibrillation group ($P>0.05$, respectively). Serum S-100B level was higher in the asphyxial group at 1, 3 and 7 days, compared with the ventricular fibrillation group ($P<0.05$, respectively). There were significantly higher histopathologic damage scores at 1, 3 and 7 days in the asphyxial group compared with the ventricular fibrillation group ($P<0.05$, respectively).
*Conclusion:* Asphyxial cardiac arrest has worse morphologic brain damage compared with ventricular fibrillation cardiac arrest, but the functional brain damage caused by asphyxial cardiac arrest is similar to that caused by ventricular fibrillation cardiac arrest.

## INTRODUCTION

Asphyxia and ventricular fibrillation (VF) are the two most prevalent causes of cardiac arrest (CA).[1] VF is the leading cause of CA in adult with ischemic heart disease.[2] Asphyxia is the common cause of CA after intoxication, drowning or trauma.[3] The rate of survival from CA remains dismally low, which closely correlates with brain damage. Brain damage is mainly caused by ischemia/reperfusion injury after cardiopulmonary resuscitation (CPR) from CA. Histopathology in brain after CA is primarily marked by ischemic neuronal changes scattered throughout most brain regions.[4]

In the two CA models of rats, Tsai *et al.*[5] demonstrated that asphyxial cardiac arrest (ACA) resulted in more diffuse myocardial injuries and more severe mitochondrial damages than ventricular fibrillation cardiac arrest (VFCA). In contrast with myocardial damage, 96 h after CA, ACA of 7 min in dogs caused worse morphologic brain damage than VFCA of 10 min, but the functional brain damage caused by ACA was similar to that caused by VFCA.[4] But brain damage at late time point between the two CA models remains unclear. In the present study, we investigated the differences of brain damage up to 7 days after return of spontaneous circulation (ROSC) between ACA and VFCA in rats with the same total insult time.

## METHODS

### *Animal preparation*

This study was approved by the Sun Yat-Sen University Institutional Animal Ethic Committee. A total of 35 male healthy Sprague-Dawley (SD) rats weighing 300-400g were obtained from Sun Yat-sen University. After an overnight fast except for free access to water, the animals were anesthetized by intraperitoneal injection of 45 mg $kg^{-1}$ pentobarbital sodium. Additional doses of 10 mg $kg^{-1}$ were administered at intervals of approximately 1 h if necessary. The tracheal

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was orally intubated with a 14 gauge cannula (Abbocath-T, North Chicago).

A gauge 4F polyethylene (PE) catheter (C-PMS-401J, Cook Critical Care; Bloomington, Indiana) was advanced through the right external jugular vein into the right atrium. A precurved guide wire was then advanced through this catheter into the right ventricle for electrically inducing VF. Through the left femoral artery, a 23 gauge PE-50 catheter (Abbocath-T, North Chicago) was advanced into the thoracic aorta for measurement of mean aortic pressure (MAP). Another 23 gauge PE-50 catheter was also advanced through the left femoral vein into the inferior vena cava for fluid and drug administration. Aortic pressure was measured with a pressure transducer (BD, Germany). Prior to insertion, the catheters were filled with physiological salt solution containing 5 IU/ml of heparin. Electrocardiogram lead II was recorded. Rectal temperature was maintained at 36.5±0.5°C with an incandescent heating lamp. The animals were mechanically ventilated with a fraction of inspired oxygen ($FiO_2$) of 21% at a tidal volume of 0.65 ml/100g animal weight and a frequency of 100 breaths/min. Peak partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was measured with a side stream infrared $CO_2$ analyzer (CAPSTAR-100, CWE Inc, USA). Hemodynamic data were recorded in a six channel recorder (Windaq acquisition system, USA).

