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ORIGINAL PAPER

Protective Effects of Catalpol on Limb Motor Function and Ultrastructure of Hippocampal Neurons in Rats with Cerebral Ischemia

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Abstract

This study aimed to examine the protective effects of catalpa on ultrastructure of hippocampal neuron and limb motor function in rats with cerebral ischemia. 90 healthy Sprague–Dawley male rats were randomly divided into control ($n=30$) and model ($n=60$) groups. Cerebral ischemia and hippocampal neurons were induced by occluding the internal carotid artery and injection of high blood glucose, respectively. Model rats were randomly divided into routine ($n=30$) and observational ($n=30$) groups. Animals in the routine group received edaravone injection (7 mg/kg/day) for 14 days, while rats in the observation group were treated with catalpol (30 mg/kg/day) for 14 days. Limb motor function score, fine motion execution capability, number of hippocampal neurons retained, and the ultrastructure of hippocampal nerve cells were considered at 3, 7, and 14 days after treatments. A significant difference was observed in the mean scores of limb motor function, fine motor execution ability, and the number of hippocampal neurons retained between groups ($p<0.001$). Repetitive treatments with catalpol significantly improved the mean number of hippocampal neurons retained ($p<0.01$), limb motor function ($p<0.001$), and fine motor execution ability scores ($p<0.01$) at 3, 7, and 14 days compared to edaravone. Catalpol treatments also improved the ultrastructure morphology of neuronal cells. Catalpa can effectively improve limb motor function and protect hippocampal neuron function in rats with cerebral ischemia.

Keywords Catalpa · Rats · Limb motor function · Cerebral ischemia · Hippocampal neurons

Abbreviations

SD	Sprague–Dawley
MCAO	Middle cerebral artery occlusion
SLMF	Scores of limb motor function
DIGC	The differences of intra-group comparison

Background

Cerebral ischemia caused by insufficient blood flow in the brain is a kind of neurological disease characterized by the decrease of microcirculatory blood volume and disorder of energy metabolism. This condition is associated with limited oxygen supply or cerebral hypoxia that leads to the death of brain tissue and the occurrence and development of cerebrovascular diseases [1]. Stroke, Parkinson's, and Alzheimer's diseases are the other common conditions that may result from cerebral ischemia. Previous studies on animal model found that middle cerebral artery occlusion (MCAO) was the main cause of ischemic stroke and symptomatic treatment could restore it [2]. If this condition left untreated for a long-term, adenosine triphosphate (ATP) in the central ischemic area will continue to decrease, seriously affecting the function closest to the cortical area until irreversible injury occurs. Furthermore, irreversible neuronal loss occurs and results in ischemic stroke, when the ischemia persists for long-term. Therefore, finding a new therapeutic strategy is urgently needed to decline the mortality rate of these patients. Neural stem cells can be considered as a potential therapeutic target for producing new neurons after stroke. They have the ability to migrate to the borders of ischemic lesions for regeneration process. This fact emphasizes that identification of new effective drugs or compounds to induce the proliferation and differentiation of neural stem cells may

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be a solution to improve hippocampal neuron function and limb motor function in these cases.

Recent evidences have revealed that catalpol, a water-soluble active and glycosides compound purified from *Rehmannia glutinosa*, has a lot of pharmacological activities such as antioxidant and anti-inflammatory properties, antiapoptotic function, and neuroprotective effect [3–5]. It has been widely used in traditional Chinese medicine for the treatment of aging diseases and stroke; however, the exact mechanism in which catalpol protects the brain cells is not well understood. This compound has been proposed to cross the blood–brain barrier (BBB), promotes angiogenesis via the JAK2/STAT3 pathway, and improves neurodegenerative brain disorders such as Parkinson's disease and Alzheimer's and can improve memory [6–9]. Some studies revealed that catalpol decreases swelling of brain capillary endothelial cells [10], but enhances the number of synapses and neuronal axon growth [6, 11]. The other studies demonstrated that catalpol protects brain cells through down-regulation of oxidative stress and inflammatory mediators such as tumor necrosis factor-α (TNF-α) and nitric oxide synthase (NOS) expression [12]. Besides, catalpol has been shown to protect brain cells by increasing the activities of antioxidants, including glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx), which decrease lipid peroxidation and consequently preserve mitochondrial membrane potential [13, 14].

