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Repetitive anodal transcranial direct current stimulation improves neurological outcome and survival in a ventricular fibrillation cardiac arrest rat model

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) modulates neuronal activity and is a potential therapeutic tool for many neurological diseases. However, its beneficial effects on post cardiac arrest syndrome remains uncertain.
Objective/hypothesis: We investigated the effects of repetitive anodal tDCS on neurological outcome and survival in a ventricular fibrillation (VF) cardiac arrest rat model.
Methods: Cardiopulmonary resuscitation was initiated after 6 min of VF in 36 Sprague-Dawley rats. The animals were randomized into three groups immediately after resuscitation (n = 12 each): no-treatment control (NTC) group, targeted temperature management (TTM) group, and tDCS group. For tDCS, 1 mA anodal tDCS was applied on the dorsal scalp for 0.5 h. The stimulation was repeated for four sessions with 1-h resting interval under normothermia. Post-resuscitation hemodynamic, cerebral, and myocardial injuries, 96-h neurological outcome, and survival were evaluated.
Results: Compared with the NTC group, post-resuscitation serum astroglial protein S100 beta and cardiac troponin T levels and 96-h neuronal and myocardial damage scores were markedly reduced in the tDCS and TTM groups. Myocardial ejection fraction, neurological deficit score, and 96-h survival rate were also significantly better for the tDCS and TTM groups. The period of post-resuscitation arrhythmia with hemodynamic instability was considerably shorter in the tDCS group, but no differences were observed in neurological outcome and survival between the tDCS and TTM groups.
Conclusions: In this cardiac arrest rat model, repeated anodal tDCS commenced after resuscitation improves 96-h neurological outcome and survival to an extent comparable to TTM by attenuating post-resuscitation cerebral and cardiac injuries.

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Introduction

With an incidence ranging from 35 to 125 cases per 100,000 people, out-of-hospital cardiac arrest (OHCA) remains a major public health problem all over the world [1]. Although initial success of cardiopulmonary resuscitation (CPR) is approximately 25%–40%, the overall survival rate remains less than 10% [2,3]. Patients resuscitated from OHCA usually develop a post cardiac arrest syndrome (PCAS), resulting in early in-hospital mortality [4]. This syndrome comprises anoxic brain injury, post cardiac arrest myocardial dysfunction, systemic ischemia/reperfusion response, and persistent precipitating pathology. The severity of these components often leads to multiple organ failure contributing to low survival rates [5].

The final link in the chain of survival, effective post-resuscitation care, is aimed at reversing the pathophysiological manifestations of PCAS [6]. Among all interventions suggested and/or recommended, therapeutic hypothermia or targeted temperature management (TTM) is the most persuasive and has proven benefits both for the brain and the heart [6,7]. Although TTM greatly improves neurological outcome after resuscitation from cardiac arrest, the beneficial effects of TTM remain controversial because recent clinical

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studies were unable to show significant improvements on long-term survival [8,9]. At the same time, the risk of adverse events may increase during TTM, since various side effects and complications have been observed in clinical trials [10,11]. Therefore, the development of alternative and widely applicable therapeutic approaches is an unmet medical need in improving the prognosis of these patients [12,13].

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that uses weak, direct electric currents applied through electrodes placed over the scalp to induce changes in cortical excitability. Compared to other invasive brain stimulation techniques (e.g., deep brain stimulation, motor cortex stimulation) that are associated with surgical risks, tDCS is much safer and less costly [14–17]. To explore the electrophysiological properties of tDCS, this technique has been studied in several rodent animal models of neurological disease, such as stroke, Alzheimer disease, Parkinson disease, schizophrenia, and depression [18]. These experimental studies demonstrated that tDCS induces effects that are polarity- and intensity-dependent, and last beyond the period of stimulation. Clinical trials also indicated that tDCS can enhance motor function in patients with stroke and improve recognition memory in patients with Alzheimer disease [19,20]. The promising outcomes obtained in various conditions has catalyzed the popularity of tDCS and its potential clinical practice [16,21]. However, whether tDCS is beneficial to PCAS is still unknown.

The objective of the present study was to investigate the effects of repeated anodal tDCS on post-resuscitation cerebral and cardiac dysfunction in an established rat model of ventricular fibrillation (VF). We hypothesized that tDCS initiated immediately after resuscitation is as effective as TTM in improving neurological outcome and survival.

Material and methods

This prospective, randomized animal study was approved by the Laboratory Animal Welfare and Ethics Committee of the Army Medical University. Forty healthy male Sprague-Dawley rats weighing between 280 and 360 g supplied by a single-source breeder (laboratory animal center of the Army Medical University) were used for this study. The animals were housed in a rodent facility at 23.0 °C ± 1.0 °C with a 12-h light-dark cycle, and had free access to tap water and regular rat chow. Six rats were housed in large cages (1584 cm^2) before the experiment, and each rat was then housed separately in small cages (350 cm^2) after resuscitation. All animals received humane care in compliance with the National Institutes of Health guidelines for the use of experimental animals.

Experimental design

The treatment time and test schedule are listed in Fig. 1-A. Among the 40 rats used, 36 were randomized to no-treatment control (NTC), TTM, and tDCS groups, and the additional four rats did not experience cardiac arrest and CPR were assigned to a sham-operated group for histological examination.

Animal preparation

All animals fasted overnight except for free access to water to reduce the risk of pulmonary aspiration of gastric contents after induction of anesthesia. Anesthesia was initiated with an intraperitoneal injection of pentobarbital sodium (45 mg/kg) [22,23]. Additional doses of 10 mg/kg were administered if signs of stress occurred, such as increases in heart rate or respiratory frequency or spontaneous movements. The trachea was orally intubated using a 14-G cannula (Abbocath-T, Abbott Laboratories, North Chicago, IL, USA). Animals were mechanically ventilated (ALC-V8, Alcott Biotech Co. Ltd, Shanghai, China) with a FiO_2 of 0.21. A PE-50 catheter (Instech Laboratories Inc., Plymouth Meeting, PA, USA) was advanced from the right femoral artery for measurement of arterial pressure and blood sampling. The left femoral vein was also cannulated for administration of fluids and drugs. All catheters were flushed intermittently with saline solution containing 2.5 IU/mL heparin. Three subcutaneous needle electrodes were inserted into the limbs to measure electrocardiography (ECG). An electroencephalogram (EEG) disc electrode (10 mm gold, Jiahongkang Electronic Technology Co., Ltd., Shenzhen, China) was secured on the midline of the dorsal surface of the shaved scalp. The electrode was filled with conductive EEG paste (Ten20, Weaver and Company, Aurora, CO, USA) to ensure maximal electrical conductance. Another self-adhesive electrode pad for electrotherapy (30 mm silicon, Jurongda Science and Technology Ltd., Shenzhen, China) was placed on the ventral torso and surrounded by gauze to avoid displacement.