*Experimental procedure*

The animals were randomly assigned to the asphyxial group (n=15), VF group (n=15), and sham group (n=5). In the VF group, VF was induced with a 2-5 mA alternating current (60 Hz) delivered to the right ventricular endocardium. The current flow was continued for 3 min to prevent spontaneous reversal of VF to a supraventricular rhythm. Mechanical ventilation was discontinued after onset of VF. Six minutes after onset of VF, precordial compression was initiated with an electrically driven mechanical chest compressor and mechanical ventilation with a $FiO_2$ of 100% was resumed. Compression rate was maintained at a rate of 200 $min^{-1}$ and synchronized with a compression/ventilation ratio of 2:1 with equal compression-relaxation duration. The depth of compression was adjusted to maintain a 1/3 decrease in the anterior-posterior chest diameter.[6] Defibrillation was delivered with a 2J direct current at 6 min after compression for a maximum of three shocks. If ROSC was not observed, compression was resumed and maintained for 30 sec before delivering a second set of up to three shocks. In the asphyxial group, the prearrest and CPR protocols were similar to the VFCA. However, instead of inducing VF, asphyxia was induced by intravenous injection of 1 mg $kg^{-1}$ vecuronium.[7] CA was determined by loss of aortic pulsation, defined as MAP equal or less than 20 mmHg, which occurred approximately 4 min after asphyxia. Compression and mechanical ventilation were initiated at 6 min after onset of CA. ROSC was defined as return of a supraventricular rhythm with a MAP ≥60 mmHg lasting for ≥5 min. Mechanical ventilation was continued for two additional hours after ROSC. $FiO_2$ was 100% at 0-0.5 h after ROSC, 50% at 0.5-1 h and 30% at 1-2 h. Sham-operated rats underwent the same operation, however, CA was not induced.

When upper airway reflexes were active, the animals were extubated and put back in their cages. Postresuscitation neurologic functions were measured at 1, 3 and 7 days after CA by an observer blinded to experimental condition. Then the animals were reanaesthetised and euthanized. Intravenous blood of 10 ml was collected from the inferior vena cava for assays of S-100B and the brains were harvested and submitted for histopathologic evaluation. There were 5 animals for every separate time point in each group.

*Neurologic functional measurement*

*Neurologic deficit scores (NDS).* The NDS as previously described[8] were evaluated. NDS quantitate neurologic deficit on a scale of 0-80. NDS are based on a composite of arousal, reflex, motor, sensory and balance responses with 0 corresponding to brain death and 80 to no deficit.

*Tape removal test (TRT).* TRT reported by Albertsmeier *et al.*[9] evaluated sensorimotor integration. The animals were trained on 5 times per day for 3 consecutive days before CA. The technique included application of two small pieces of 1 cm by 1.2 cm adhesive tapes to both forepaws in random order. The time from attachment of adhesive tapes to the front paws until the animals completely removed them using their teeth was recorded on each of three trials lasting up to 180 sec. After training, most unimpaired animals tore off adhesive tapes within less than 20 sec.

*Serum S-100B concentrations*

Serum S-100B concentrations were determined from rats at 1, 3 and 7 days after CA using the rat S-100B ELISA kit (CUSABIO, USA) according to the directions of the manufacturer.

**Table 1: Baseline characteristics of study subjects**

| Variables | Sham (n=5) | ACA (n=15) | VFCA (n=15) |
|---|---|---|---|
| Body weight (g) | 349.5±34.5 | 338.5±32.1 | 365.8±29.1 |
| Heart rate (beats/min) | 337±31.2 | 335±17.6 | 395±29.1 |
| Breath rate (beats/min) | 80±8.2 | 85±7.1 | 90±9.5 |
| Systolic blood pressure (mmHg) | 157±12.7 | 155±11.3 | 144±13.9 |
| Diastolic blood pressure (mmHg) | 118±10.6 | 119±8.4 | 110±12.5 |
| $P_{ET}CO_2$ (mmHg) | 35.6±2.9 | 36.8±2.1 | 36.1±1.2 |

Values as means ± standard deviation for each group. ACA, asphyxial cardiac arrest; VFCA, ventricular fibrillation cardiac arrest; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide.

*Histopathologic evaluation*

The brains were removed, post-fixed in 4% paraformaldehyde and cut into 5 to 7 coronal sections. Each coronal section was embedded in paraffin, cut into 5 μm coronal sections and then stained with hematoxylin and eosin. Each coronal section for each animal was examined by an observer blinded to experimental condition according to the histopathologic damage scoring system established previously.[10] In brief, nine brain regions were examined separately by light microscopy for edema, ischemic neuronal changes and microinfarcts. Viable neurons were defined by a defined cellular outline with round and vesicular nuclei. The severity score of lesions was assessed on a four-point scale (minimal = 1 +, moderate = 2 +, severe = 3 + and maximal = 4 +). The severity score was then multiplied by a weighing factor according to the type of damage (edema × 1, ischemic neuronal changes × 2 and microinfarcts × 4). Total scores were the sum of scores of 9 bilateral regions.