Catalpol has been proposed as a potential drug for the treatment of cerebral ischemia through protecting vascular volatilization, but its effects on hippocampal neurons have not been clarified yet [15–17]. An experiment study on rat models confirmed that catalpol promoted axonal regeneration and neovascularization of neurons and exhibited a protective effect on neurovascular units [18]. Since limb motor and hippocampal neuron functions may be affected in cerebral ischemia, we designed this study to evaluate the protective effect of catalpol against cerebral ischemia by analyzing the morphology and structure of hippocampal neurons, as well as limb motor function in rat models.

Materials and Methods

Animals

Ninety healthy male Sprague–Dawley, SD, rats (260–290 g) were purchased from Shanghai Biotechnology Co., Ltd. (*Shanghai*, China) in January 2018. This experimental study was approved by the Animal Care and Use Committee at Xinjiang Medical University (*Ürümqi*, China). Rats were adapted with lab environment for 1 week and then housed 3 per cage (30 × 15 × 15 cm) in a controlled room with adequate food and water, standard temperature and humidity (22 ± 2 °C and humidity $50\% \pm 5\%$, respectively), as well as a 12:12 light/dark cycle.

Induction of Cerebral Ischemia Model and Animal Grouping

Induction of cerebral ischemia was performed using the standard method under sterile conditions [19]. Briefly, 60 SD rats were randomly selected and anesthetized with 4% chloral hydrate through intraperitoneal injection and then a longitudinal midline ventral incision was made (2 cm to 3 cm in length) in the throat to expose the right common carotid artery, internal and external carotid arteries. Cerebral ischemia was created by occluding the internal carotid artery with processed fishing nylon. The external and internal carotid artery at the proximal end of the heart was ligated and then the distal end of the internal carotid artery was closed. A small incision was made at the intersection of the two and the middle cerebral artery was occluded with fishing nylon line leading to cerebral ischemia. After cerebral ischemia, the embolus was removed to allow the reperfusion of blood through carotid arteries. Rats were kept in pre-warmed cages and then returned to their cages and given food and water ad libitum. The other 30 SD rats were included in the sham group.

Hippocampal neuronal injury in model group was induced with injection of high blood glucose (250 mg/kg) [20], but animals in the sham group were injected with sodium citrate solution (250 mg/kg). High blood glucose-induced hippocampal neuronal injury was considered to be successful when animals in model group exhibited blood glucose > 1.1 mmol/L after 24 weeks.

Treatments

Rats in the sham group received saline perfusion (30 mg/kg/day) for 14 days. Animals in the ischemic group were randomly divided into observation ($n = 30$) and routine groups ($n = 30$). Rats in the routine group were injected with edaravone (7 mg/kg/day), while rats in the observation group were treated with catalpol (30 mg/kg/day) for 14 days.

Evaluation Indicators

The Sensation of the Limb Motor Function

The sensory status of nerve function was evaluated at 3, 7, and 14 days after catalpol treatments by horizontal wooden walking test. The higher the score, the better the limb motor function [21].

The Fine Motion Execution Capability

Tactile stimulation experiment was used to examine the fine motion execution capability of rats at the 3, 7, and 14 days after catalpol treatments [22]. The ventral side of the wrist of the ischemic contralateral forelimb was glued to the medical adhesive tape, and the time of removing the adhesive tape was recorded. A short time scale indicated the better somatosensory and fine motion execution capability.

Retention of Hippocampal Neurons

SD rats were sacrificed immediately after the treatment, and 1 mm^3 tissue lesion was selected for morphological and histological parameters by light microscope. Samples were dehydrated in graded series of ethanol, then embedded in paraffin, and finally sectioned using an microtome at 5 mm thickness. The sections then were stained with hematoxylin–eosin and investigated and photographed with a regional visual field, and the morphology, structure, and existence of rat neurons were observed within 200 times visual field.

