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Brief Report
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Effect of Tempol on Cerebral Resuscitation Caused by Asphyxia-Induced Cardiac Arrest

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Background: This study was conducted to investigate the effect and mechanism of the nitrogen oxide 4-hydroxy-2,2,6,6-tetramethylpiperidine (Tempol) on cerebral resuscitation caused by asphyxia-induced cardiac arrest.

Methods: Airway occlusion-induced asphyxia at the end of expiration was used to establish the rat cerebral ischaemia-hypoxia injury model. A total of 90 adult male Sprague-Dawley rats were randomly divided into the three groups. The Tempol and conventional cardiopulmonary resuscitation (CPR) groups were further divided into four subgroups according to different time points.

Results: After cerebral ischaemia, independent heart rate following asphyxia appeared earlier, and the success rate of primary recovery and the neurological function score of rats were higher in the Tempol group than in the conventional CPR group. The serum neuron-specific enolase (NSE) levels in the Tempol and conventional CPR groups were significantly higher within 6 to 48 h than that in the blank control group. The serum NSE level was significantly lower in the Tempol group than the conventional CPR group.

Conclusions: After global cerebral ischaemia-hypoxia, the antioxidant Tempol improved cerebral resuscitation by reducing oxidative stress injuries and post-CPR cerebral damage. The NSE level can be used as an early detection index in the diagnosis of global cerebral ischaemia-hypoxia injuries.

Key Words: Cerebral ischemia • Neuron-specific enolase • Rats • Tempol

INTRODUCTION

Cerebral recovery is the return of consciousness after positive cardiopulmonary resuscitation (CPR) by various means. This typically involves respiratory arrest and an induced heartbeat, which is the key to measuring successful recovery. Early diagnosis and proper valuation of the severity of brain cell damage is very important. The antioxidant 4-hydroxy-2,2,6,6-tetramethylpiperidine (Tempol) has similar activities as superoxide dismutase towards reactive oxygen species. Tempol is a strong, effective and membrane-permeable oxygen radical scavenger that can penetrate the blood-brain barrier and can exhibit significant antioxidant capacity in injuries.[1-4] In cases of brain damage, changes in specific proteins or metabolites in blood and cerebrospinal fluid are customarily observed.[5] The neuron-specific enolase (NSE) in blood and cerebrospinal fluid is a valuable marker of post-CPR ischaemia-hypoxic brain damage and thus efficacious for prognostic evaluation.[6] The NSE level abnormally increases in early ischaemia-hypoxic brain damage, and the extent of increase in the cerebrospinal fluid is higher than that in the blood. A good correlation in the variation between blood and cerebrospinal fluid exists; thus, the serum NSE level can be detected in clinical settings.[7] In this experiment, a rat CPR model[8] was established to dynamically monitor the effect of Tempol on NSE concentration, the post-CPR

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cerebral oedema and the damage index. We aimed to further explore the effects of Tempol on the improvement of rat CPR caused by asphyxia-induced cardiac arrest. The protective effects of Tempol and the underlying mechanisms of these effects on post-CPR brain injuries are also determined.

METERIALS AND METHODS

Animals

A total of 90 clean, healthy adult male Sprague-Dawley rats were used, which were provided by the Experimental Animal Centre of China Medical University. The rats were 70 to 90 days of age, with body weights ranging from 260 to 320 g. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shengjing Hospital of China Medical University.

Animal grouping

A total of 90 rats were randomly divided into three groups, namely: the Tempol group (T group), the conventional CPR group (C group) and the blank control group (S group), with 40, 40 and 10 rats in each group, respectively. Arteriovenous catheters and endotracheal intubation were performed on the S group, and the rats were sacrificed 30 min after the surgical operation for the blood sample without undergoing asphyxia, cardiac arrest and CPR. The C and T groups were divided into four subgroups (C6, C12, C24 and C48; and T6, T12, T24 and T48, respectively) corresponding to four time points (6, 12, 24 and 48 h), with 10 rats in each subgroup. Arteriovenous catheters, endotracheal intubation, asphyxia, cardiac arrest and CPR were performed in the C and T groups, and the blood samples were obtained at 6, 12, 24 and 48 h after spontaneous breathing was restored. The blood samples were left to stand and centrifuged to obtain the serum, which was stored at -80 °C.