Experimental procedures

After collection of baseline data, VF was induced through high-frequency transesophageal cardiac pacing with an alternating current of 3–5 mA [24]. Mechanical ventilation was discontinued when cardiac pacing was started. After 6 min of untreated cardiac arrest, CPR, including chest compression and ventilation, was begun. Mechanical chest compression was delivered by a pneumatically driven compressor at a stroke rate of 200/min with a depth of 25%–30% of the anterior posterior diameter of the animal's chest. Coincident with the start of precordial compression, animals were mechanically ventilated at a frequency of 80/min with a tidal volume 0.6 mL/100g and a FiO_2 of 1.0. A dose of epinephrine (0.02 mg/kg) was injected 1 min after the start of CPR and repeated at 3-min intervals as needed. After 3 min of CPR, defibrillation was attempted with a single 2-J biphasic shock (M Series, Zoll Medical Corporation, Chelmsford, MA, USA). If restoration of spontaneous circulation (ROSC) was not achieved, a 30-s interval of CPR was performed before another 2-J shock was attempted. ROSC was defined as the return of an organized rhythm with a mean arterial pressure (MAP) of 60 mmHg or greater for an interval 5 min or longer.

Immediately after ROSC, animals were randomized into three groups (n = 12 per group) by the sealed envelope method: a NTC group, a TTM group and a tDCS group. For the NTC group, core temperature was maintained at 37.0 °C ± 0.3 °C. For the TTM group, rapid cooling was started immediately after ROSC and induced with ice packs and an electric fan. Once the target temperature reached 33.5 °C, it was maintained for 2 h and then gradually returned to 37.0 °C over a rewarming period of 2 h [22]. For the tDCS group, 1 mA anodal tDCS was applied on the dorsal scalp (Fig. 1-B) for 0.5 h with a constant direct current generator (HSPY-600, Hanshengpuyuan Science and Technology Ltd., Beijing, China). The stimulation was repeated for four sessions with a resting interval of 1 h under normothermia. The stimulation intensity and duration in each session were selected based on reports of previous studies (i.e., constant current of 0.5–2.0 mA and each session lasting 20–30 min was the effective and safe dosage range) [25–29]. Considering that the after-effects elicited by tDCS can be sustained for up to 1 h, the resting interval between each stimulation session was set for 1 h [30]. For the NTC and TTM groups, the electrodes were placed in the same position, but no current was delivered during the experiment.

Following ROSC, all animals were anesthetized and mechanically ventilated with 100% oxygen for 2 h and then with room air for another 4 h to avoid hypoxia and hyperoxia [22,23]. All catheters, including the endotracheal tube, were removed, and wounds were

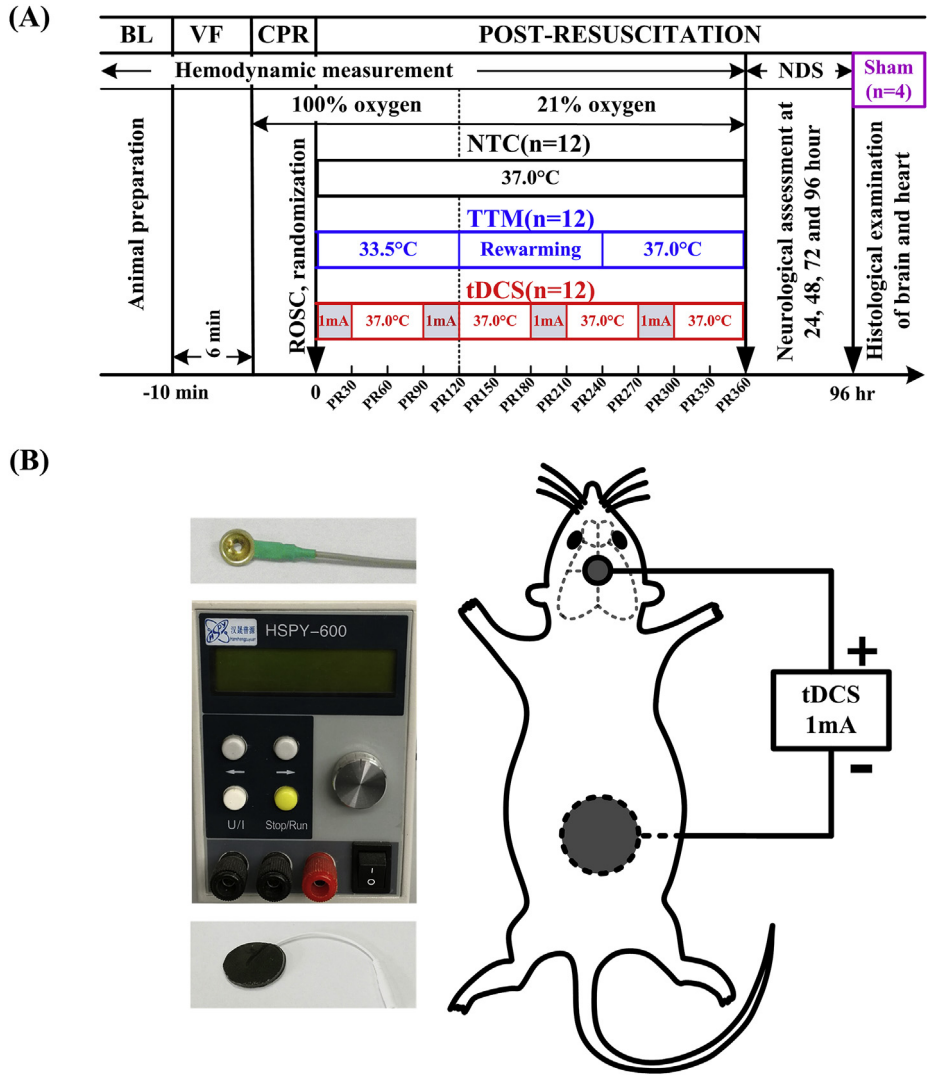

Fig. 1. Experimental time line of the study (A) and transcranial direct current stimulation (tDCS) in cardiac arrest rat model using a EEG disc electrode for the active and a self-adhesive electrode pad for the reference (B). BL: baseline; VF: ventricular fibrillation; CPR: cardiopulmonary resuscitation; ROSC: restoration of spontaneous circulation; NTC: no-treatment control; TTM: targeted temperature management; NDS: neurologic deficit score.

surgically sutured 6 h after resuscitation. The animals were then returned to their cages and observed for 96 h.

Measurements

Arterial pressure and ECG were measured via a multiparameter patient monitor (Model 90369, Spacelabs, Snoqualmie, WA, USA). Core temperature was monitored by a thermocouple probe (TH-212, Bjhocy science and technology Co. Ltd., Beijing, China) that was placed into the esophagus and maintained by a heating lamp throughout the experiment to ensure appropriate temperature management. The duration of post-resuscitation ventricular arrhythmias with hemodynamic instability was quantified. Cardiac function was noninvasively assessed at baseline and at hourly intervals after resuscitation with an echocardiograph system (DC-6, Mindray Medical International Limited, Shenzhen, China).