Statistical Analysis

All quantitative data are presented as mean ± SD. One-way ANOVA: post hoc-Tukey test was used to compare the mean of all quantitative data with normal distribution between three groups. Data were analyzed using SPSS software (version 19) and a $p < 0.05$ was considered as significant.

Results

Limb Motor Function

Comparison of the sensory scores of limb motor function (SLMF) is shown in Table 1. A significant difference was observed in the mean score of limb motor function between groups. Control group exhibited the highest mean scores compared to the other groups. While no significant difference was found in the mean score of limb motor function from the day 3 to 14 in the control group, the mean SLMF in the both routine and observation groups were gradually improved. However, the sensory score of limb motor function in observation group was higher than that of the routine group in day 7 and 14 ($p < 0.001$).

Fine Exercise Execution Ability

Comparison of mean score of fine motor execution ability between each group is shown in Table 2. There was a significant difference in the mean score of fine motor execution ability between groups. While control group had significantly lower fine motor execution ability compared to the other groups in day 3 and 7 ($p < 0.01$), animals in observation group exhibited lowest fine motor execution ability in day 14 than other groups ($p < 0.01$). There were no significant differences in the time of releasing adhesive tape from day 3 to 14 ($p = 0.42$); in contrast, the time of releasing adhesive tape in the both routine and observation groups was gradually reduced ($p < 0.001$). Additionally, the time of releasing adhesive tape in the observation group at 3, 7,

Table 1 Comparison of sensory scores of limb motor function in each group

Groups	*n*	The feeling of the function of the limb			*F*	*p*
		3 days	7 days	14 days		
Control group	30	5.61 ± 0.24	5.58 ± 0.32	5.51 ± 0.21	1.16	0.3179
Routine group	30	2.26 ± 0.57	3.21 ± 0.33	3.47 ± 0.35	80.19	< 0.001
Observation group	30	2.35 ± 0.52	4.96 ± 0.38	5.12 ± 0.32	123.54	< 0.001
F		540.42	325.04	102.69		
p		< 0.001	< 0.001	< 0.001		

Table 2 Comparison of fine motor execution ability of rats in each group

Groups	*n*	Time of taking off the tape			*F*	*p*
		3 days	7 days	14 days		
Control group	30	1.61 ± 0.20	1.53 ± 0.31	1.58 ± 0.09	1.02	0.421
Routine group	30	4.26 ± 0.23	4.01 ± 0.36	3.42 ± 0.08	32.51	< 0.001
Observation group	30	4.13 ± 0.25	2.69 ± 0.34	1.25 ± 0.31	63.72	< 0.001
F		417.41	252.14	512.57		
p		< 0.01	< 0.01	< 0.01		

Table 3 Comparison of the number of hippocampal neurons retained in each group

Groups	*n*	Number of hippocampal neurons retained
Control group	30	15.69 ± 2.69
Routine group	30	10.25 ± 2.62
Observation group	30	13.87 ± 2.57
F		32.92
p		< 0.01

and 14 days was significantly lower than that in the routine group ($p < 0.01$).

The Number of Hippocampal Neurons Retained

Comparison of the mean number of hippocampal neurons retained between each group is summarized in Table 3. The mean number of hippocampal neurons retained was significantly higher in control group than that in observation and routine groups ($p < 0.01$). The mean number of hippocampal neurons retained in routine group was significantly lower in the observation group than that in the routine group ($p < 0.01$).

Morphological and Structural Analysis of Hippocampal Neurons

Histopathological examination of hippocampal neurons revealed that the cells were basically normal with partial disappearance of nucleus in the routine group, but the extracellular model was intact, and the mitochondria had phagocytic vesicles (Fig. 1). The hippocampal cell structure of the rats in the observation group was basically normal. The extracellular model and nuclear membrane were intact with only slightly deformed, and mitochondria were normal with only a few high-electron dense particles (Fig. 1). In the control group, the hippocampal nerve cells had unclear external model and severely damaged with nuclear deformation. Additionally, nuclear membrane was partially disappeared and revealed local edema in some parts. The organelle structures were difficult to recognize (Fig. 1).