Preparation of animal model

The improved method of airway-occlusion-induced suffocation at the end of expiration was adapted to establish the rat CPR model. The experimental rats were fasted 12 h before the surgery, but were given free access to water. After weighing, the rat was intraperitoneally injected with 10% chloral hydrate (3.5 mL/kg) and fixed supinely on the operating table. The BL-420 Biological Experimental System was used to monitor the vital signs, and body temperature was regulated at 37.0 $\pm$ 0.5 °C with a heating lamp. Endotracheal intubation was performed, and a small animal ventilator was connected for mechanical ventilation (pure oxygen). Catheters were inserted in the left intrailiac artery and right intrailiac veins to monitor mean arterial pressure, electrocardiogram (ECG), heart rate, end-expiratory CO_2, anal temperature and blood gas. Postsurgical stabilization was performed at room temperature for 10 min. The C group was intraperitoneally injected with 0.5 mL of saline 5 min before asphyxia. The T group was intraperitoneally injected with Tempol (100 mg/kg, Sigma Aldrich Companies, St. Louis, Missouri, USA) dissolved in 0.5 mL of saline 5 min before asphyxia. The endotracheal tube was occluded for 8 min towards the end of expiration to induce cardiac arrest (CA).[8-11] Subsequently, standard CPR was performed, and 20 μg/kg of epinephrine and fluid infusion was injected through the intrailiac vein. The sustained appearance of spontaneous cardiac rhythm, pulse wave and systolic blood pressure $\geq$ 60 mm Hg for more than 10 min was considered the return of spontaneous circulation (ROSC). After ROSC, the ventilator was withdrawn, the endotracheal intubator and the arteriovenous catheters were extubated and the wound was sutured. The rat was placed in a hypoxic chamber at 37 °C for inhalation of low-concentration oxygen for 2 h. Blood was collected from the T and C groups at 6, 12, 24 and 48 h after ROSC and centrifuged for serum, which was subsequently frozen.

CA standard

Cardiac arrest was deemed to have occurred when the ECG exhibited electrocardiac silence, ventricular fibrillation and cardiac mechanical separation. Additionally, the heart beat in the apical region disappeared, and the animal skin and mucous membrane exhibited obvious cyanosis.

ROSC criteria

The ROSC criteria were as follows: ECG exhibited

normal QRS waves, with the ventricular pressure maintained at ≥ 60 mm Hg for more than 10 min; obvious and palpable apical beat was observed, and the cyanosis of animal skin and mucous membrane was significantly reduced.

Neurological deficit score

This experiment was performed according to the neurological severity scores (NSS) method of Li et al.[12] to assess the neurological function of rats, including the perception level, activity, gripping reaction, placement reaction and balancing test (the maximum score was 15 points). A high score indicates serious injury.

Determination of serum NSE concentration

The rats in the C and T groups were anaesthetised with an intraperitoneal injection of 10% chloral hydrate at 6, 12, 24 and 48 h, and fixed and sampled for blood from the abdominal aorta. Blood samples were placed in a procoagulant tube at low temperature and left to stand for 1 h. The blood samples were centrifuged at 3000 rpm for 10 min, and the supernatant was placed in an Eppendorf tube and stored at -80 °C for the test. The rats in the S group were sacrificed 30 min after serum sampling. An ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to determine the serum NSE concentrations according to the manufacturer's instructions.

Statistical analysis

SPSS 17.0 software was used for data analysis, and the measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The comparison of the mean time of asphyxia to CA and the time for autonomic rhythm of the heart between two groups were performed using a two-sample test. Intergroup comparisons were performed with one-way ANOVA analysis. The statistical significance was determined by pairwise comparison with the least significant difference method and Tukey's test. The counting data were tested with Chi-square test (χ^2 test). Subsequently, the primary success rate of recovery was determined by χ^2 test. The neurological severity scores were expressed as median (interquartile range), and the Wilcoxon rank sum test was performed at a test level of $\alpha = 0.05$.