*Statistical analysis*

SPSS 13.0 software was used for statistical analysis. Continuous variables are presented as means ± standard deviation. Comparisons between two groups were made by unpaired *Student's t* test. Multiple comparisons were made by one-way ANOVA, followed by Bonferronni' *post hoc* test. Data between two groups of each measurement time were compared by analysis of variance for repeated measures. *P* values of less than 0.05 were considered significant.

## RESULTS

There were no significant differences in baseline hemodynamic variables among three groups before inducing CA (Table 1). Mean CPR time required for ROSC was significantly longer in the VF group, compared with that in the asphyxial group (7′42″±1′13″ vs. 2′53″±14″, $P<0.05$). There was no significant difference in total insult time between two groups (Table 2).

All unimpaired animals had normal NDS at all time points (NDS 80). One day after CA, NDS were lowest and then increased with time. But differences between two groups were not significant at 1, 3 and 7 days after CA (Figure 1). Before CA, all animals tore off the tapes rapidly. After ROSC, all animals had an obvious sensorimotor deficit on all testing days until 7 days. Throughout the observation period, the sensorimotor deficits were increasingly improved.

**Table 2 Resuscitation after asphyxial or ventricular fibrillation cardiac arrest**

| Group | Asphyxia time to arrest (min, sec) | Arrest time (min) | CPR time to ROSC (min, sec) | Total insult time (min, sec) |
|---|---|---|---|---|
| ACA (n=15) | 4'39" ±26" | 6' | 2'53" ±14" | 13'32" ±1'14" |
| VFCA (n=15) | - | 6' | 7'42" ±1'13"* | 13'42"± 2'14" |

Values as means ± standard deviation for each group. ACA, asphyxial cardiac arrest; VFCA, ventricular fibrillation cardiac arrest; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; *$P<0.05$ vs. ACA.

But time needed for tape removal in the asphyxial group was similar to that in the VF group at 1, 3 and 7 days after CA (Figure 2).

Serum S-100B levels at 1, 3 and 7 days in the asphyxial group were significantly higher than those in the VF group (Figure 3). Histopathologic evaluation revealed that the most obvious abnormalities for ACA and VFCA were cortical and hippocampal ischemic neuronal changes, which were defined by shrunken eosinophilic neurons. Edema and microinfarcts were not seen in brains (Figure 5). There were significantly higher histopathologic damage scores at 1, 3 and 7 days in the asphyxial group compared with those in the VF group (Figure 4).

## DISCUSSION

In the present study, our results demonstrated ACA of 6 min in rats caused worse morphologic brain damage, compared with VFCA of 6 min, although the functional brain damage caused by ACA was similar to that caused by VFCA. The inconsistence may be elucidated by the fact that NDS assess brain damage from the functional perspective, however, histopathology directly assesses brain damage at the cellular structure. The correlation between NDS and histopathology has been evaluated in a study[4] in which NDS do not correlate with histopathology after ACA, as NDS do after VFCA. Small lesions in functionally important areas may cause worse functional brain damage marked by lower NDS. Contrarily, large lesions in functionally less important areas may result in less functional brain damage marked by higher NDS. The evidence may at least in part explain our inconsistent results.

The obvious differences between ACA and VFCA are that asphyxia causes hypoxemia, hypercarbia and hypotension with incomplete ischemia for several minutes preceding the onset of pulselessness, on the contrary, oxygenation and blood pressure are usually normal before inducing VF. During the complete ischemia, asphyxia further exacerbates brain acidosis, which results in irreversible neuronal injuries.[11] Therefore, ACA causes more brain acidosis and edema, which may contribute to worse ischemic-anoxic damage compared with that of VF of the same duration. Once cerebral acidosis is mildly attenuated or neutralized, neuronal cell death in the hippocampus is decreased.[7] Brain histopathology and serum S-100B can reflect these pathophysiologic changes. After CA, the whole body ischemia occurs. So whole brain histopathologic damage scores were used to evaluate brain damage. A total of 9 regions including frontal cortex, parieto-occipital cortex, temporal cortex, hippocampus, basal ganglia, thalamus, midbrain, cerebellum and caudate putamen were examined.[10] Brain histopathology showed that the asphyxial group had higher histopathologic damage scores suggesting ACA caused worse morphologic brain damage. Serum S-100B is a specific marker for central nervous injuries. Serum S-100B has been shown to be primarily synthesized and secreted by neuroglial