Arterial blood samples were drawn at baseline, and at 3 and 6 h after ROSC. Serum concentration of astroglial protein S100 beta (S100B) and cardiac troponin T (cTnT) were quantified with an enzyme-linked immunoassay (Elisa Kit, Cusbio Biotech Co. Ltd., Wuhan, China) according to the manufacturer's instructions. The sensitivity was 0.78 pg/mL and 3.12 pg/mL for S100B and cTnT, respectively. The astrocyte-derived neurotrophic protein S100B is a common biomarker of brain injury after cardiac arrest and has the potential to assist in the early detection and quantification of the severity of brain injury, response to therapeutic interventions, and prediction of outcome. cTnT, one of three subunits of the troponin complex, plays an important role in the regulation of cardiac muscle contraction and is the most sensitive and specific biomarker for myocardial damage [31].

Neurological deficit score (NDS) was examined every 24 h and confirmed by two investigators. Consciousness and breathing, cranial nerve reflexes, motor function, sensory function, and coordination were scored according to an NDS system (0–500 scale; 0 no observed neurological deficit, 500 death or brain death) that was developed to evaluate neurological outcome after global cerebral ischemia in rats [32].

Histological examination

Four days after ROSC, after assessment of the final NDS, the surviving animals were re-anaesthetized with pentobarbital

sodium. The brains and hearts were removed and immersion fixed in 10% neutral buffered formalin. The organs were histologically compared with those of sham-operated rats. The organs were embedded in paraffin and sectioned (5 μm) on a microtome. Sections were stained with hematoxylin and eosin (H&E) for histopathological evaluation. In each section, 10 random slide fields were examined under an optical microscope at ×400 magnification. Neuronal damage was diagnosed based on the prevalence of dystrophic neurons, including nuclear pyknosis, karyorrhexis, and vacuolization in the CA1 region of the hippocampus. Myocardial damage was assessed based on the prevalence of infiltration of immune cells, myocytolysis and contraction band necrosis of the selected left ventricular sections. Lesions were graded as normal (score 0), minimal (score 1), mild (score 2), moderate (score 3), and severe (score 4), respectively, as was previously described [33,34].

Blinding

To control possible measurement bias, the primary investigator randomly chose one animal from the housing cages and was unaware of the group to which it would be allocated during animal preparation, cardiac arrest induction, and CPR. The treatment randomization was made by an assistant professor independent of the research team after the animal was successfully resuscitated. Masking a rat's group assignment from the primary investigator was not possible, since he gave the intervention. The co-investigators who collected, quantified, assessed, and recorded the experimental measurements and outcomes were blinded to the intervention. The results were incorporated in a final dataset for analysis after the experiment was fully finished.

Statistical analysis

Normal distribution of the continuous variables was confirmed using the Kolmogorov-Smirnov test and presented as mean ± standard deviation. A two-tailed Student *t*-test was used for single comparison. One-way analysis of variance (ANOVA) followed by a Bonferroni correction for post-hoc test was performed for multiple comparisons. Physiological variables were examined by repeated-measures analysis comprising treatment group, time, and treatment-by-time interaction. The Fisher's exact test was performed for categorical data. The Kaplan-Meier analysis and the log-rank test were used for survival analysis and comparisons. A $p < 0.05$ was regarded as statistically significant.

Results

Baseline and resuscitation data

No differences were observed in baseline measurements and resuscitation data among the groups using ANOVA or Fisher's exact test (Table 1). All animals were successfully resuscitated and survived the 6-h post-resuscitation monitoring period.

Post-resuscitation hemodynamics

Fig. 2 shows the esophagus temperature, heart rate, MAP, and left ventricular ejection fraction (EF) measurements before and after cardiac arrest. The target core temperature was obtained within 9.5 ± 1.4 min and was returned to 37.0 °C after 2 h of rewarming for hypothermic animals; but, there were no significant changes in NTC and tDCS groups (Fig. 2-A). Heart rates measured at 30 and 60 min post-resuscitation in the TTM group were significantly lower compared with the NTC and tDCS groups using repeated-measures analysis (Fig. 2-B). MAP measured at 60, 120, and 180 min post-resuscitation in the TTM group and measured at 60 and 120 min post-resuscitation in the tDCS group were significantly higher than in the NTC group (Fig. 2-C). EF measured 30 min after ROSC in the TTM group and 120 min after ROSC in the tDCS group were statistically higher than in the NTC group (Fig. 2-D). In addition, EF measurement at 30 min post-resuscitation was considerably lower in tDCS compared with that of TTM.

Table 1
Baseline characteristics and resuscitation variables.

Group	NTC (n = 12)	TTM (n = 12)	tDCS (n = 12)
Body weight, g	330.2 ± 23.7	337.9 ± 28.6	337.9 ± 28.6
Heart rate, beat/min	401.6 ± 45.4	395.3 ± 24.2	405.8 ± 32.9
MAP, mmHg	125.2 ± 8.8	128.8 ± 9.9	126.6 ± 7.6
Temperature, °C	37.0 ± 0.3	37.0 ± 0.3	37.0 ± 0.2
Duration of CPR, second	227.3 ± 49.9	222.0 ± 24.0	225.3 ± 58.3
DBP at CPR1, mmHg	26.1 ± 4.9	27.9 ± 4.2	25.5 ± 3.7
DBP at CPR2, mmHg	36.8 ± 5.5	36.5 ± 6.3	37.6 ± 6.1
DBP at CPR3, mmHg	31.9 ± 5.7	33.2 ± 5.5	32.7 ± 5.3
Number of shocks, n	2.7 ± 1.6	2.7 ± 2.5	2.6 ± 1.8
ROSC rate, %	100	100	100

NTC: no-treatment control; TTM: targeted temperature management; tDCS: transcranial direct current stimulation; MAP: mean arterial pressure; CPR: cardiopulmonary resuscitation; DBP: diastolic blood pressure; CPR1-3: 1–3 min after CPR; ROSC: restoration of spontaneous circulation.

Successful resuscitation was immediately followed by a period of severe arrhythmias, including ventricular premature beat and ventricular tachycardia in all of the animals. However, the duration of post-resuscitation ventricular arrhythmia with hemodynamic instability was significantly longer in the TTM group [$F(2,33) = 5.590$, $p = 0.008$], when compared with the NTC group [526 ± 512 vs. 136 ± 149 s, $F(1,22) = 6.424$, $p = 0.019$] and compared with the tDCS group [526 ± 512 vs. 160 ± 153 s, $F(1,22) = 5.632$, $p = 0.027$] using ANOVA followed by Bonferroni correction for post-hoc test.

Post-resuscitation cerebral and cardiac injury

After ROSC, an significant increase in serum S100B (more than 9-, 3-, and 4-fold) and cTnT (more than 6-, 2-, and 4-fold) levels was seen in the NTC, TTM and tDCS groups compared with their pre-arrest values using repeated-measures analysis. (Fig. 3). Compared with the NTC group, these biomarkers measured at 3 and 6 h post-resuscitation were significantly lower in both the TTM (0.3-fold for S100B and 0.4-fold for cTnT) and tDCS (0.3-fold for S100B and 0.6-fold for cTnT) groups. At the same time, no statistical difference in S100B was observed between the tDCS and TTM groups (Fig. 3-A), but cTnT level in the tDCS group was significantly higher than that with the TTM (1.6-fold) group (Fig. 3-B).