RESULTS

Occurrence time of independent heart rate and primary recovery success rate of rats

After standard CPR, the occurrence time of independent heart rate in the T group was significantly shorter than that in the C group ($p < 0.05$). The primary recovery success rate in the T group was significantly higher than in the C group ($p < 0.05$) (Table 1).

Neurological function score

Performing the neurological function score test at 6 h after recovery was difficult because of the aftereffects of anaesthesia and surgery. Thus, the test was performed at 12, 24 and 48 h. The neurological function score of the T group was significantly higher than in the C group ($p < 0.05$) (Figure 1).

Serum NSE level after global cerebral ischaemia-hypoxia

After brain ischaemia-hypoxia, the serum NSE levels in the T and C groups at each time point were significantly higher than in the S group ($p < 0.05$). The NSE level in the T group was significantly lower than in the C group ($p < 0.05$) (Table 2 and Figure 2).

Table 1. Related parameters of rats after asphyxia ($\bar{x} \pm s$)

Group	The time from asphyxia to CA (s)	The time for autonomic rhythm of the heart (s)	The primary success rate of recovery (%)
Group C	217 ± 22	110 ± 23	75
Group T	219 ± 21	88 ± 31*	88*

Note: compared with C group: * $p < 0.05$. CA, cardiac arrest.

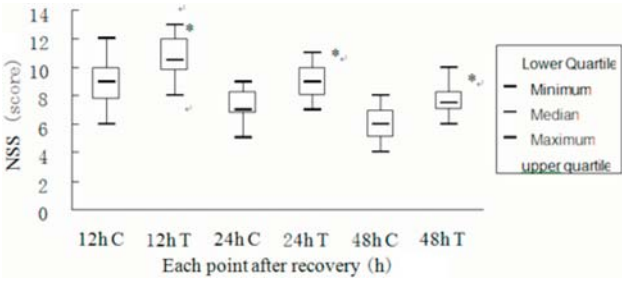

Figure 1. *Neurological severity scores of two groups at each point. Note: Compared with C group, * $p < 0.05$. NSS, neurological severity scores.*

Table 2. Changes of NSE levels after cerebral ischemia hypoxia ($\bar{x} \pm s$)

Group	NSE (μg/L)			
	6 h	12 h	24 h	48 h
Group S	90.12 ± 12.61	85.32 ± 15.60	88.65 ± 10.61	93.21 ± 15.30
Group C	754.79 ± 24.85*	1034.42 ± 18.37*	1375.51 ± 22.07*	2009.25 ± 20.56*
Group T	680.19 ± 19.61*#	811.21 ± 15.60*#	1133.39 ± 10.61*#	1779.78 ± 25.30*#

Note: Compared with S group, * $p < 0.05$; compared with C group, # $p < 0.05$. NSE, neuron specific enolase.

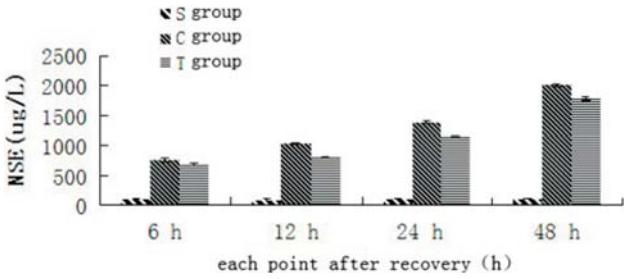

Figure 2. *Changes of NSE levels after cerebral ischemia hypoxia at each time. Note: Compared with S group, * $p < 0.05$; compared with C group, # $p < 0.05$. NSE, neuron specific enolase.*

DISCUSSION

CPR can save the lives of patients, but might simultaneously cause ischaemia-reperfusion injury in ischemic tissues, among which the brain tissue is the most sensitive to ischaemia and hypoxia. After cardiac arrest, the brain tissue cannot tolerate the low-oxygen environment because of the sudden drop in cerebral blood supply. Lack of oxygen and nutrients would cause accumulation of metabolites and brain damage.