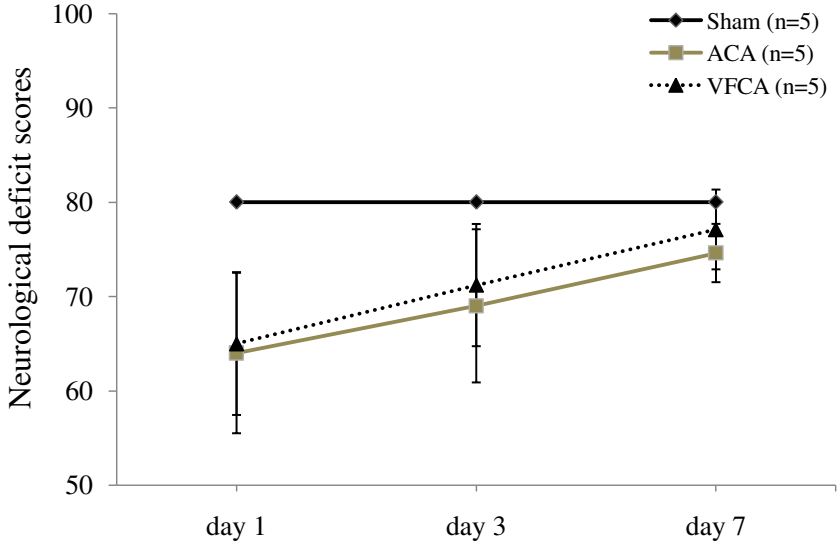


Figure 1. Neurological deficit scores. ACA, asphyxial cardiac arrest; VFCA, ventricular fibrillation cardiac arrest

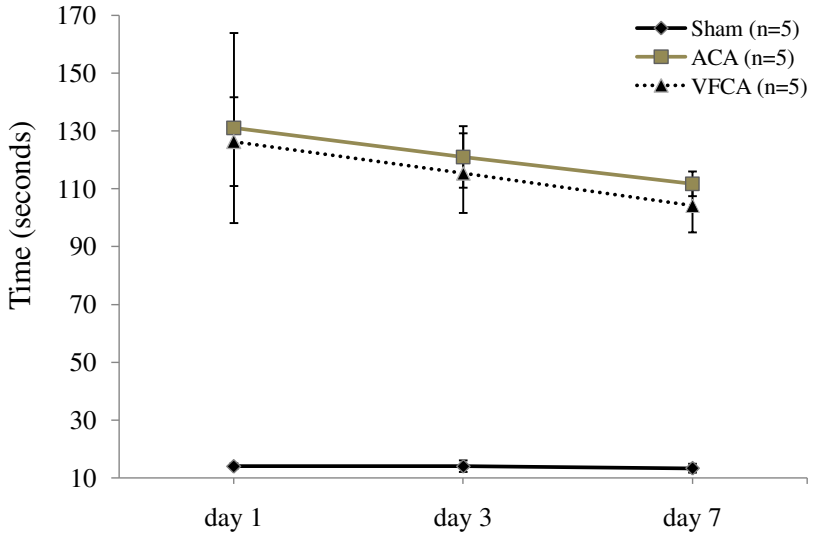


Figure 2. Tape removal test. ACA, asphyxial cardiac arrest; VFCA, ventricular fibrillation cardiac arrest

cells, especially astrocytes and oligodendrocytes. Serum S-100B also can evaluate the extent of brain injuries and prognosis of brain injuries.[12] Asphyxia caused higher S-100B, which indicated that ACA caused worse brain damage compared with VFCA. The results were consistent with brain histopathology.