Discussion

In this study, we investigated the protective effect of catalpa in improving limb motor function and hippocampal neuron function in rats with cerebral ischemia. Firstly, the catalpol effects on severe disability recovery were investigated. Our findings showed that repeated treatment with catalpa significantly improved limb motor function scores at 3, 7, and 14 days after cerebral ischemia, which indicates catalpol treatment in the early stage of cerebral ischemia can significantly improve the neurological function and consequently limbs motor function. Interestingly, with the time extending, the limited limb motor and sensory function was found to be restored in the model showing by the gradually reducing scores, which indicated that model rats exhibited an ability for self-repair and that catalpol could promote significant recovery of balance function in rats with cerebral ischemia. The results also shown that fine exercise execution ability of rats after repetitive treatment with catalpa was significantly improved, which emphasizes that catalpol can enhance the tactile sensitivity and promote the motor coordination of the model rats with cerebral ischemia. We also found that catalpol is more effective than conventional drugs in promoting neurological dysfunction in ischemic injury. Although a slight improvement was found in the mean number of hippocampal neurons after treatment with edaravone, the number of hippocampal neurons retained in the observation group was significantly more than that in the routine group. This data indicates that catalpol has strong effects in protecting the structure and quantity of hippocampal neurons against ischemic injury. The histopathological findings revealed that the hippocampal nerve structure of animals in catalpol group was normal and the nuclear membrane was complete and clear. Furthermore, the structure of mitochondria was in good condition. These data implicate that catalpol is a potential compound that protects the hippocampal nerve cell structure and function, prevents mitochondrial damage, and is beneficial for the recovery of cerebral ischemia. To support these findings, some studies reported the neuroprotective effect of catalpol. Wang et al. [23] showed that repeated treatments with catalpol not only improved neurological score but also enhanced neuronal cell activity, axonal regeneration, survive rate and promoted axonal growth of the neurons after stroke. They proposed that catalpol might improve neuronal cell activity and axonal regeneration by regulating PI3K/AKT/mTOR pathway. In another experimental study, Yang et al. [18] demonstrated that catalpol improved neurological function scores, attenuated inflammatory cell infiltration and demyelination by promoting the regeneration of mature oligodendrocytes via upregulation of Olig1 and Olig2 transcription factors in mice with multiple sclerosis. Zheng et al. [24] proposed that catalpol exerts neuroprotective effects through decreasing oxidative reactions, prohibiting apoptosis, and suppressing inflammatory reactions and autophagy in experimental rats with acute focal ischemic stroke. A previous study revealed that repeated treatments with catalpol significantly declined neurological deficits and significantly improved angiogenesis, but improved brain levels of erythropoietin and vascular endothelial growth factor without worsening BBB edema in rat model of stroke. Catalpol therapy has been also