96-h neurological outcome and survival

The NDS values measured during the 4 days following resuscitation are listed in Fig. 4-A. The NDS values were significantly lower in the TTM and tDCS groups compared with the NTC group, but no differences were observed between the TTM and tDCS groups during the entire observation period using repeated-measures analysis.

Four animals in the NTC group survived to 96 h, compared with 10 and 11 animals in the TTM and tDCS groups, respectively. As shown in Fig. 4-B, the cumulative 96-h survival was significantly higher in the TTM (83.3% vs. 33.3%, $\chi^2 = 7.244$, $df = 1$, $p = 0.007$) and tDCS (91.7% vs. 33.3%, $\chi^2 = 8.969$, $df = 1$, $p = 0.003$) groups when compared with the NTC group using log-rank test. There was also

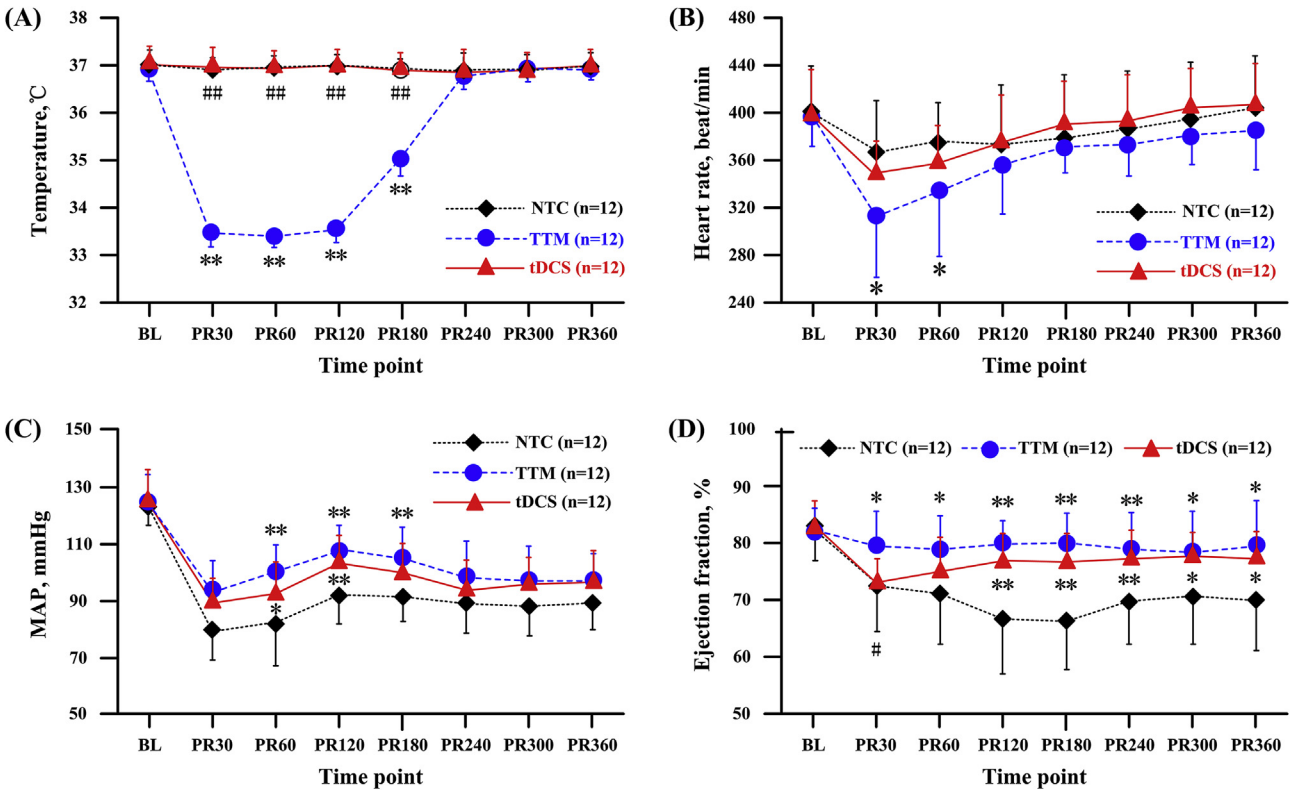

Fig. 2. Esophageal temperature (A), heart rate (B), mean arterial pressure (C), and left ventricular ejection fraction (D) measurements before and after cardiac arrest. BL: baseline; PR: post-resuscitation; NTC: no-treatment control; TTM: targeted temperature management; tDCS: transcranial direct current stimulation; * and **: $p < 0.05$ and $p < 0.01$ compared with NTC; # and ##: $p < 0.05$ and $p < 0.01$ compared with TTM.

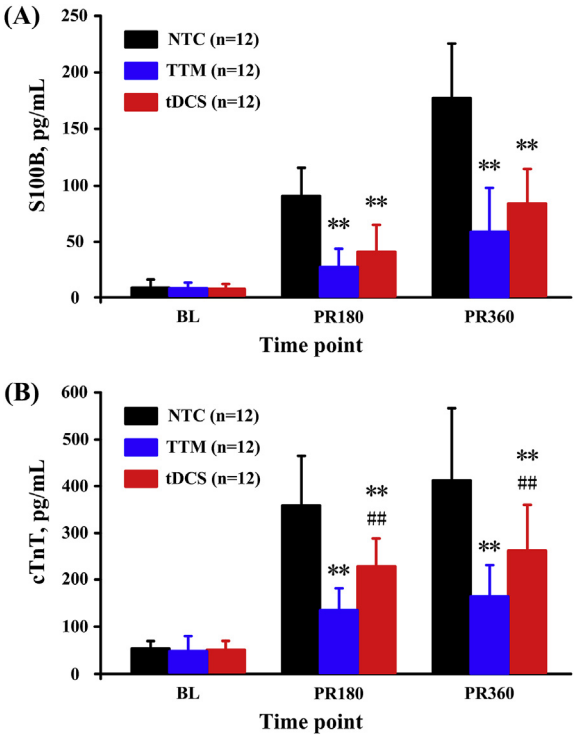

Fig. 3. Circulating levels of protein S100B (A) and cardiac troponin T (cTnT) (B) measured at baseline, 180 and 360 min post-resuscitation (PR180 and PR360). NTC: no-treatment control; TTM: targeted temperature management; tDCS: transcranial direct current stimulation; **: $p < 0.01$ compared with NTC; ##: $p < 0.01$ compared with TTM.