In our study, sudden and complete brain ischemia (as in VFCA) reversed by prompt CPR resulted in predominantly diffuse ischemic neuronal changes throughout most brain regions. These were most pronounced in the cortices and hippocampus. These histopathologic changes were consistent with previous studies in rats and dogs.[13-15] In this model of ACA, ischemic neuronal changes were more severe compared with those in VFCA model. Evidence indicated that ACA also caused microinfarctions in most cortical regions.[4] Microinfarctions might be due to hypoxic acidosis and hemodynamic changes before inducing CA. However, microinfarctions were not found in our study, which may be the result of shorter asphyxia

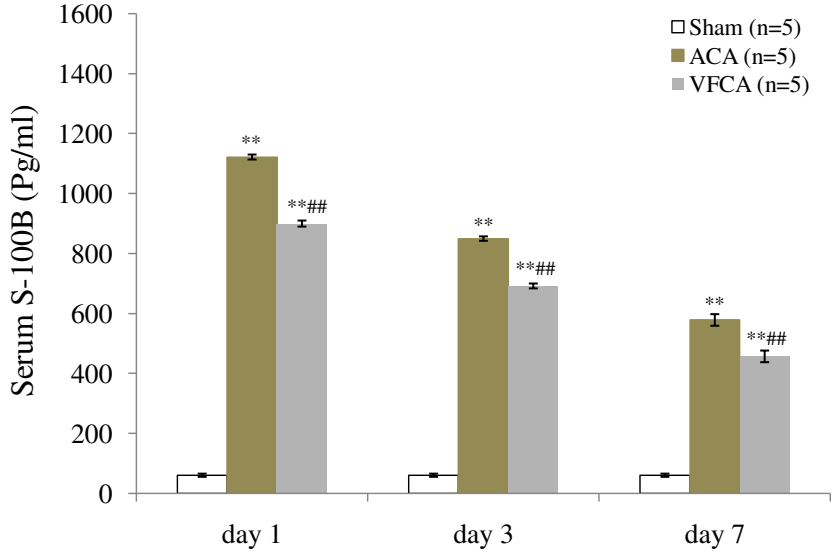


Figure 3. Serum S-100B levels at 1, 3 and 7 days after asphyxial cardiac arrest (ACA) or ventricular fibrillation cardiac arrest (VFCA) in rats. Mean±SEM. $^{**}P<0.01$ vs. Sham; $^{\#\#}P<0.01$ vs. ACA.

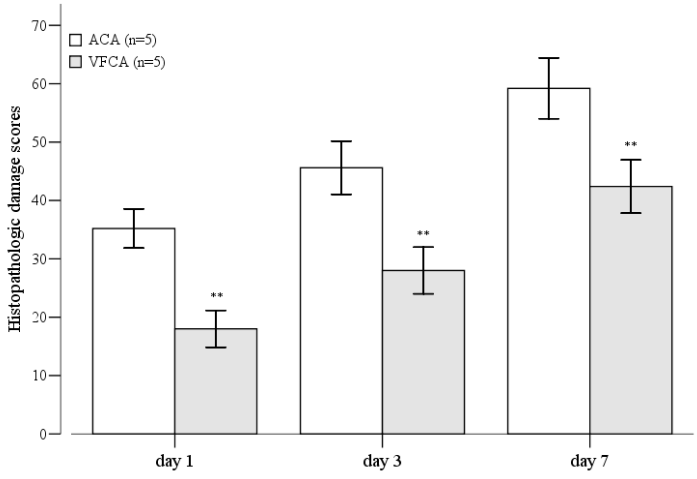


Figure 4. Whole brain histopathologic damage scores at 1, 3 and 7 days after asphyxial cardiac arrest (ACA) or ventricular fibrillation cardiac arrest (VFCA) in rats. Mean±SEM. $^{**}P<0.01$ vs. ACA.