Oedema of cerebral glial cells, degenerated necrosis and blood-brain barrier destruction occur in a short time. When circulation is restored, the brain tissues would enter the stages of no-reflow, reactive hyperaemia and delayed hypoperfusion, which are the most important stages in cerebral ischaemia-hypoxic injury; the individual might remain in these stages for 2 to 12 h.[13] Currently, the most promising nitroxide is Tempol, which is a low molecular weight, stable compound with good membrane permeability and no toxicity. Tempol is a strong and effective membrane-permeable-type oxygen radical scavenger that can penetrate the blood-brain barrier. Previous experimental results showed that Tempol had significant antioxidant activities,[1-3] which could scavenge free radicals and inhibit lipid peroxidation, thereby inhibiting oxidative damage to vascular endothelial cells and nerve cells, reducing inflammation and brain oedema and reducing reperfusion injury. Some scholars used rat models of myocardial infarction in the chaemia-reperfusion model to study the function of Tempol in ischaemia-reperfusion injuries. Studies confirmed that 5 min pre-intraperitoneal administration of Tempol is sufficient for the drug to enter cells and generate antiarrhythmic disorder and anti-ischemic effects.[4] This experiment showed that the early application of Tempol could shorten the occurrence time of the autonomic heart rate after asphyxia and increase the success rate of primary recovery, whose mechanism may arise from Tempol acting as a oxygen radical scavenger. Thus, Tempol has an anti-ischemic cardioprotective function and promotes ROSC. Moreover, after a successful recovery, the neurological function scores at 12, 24 and 48 h in the T group were higher than in the C group, indicating that the Tempol intervention could reduce the degree of brain injury and improve brain recovery.

NSE is the isoenzyme of glycolysis enolase that is specifically distributed in the nerve and neuroendocrine cells and is the specific marker of the neuron.[14,15] Under normal circumstances, a small amount of NSE is present in the cerebrospinal fluid and serum. When the brain is ischemic, hypoxic, poisoned or under trauma, the integrity of the nerve cell membrane is damaged and the permeability of the blood-brain barrier increases. NSE protein would then leak into the blood or cerebrospinal fluid, and the level of the NSE protein would show significant changes.[16,17] Previous studies have shown that the serum NSE level was highly correlated with the NSE level of cerebrospinal fluid.[7] Therefore, the detected serum NSE may accurately reflect the degree of brain tissue damage and can be used as an early diagnostic index in the evaluation of post-CPR brain damage.[6,18] Serum NSE level can help predict the severity of brain injuries of CPR patients, the hospital-discharging survival rate and the recovery of neurological function. Serum

NSE concentration was significantly high after ROSC and continued to increase at 6, 12, 24 and 48 h, which is consistent with results in previous reports.[19-21] The serum NSE protein dynamics could indicate changes and reflect nerve cell damage. The changes of serum NSE level after cerebral ischaemia-hypoxia can be used as a valuable marker of post-CPR ischaemia-hypoxic brain damage and for prognostic evaluation. The serum NSE level in the T group was significantly lower than that in the C group after ROSC, indicating that Tempol can reduce the occurrence of ischaemia-hypoxic brain damage and can protect against brain injury caused by asphyxia-induced ischaemia-hypoxia. The underlying mechanism for these activities may involve stabilizing cells and microsomal membranes, scavenging reactive oxygen species, reducing blood-brain barrier permeability and decreasing serum NSE level. Tempol reduces the oxidative stress damages of nerve cells caused by CPR, reduces brain tissue damage induced by ischaemia-hypoxia and inhibits the occurrence of brain damage after CPR.

After Tempol intervention in the ischaemia and hypoxia situation, the changes of serum NSE level in the T group were lower than those in the C group, rat survival rate improved and neurological damage decreased. These results clarify the function of Tempol in brain protection after CPR, which is expected to increase the success rate of recovery or prolong the survival duration. Our results provide important clinical research ideas on post-CPR brain resuscitation in part by further demonstrating that antioxidant intervention might be a new and effective post-CPR treatment.

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