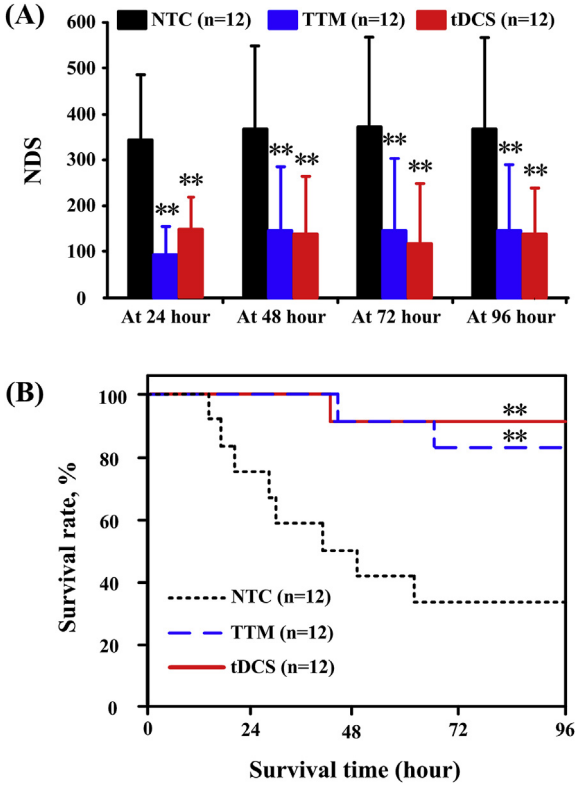

Fig. 4. Neurologic deficit score (NDS) (A) and Kaplan-Meier survival curve (B). NTC: no-treatment control; TTM: targeted temperature management; tDCS: transcranial direct current stimulation; **: $p < 0.01$ compared with NTC.

no statistical difference in survival rate between the TTM and tDCS groups (83.3% vs. 91.7%, $\chi^2 = 0.305$, df = 1, $p = 0.581$).

Histological analysis

A total of 16 animals (n = 4 in each group) underwent neuronal and myocardial sampling for histopathology analysis because only four animals survived to 96 h in the NTC group. Fig. 5-A shows representative sections from the CA1 region of the hippocampus. The number of degenerated neurons was significantly higher in the NTC, TTM and tDCS groups than that in the sham-operated rats. On the contrary, the number of remaining viable neurons was markedly preserved in the TTM and tDCS groups when compared with the NTC group. Representative photomicrographs of the H&E-stained left ventricle are presented in Fig. 5-B. Compared with the sham-operated rats, animals in the NTC group exhibited distinctive contraction bands and infiltration of a large number of immune cells. Compared with the NTC group, minor irregularities and few immune cells were observed in the TTM and tDCS groups. The overall neuronal damage score and myocardial damage score were markedly reduced in the TTM and tDCS groups compared with the NTC group using ANOVA followed by a Bonferroni correction for post-hoc test. (Fig. 6). However, no statistical differences were observed between the TTM and tDCS groups in neuronal and myocardial damage scores.

Discussion

The present study demonstrated that repeated anodal tCDS commenced immediately after ROSC significantly improves neurological outcome and survival in a rat model of cardiac arrest and CPR. The beneficial effects of repeated anodal tCDS on PCAS was through attenuating post-resuscitation cerebral and cardiac injuries and to an extent comparable to TTM.

In cardiac arrest and resuscitation, the whole body is subjected to a transient period of complete ischemia followed by reperfusion. Although the cessation of blood flow initially causes global tissue and organ injury, additional damage occurs during and after reperfusion [35]. Post-resuscitation brain injury is the major cause of morbidity and mortality in patients who initially achieve ROSC. It has been reported that a number of processes ultimately lead to cerebral injury and neuron death following ischemia/reperfusion, including excitotoxicity, disrupted calcium homeostasis, free radical formation, pathological protease cascades, neuro-inflammation, and delayed neuro-degeneration [36]. Post cardiac arrest myocardial dysfunction, which results from the exacerbated reactive oxygen species production and oxidative stress in myocardial tissue, also contributes to the low survival rate after ROSC [37]. In the current study, in agreement with previous animals studies, we confirmed that the high mortality rate in the NTC group are due largely to the ischemia/reperfusion-induced cerebral and cardiac dysfunction [22,37,38]. The severity of brain injury was exhibited by the significantly increased serum S100 level and neuronal damage score; the severity of myocardial injury was reflected by the decreased left ventricular EF, increased cTnT level, and myocardial damage score following resuscitation.

In the present study, post-resuscitation cerebral and myocardial injuries were significantly attenuated for animals treated with hypothermia. As a result, the neurological outcome and survival rate in the TTM group were dramatically improved when compared with the NTC group. The result is consistent with previous laboratory studies using same animal model [22,23], but contradicts recent clinical studies that therapeutic hypothermia did not confer a significant benefit in survival with a favorable functional outcome for both OHCA and in-hospital cardiac arrests [8,9]. In animal studies, hypothermia can be induced immediately after ROSC, and target temperature can be achieved within a couple of minutes. However, the pre-induction time and induction time are typically up to a couple of hours in clinical studies since TTM requires significant human and material resources and can only be performed by experienced teams in the intensive care unit [39]. This may, in part, explain the potent protection of TTM observed in laboratory animals compared with the lack of benefit using the same methods in the clinical arena [40].

tDCS, which was originally developed to help patients with neuropsychiatric disorders such as melancholia, schizophrenia and depression, produces profound results for improving functional recovery from ischemic stroke [30]. Using a middle cerebral artery occlusion rat model, laboratory studies demonstrated that tDCS significantly promotes behavioral and cognitive functions after stroke [25–27]. Clinical investigations also confirmed that tDCS improves activities of daily living in the majority of patients with stroke [19,41]. These findings may have translational relevance in post-resuscitation neuroprotection because the pathophysiologic processes of hypoxic-ischemic brain injury occurring in cardiac arrest also are characteristic of ischemic stroke [42]. However, cardiac arrest produces complete cessation of normal circulation and global brain ischemia; this is different from the focal cerebral ischemia induced by local reduction of blood flow in ischemic stroke. In addition, the post-resuscitation ischemic/reperfusion injury affects not only the brain but all organs of the body, which may interfere with the recovery of cerebral function in various ways [43]. As expected, the neurological outcome and survival were considerably improved when repetitive anodal stimulations were applied to the animals subjected to the tDCS group immediately after ROSC in the current study.