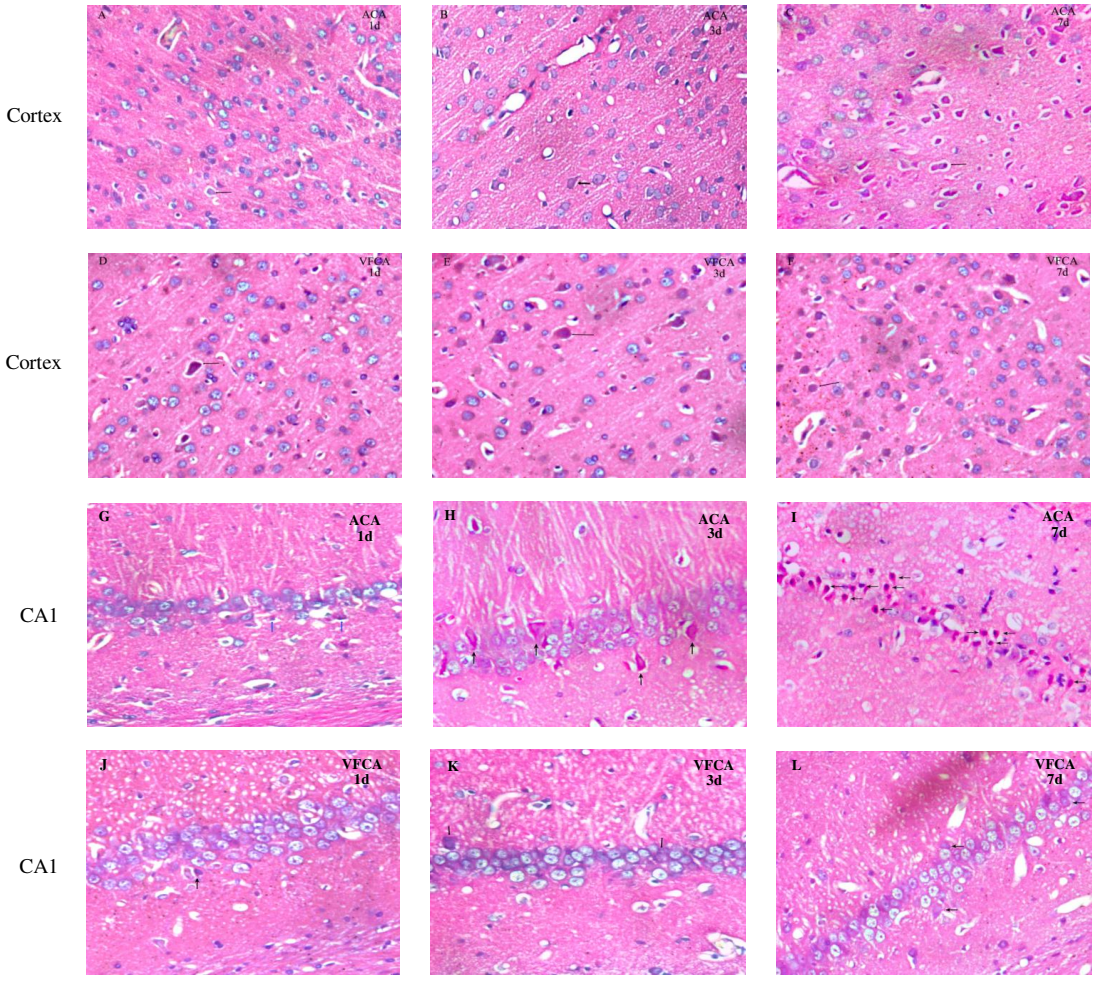


Figure 5. H-E staining in the frontal cortex and CA1 region of the hippocampus at 1, 3 and 7 days after asphyxial cardiac arrest (ACA) or ventricular fibrillation cardiac arrest (VFCA) in rats. Ischemic neuronal changes are indicated by arrow. n=5/group. Magnification: 40×.

time in our study. In addition, brain edema was not observed, irrespective of ACA or VFCA. The potentiating reason was that our observation time point had been over 12 hours because the previous study demonstrated that brain edema mainly occurred within 12 hours after ischemia.[10]

A limitation of the present study is that healthy animals are used as materials in this study. In fact, VF in CA patients is mostly caused by ischemic heart disease, and ACA usually accompanies intoxication and trauma. Therefore, the findings of this study should be interpreted with caution when applied into clinical practice.

In conclusion, based on the fact that the differences in morphologic brain damage resulting from ACA and VFCA, the interventions involved in cerebral resuscitation from CA caused by asphyxia or VF may be differently considered.

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## DISCLOSURE

Conflict of interest: None

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