This is the first study to evaluate the effects of repetitive anodal tDCS on PCAS and compare them with TTM in a rat model of VF and CPR. We observed that repeated anodal tCDS improves neurological outcome and survival to an extent comparable with TTM. This result is notable because the mechanisms of action are different between the two interventions. The physiological effects of hypothermia are thought to be multifactorial, including the suppression of free radicals, enzymes, and excitotoxic and inflammatory reactions, in addition to the direct physical protection of membranes [44]. By contrast, the most accepted effects of tDCS is its ability to induce changes in cortical excitability and modulate neuronal plasticity [45,46]. At the neuronal level, the primary mechanism of tDCS is to manipulate the resting membrane potential of cortical neurons by modulating sodium and calcium channels [30,45,46]. In addition, the direct current electric fields generated in the stimulated cerebral tissue can also modify the synaptic microenvironment and further influence physiological processes, including inflammation, neurogenesis, neuroplasticity, and angiogenesis [45,46]. This increase in S100B reflected the combination of brain edema related to hypoxia during the arrest period and brain tissue damage via generation of free radicals and other mediators after resuscitation. The therapeutic effect of TTM was to interrupt one or more of the pathways participating in the genesis of anoxic brain injury and therefore greatly decrease S100B levels [47]. The dramatically decreased glial-specific S100B level and lower degree of neuron degeneration indicate that the beneficial effect of tDCS may be mediated via attenuation of the detrimental effects of such high S100B release secondary to the initial insult and subsequent resuscitation. Surprisingly, the protective effect of tDCS for PCAS is not unique to brain tissue. The significantly decreased cTnT concentration and myocardial damage score demonstrate that tDCS may also play a cardioprotective role. Following myocardial ischemia, the ensuing necrosis of cardiac myocytes results in elevation of serum cTnT. TTM may reduce myocardial damage

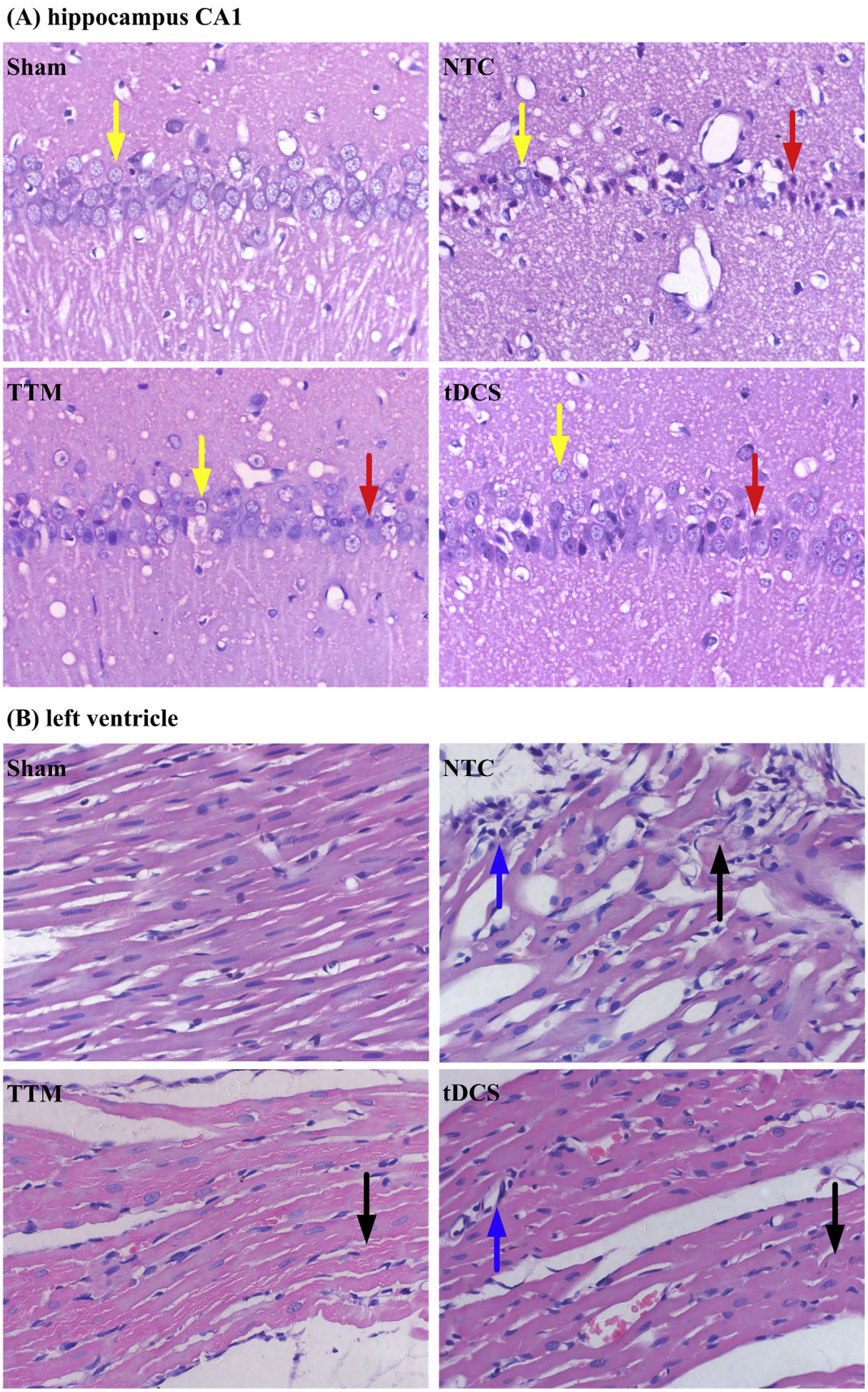

Fig. 5. Representative micrographs (magnification, ×400) of the hematoxylin and eosin-stained hippocampus CA1 (A) and left ventricle (B) at 96 h after resuscitation in sham-operated (Sham), no-treatment control (NTC), targeted temperature management (TTM), and transcranial direct current stimulation (tDCS) groups. Yellow arrows indicates viable neurons, red arrow indicates degenerated neurons, blue arrows indicates immune cells, black arrow indicates myocytolysis and contraction band necrosis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

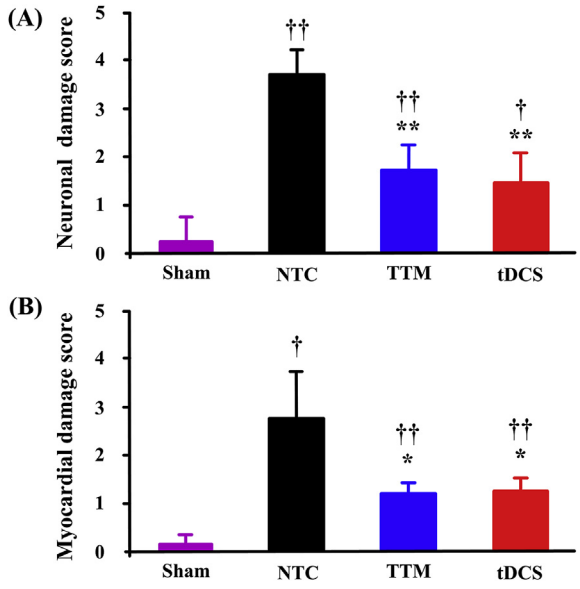

Fig. 6. The overall neuronal damage score (A) and myocardial damage score (B) at 96 h after resuscitation. Sham: sham-operated; NTC: no-treatment control; TTM: targeted temperature management; tDCS: transcranial direct current stimulation. † and ††: $p < 0.05$ and $p < 0.01$ compared with Sham; * and **: $p < 0.05$ and $p < 0.01$ compared with NTC. n = 4 in each group.

through its anti-inflammatory and anti-apoptotic effects [44]. tDCS, however, may decrease cTnT indirectly via the favorable effects on the autonomic nervous system, as reported in other brain stimulation methods [48]. The compelling evidence of neuroprotective and cardioprotective effects together can at least partially explain the improved 96-h survival rate seen in the tDCS-treated animals.

The findings of the current study open a new avenue for the management of PCAS in humans. On the one hand, the therapeutic window for neuronal protection after cardiac arrest is limited. The practical advantages of using tDCS as a therapy are readily apparent because it is tolerable, inexpensive, simple to operate, and easy to combine with other treatments [41]. Precise temperature control, however, requires specialized equipment and all members of the medical team, from emergency medical technicians to intensive care staff, to be coordinated in the use of TTM [39]. On the other hand, tDCS is relatively safe, and the only reported potential side effect is skin lesion under the electrode. The occurrence of lesions may depend on the intensity and duration of tDCS as well as on the impedance between the electrode and the skin [21]. On the contrary, a number of adverse effects have been reported in hypothermia, including pneumonia, sepsis, arrhythmia, electrolyte imbalance, bleeding, shivering, and coagulopathy [11,49]. These advantages together with its effectiveness suggest that tDCS may have promising advantages for application in post-resuscitation management.

Our study has some limitations. First, we studied a single duration of cardiac arrest caused by VF in healthy animals without comorbidities, such as acute coronary syndrome and acute myocardial infarction that are commonly associated with cardiac arrest in humans. Second, the small rodent brain has different metabolic and physiologic properties from the complex human brain. Third, this study does not address the mechanisms by which tDCS reduces cerebral and myocardial injuries.

Conclusion

In this rat model of cardiac arrest, repeated anodal tDCS started after ROSC resulted in less severe cerebral injury as determined by the decreased release of serum S100B and lower degree of neuron degeneration. Anodal tDCS also attenuated post-resuscitation myocardial damage as reflected by the decreased level of serum cTnT and reduced infiltration of immune cells in the myocardium. The improvements in the 96-h neurological outcome and survival were comparable to those of TTM. Our findings suggest that anodal tDCS may be a potentially feasible and easily applicable solution for the management of PCAS. However, these findings will need further evaluation on long-term outcome among survivors of OHCA.

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Conflicts of interest

The authors have no conflict of interest for publishing this paper.

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References

[1] Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, et al. Resuscitation Outcomes Consortium Investigators: regional variation in out-of-hospital cardiac arrest incidence and outcome. JAMA 2008;300:1423–31.
[2] Wachelder EM, Moulaert VR, van Heugten C, Verbunt JA, Bekkers SC, Wade DT. Life after survival: long-term daily functioning and quality of life after an out-of-hospital cardiac arrest. Resuscitation 2009;80:517–22.
[3] Kazaure HS, Roman SA, Sosa JA. Epidemiology and outcomes of in-hospital cardiopulmonary resuscitation in the United States, 2000-2009. Resuscitation 2013;84:1255–60.
[4] Mongardon N, Dumas F, Ricome S, Grimaldi D, Hissem T, Pène F, et al. Post-cardiac arrest syndrome: from immediate resuscitation to long-term outcome. Ann Intensive Care 2011;1:45.
[5] Stub D, Bernard S, Duffy SJ, Kaye DM. Post cardiac arrest syndrome: a review of therapeutic strategies. Circulation 2011;123:1428–35.
[6] Callaway CW, Donnino MW, Fink EL, Geocadin RG, Golan E, Kern KB, et al. Part 8: post-cardiac arrest care: 2015 American heart association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2015;132:S465–82.
[7] Donnino MW, Andersen LW, Berg KM, Reynolds JC, Nolan JP, Morley PT, et al., ILCOR ALS Task Force. Temperature management after cardiac arrest: an advisory statement by the advanced life support task force of the international Liaison committee on resuscitation and the American heart association emergency cardiovascular care committee and the council on cardiopulmonary, critical care, perioperative and resuscitation. Circulation 2015;132:2448–56.
[8] Chan PS, Berg RA, Tang Y, Curtis LH, Spertus JA. American Heart Association's Get with the Guidelines-Resuscitation Investigators: association between therapeutic hypothermia and survival after in-hospital cardiac arrest. JAMA 2016;316:1375–82.
[9] Martinell L, Herlitz J, Karlsson T, Nielsen N, Rylander C. Mild induced hypothermia and survival after out-of-hospital cardiac arrest. Am J Emerg Med 2017;35:1595–600.
[10] Xiao G, Guo Q, Shu M, Xie X, Deng J, Zhu Y, et al. Safety profile and outcome of mild therapeutic hypothermia in patients following cardiac arrest: systematic review and meta-analysis. Emerg Med J 2013;30:91–100.
[11] Kirkegaard H, Søreide E, de Haas I, Pettilä V, Taccone FS, Arus U, et al. Targeted temperature management for 48 vs 24 hours and neurologic outcome after out-of-hospital cardiac arrest: a randomized clinical trial. JAMA 2017;318:341–50.
[12] Nielsen N, Friberg H, Gluud C, Herlitz J, Wetterslev J. Hypothermia after cardiac arrest should be further evaluated-a systematic review of randomised trials with meta-analysis and trial sequential analysis. Int J Cardiol 2011;151:333–41.
[13] Wise MP, Horn J, Aneman A. Targeted temperature management after out-of-hospital cardiac arrest: certainties and uncertainties. Crit Care 2014;18:459.
[14] Hummel FC, Cohen LG. Non-invasive brain stimulation: a new strategy to improve neurorehabilitation after stroke? Lancet Neurol 2006;5:708–12.

[15] Palm U, Ayache SS, Padberg F, Lefaucheur JP. Non-invasive brain stimulation therapy in multiple sclerosis: a review of tDCS, rTMS and ECT results. Brain Stimul 2014;7(6):849–54.
[16] Krishnan C, Santos L, Peterson MD, Ehinger M. Safety of noninvasive brain stimulation in children and adolescents. Brain Stimul 2015;8(1):76–87.
[17] Yosephi MH, Ehsani F, Zoghi M, Jaberzadeh S. Multi-session anodal tDCS enhances the effects of postural training on balance and postural stability in older adults with high fall risk: primary motor cortex versus cerebellar stimulation. Brain Stimul 2018. pii: S1935-861X(18)30248-1.
[18] Brunoni AR, Fregni F, Pagano RL. Translational research in transcranial direct current stimulation (tDCS): a systematic review of studies in animals. Rev Neurosci 2011;22:471–81.
[19] Boggio PS, Nunes A, Rigonatti SP, Nitsche MA, Pascual-Leone A, Fregni F. Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. Restor Neurol Neurosci 2007;25:123–9.
[20] Ferrucci R, Mameli F, Guidi I, Mrakic-Sposta S, Vergari M, Marceglia S, et al. Transcranial direct current stimulation improves recognition memory in Alzheimer disease. Neurology 2008;71:493–8.
[21] Russo C, Souza Carneiro MI, Bolognini N, Fregni F. Safety review of transcranial direct current stimulation in stroke. Neuromodulation 2017;20:215–22.
[22] Ye S, Weng Y, Sun S, Chen W, Wu X, Li Z, et al. Comparison of the durations of mild therapeutic hypothermia on outcome after cardiopulmonary resuscitation in the rat. Circulation 2012;125:123–9.
[23] Hayashida K, Sano M, Kamimura N, Yokota T, Suzuki M, Maekawa Y, et al. (2) gas improves functional outcome after cardiac arrest to an extent comparable to therapeutic hypothermia in a rat model. J Am Heart Assoc 2012;1(5):e003459.
[24] Chen MH, Liu TW, Xie L, Song FQ, He T, Zeng ZY, et al. Ventricular fibrillation induced by transoesophageal cardiac pacing: a new model of cardiac arrest in rats. Resuscitation 2007;74:546–51.
[25] Yoon KJ, Oh BM, Kim DY. Functional improvement and neuroplastic effects of anodal transcranial direct current stimulation (tDCS) delivered 1 day vs. 1 week after cerebral ischemia in rats. Brain Res 2012;1452:61–72.
[26] Braun R, Klein R, Walter HL, Ohren M, Freudenmacher L, Getachew K, et al. Transcranial direct current stimulation accelerates recovery of function, induces neurogenesis and recruits oligodendrocyte precursors in a rat model of stroke. Exp Neurol 2016;279:127–36.
[27] Pikhovych A, Stolberg NP, Jessica Flitsch L, Walter HL, Graf R, Fink GR, et al. Transcranial direct current stimulation modulates neurogenesis and microglia activation in the mouse brain. Stem Cells Int 2016;2016:2715196.
[28] Wachter D, Wrede A, Schulz-Schaeffer W, Taghizadeh-Waghefi A, Nitsche MA, Kutschenko A, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. Exp Neurol 2011;227:322–7.
[29] Yu X, Li Y, Wen H, Zhang Y, Tian X. Intensity-dependent effects of repetitive anodal transcranial direct current stimulation on learning and memory in a rat model of Alzheimer's disease. Neurobiol Learn Mem 2015;123:168–78.
[30] Brunoni AR, Nitsche MA, Bolognini N, Bikson M, Wagner T, Merabet L, et al. Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. Brain Stimul 2012;5:175–95.
[31] Scolletta S, Donadello K, Santonocito C, Franchi F, Taccone FS. Biomarkers as predictors of outcome after cardiac arrest. Expert Rev Clin Pharmacol 2012;5:687–99.
[32] Hendrickx HH, Safar P, Miller A. Asphyxia, cardiac arrest and resuscitation in rats. II. Long term behavioral changes. Resuscitation 1984;12:117–28.
[33] Neigh GN, Kofler J, Meyers JL, Bergdall V, La Perle KM, Traystman RJ, et al. Cardiac arrest/cardiopulmonary resuscitation increases anxiety-like behavior and decreases social interaction. J Cereb Blood Flow Metab 2004;24:372–82.
[34] Tsai MS, Huang CH, Tsai SH, Tsai CY, Chen HW, Cheng HJ, et al. The difference in myocardial injuries and mitochondrial damages between asphyxial and ventricular fibrillation cardiac arrests. Am J Emerg Med 2012;30:1540–8.
[35] Neumar RW, Nolan JP, Adrie C, Aibiki M, Berg RA, Böttiger BW, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A consensus statement from the international Liaison committee on resuscitation (American heart association, Australian and New Zealand council on resuscitation, European resuscitation council, heart and stroke foundation of Canada, InterAmerican heart foundation, resuscitation council of asia, and the resuscitation council of southern africa); the American heart association emergency cardiovascular care committee; the council on cardiovascular surgery and anesthesia; the council on cardiopulmonary, perioperative, and critical care; the council on clinical cardiology; and the stroke council. Circulation 2008;118:2452–83.
[36] Martin LJ, Al-Abdulla NA, Brambrink AM, Kirsch JR, Sieber FE, Portera-Cailliau C. Neurodegeneration in excitotoxicity, global cerebral ischemia, and target deprivation: a perspective on the contributions of apoptosis and necrosis. Brain Res Bull 1998;46:281–309.
[37] Hackenhaar FS, Fumagalli F, Li Volti G, Sorrenti V, Russo I, Staszewsky L, et al. Relationship between post-cardiac arrest myocardial oxidative stress and myocardial dysfunction in the rat. J Biomed Sci 2014;21:70.
[38] Liu S, Zhang Y, Zhao Y, Cui H, Cao C, Guo J. Effects of hypothermia on S100B and glial fibrillary acidic protein in asphyxia rats after cardiopulmonary resuscitation. Cell Biochem Biophys 2015;71:401–6.
[39] Merchant RM, Soar J, Skrifvars MB, Silfvast T, Edelson DP, Ahmad F, et al. Therapeutic hypothermia utilization among physicians after resuscitation from cardiac arrest. Crit Care Med 2006;34:1935–40.
[40] Lee BK, Jeung KW, Jung YH, Lee DH, Lee SM, Cho YS, et al. Relationship between timing of cooling and outcomes in adult comatose cardiac arrest patients treated with targeted temperature management. Resuscitation 2017;113:135–41.
[41] Elsner B, Kugler J, Pohl M, Mehrholz J. Transcranial direct current stimulation (tDCS) for improving activities of daily living, and physical and cognitive functioning, in people after stroke. Cochrane Database Syst Rev 2016;3:CD009645.
[42] Ghanekar S, Corey S, Lippert T, Borlongan CV. Pathological links between stroke and cardiac arrest. Chin Neurosurg J 2016;2:40.
[43] Jentzer JC, Chonde MD, Dezfulian C. Myocardial dysfunction and shock after cardiac arrest. BioMed Res Int 2015;2015:314796.
[44] González-Ibarra FP, Varon J, López-Meza EG. Therapeutic hypothermia: critical review of the molecular mechanisms of action. Front Neurol 2011;3(2):4.
[45] Pelletier SJ, Cicchetti F. Cellular and molecular mechanisms of action of transcranial direct current stimulation: evidence from in vitro and in vivo models. Int J Neuropsychopharmacol 2014;18. pyu047.
[46] Giordano J, Bikson M, Kappenman ES, Clark VP, Coslett HB, Hamblin MR, et al. Mechanisms and effects of transcranial direct current stimulation. Dose Response 2017 Feb 9;15(1). 1559325816685467.
[47] Hachimi-Idrissi S, Zizi M, Nguyen DN, Schiettecate J, Ebinger G, Michotte Y, et al. The evolution of serum astroglial S-100 beta protein in patients with cardiac arrest treated with mild hypothermia. Resuscitation 2005;64(2):187–92.
[48] Samniang B, Shinlapawittayatorn K, Chunchai T, Pongkan W, Kumfu S, Chattipakorn SC, et al. Vagus nerve stimulation improves cardiac function by preventing mitochondrial dysfunction in obese-insulin resistant rats. Sci Rep 2016;6:19749.
[49] Soleimanpour H, Rahmani F, Golzari SE, Safari S. Main complications of mild induced hypothermia after cardiac arrest: a review article. J Cardiovasc Thorac Res 2014;6:1–8.