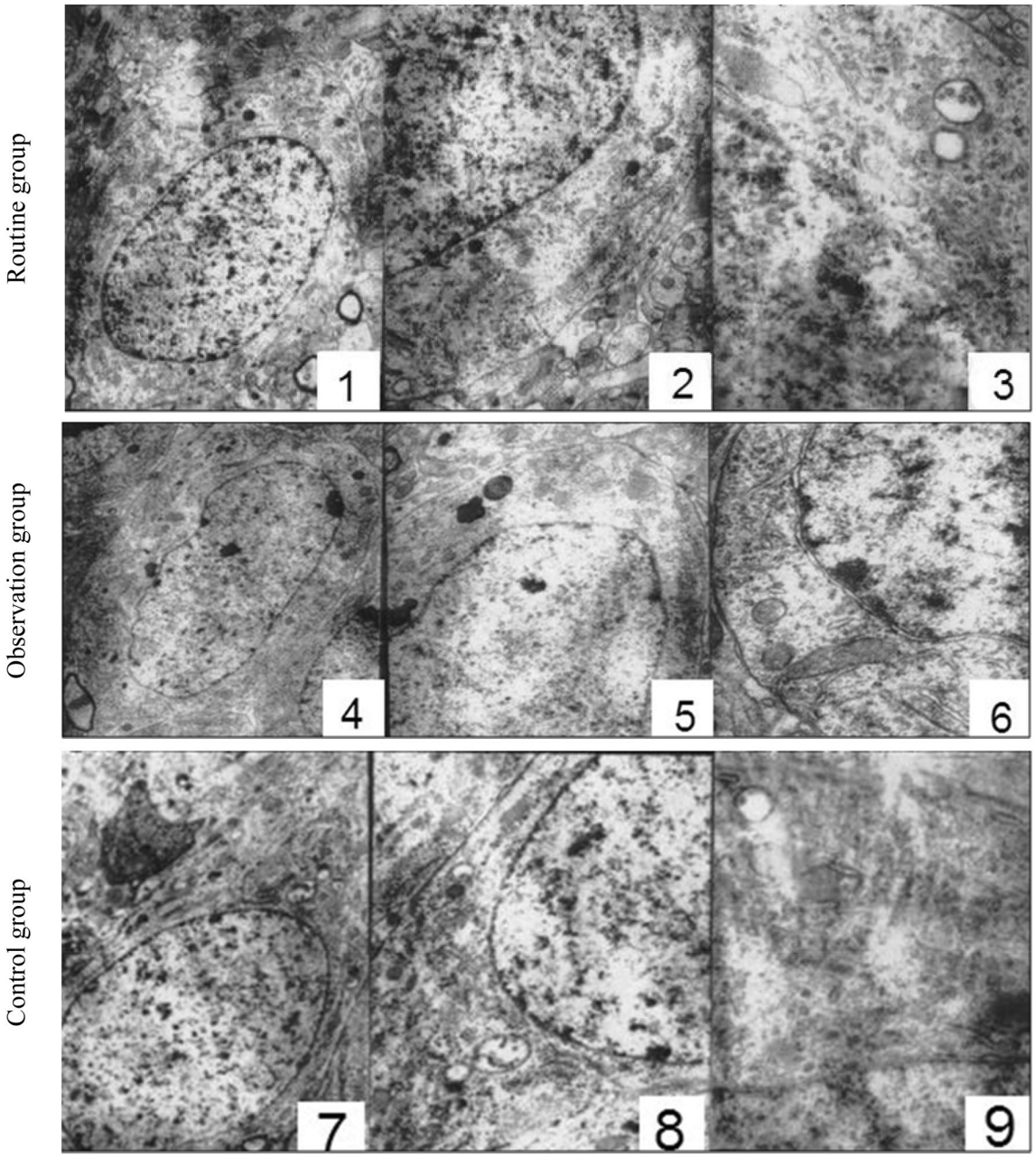

Fig. 1 Morphology and ultrastructure of hippocampal neurons in different groups. (1) Local disappearance of nuclear membrane, (2) less cell network, (3) vacuolated mitochondria, (4) cell with normal basic structure, (5) extracellular membrane, and nuclear membrane were intact and the nucleus was slightly deformed, (6) mitochondria without vacuoles, (7) most cells were deformed and a few of them were normal, (8) reduction in endoplasmic reticulum, and (9) there were more vacuoles and blurring in the mitochondria

shown to preserve neural function and pathological changes through mitigating of oxidative stress in mice with Alzheimer's disease [25]. A more recent study has demonstrated that catalpol treatment significantly improved the locomotor functional recovery, suppressed neuronal cells apoptosis, and alleviated inflammatory and oxidative response in mice with spinal cord injury [26]. Xu et al. [27] reported that catalpol treatment significantly improved the whole-body muscle health which was accompanied by enhanced grip strength, reduced fibrosis, reduced muscle fatigue, promoted muscle recovery, increased myoblast differentiation, and subsequently improved the function of dystrophic skeletal muscles in mice models with Duchenne muscular dystrophy. Therefore, according to the accomplished studies and our findings, catalpol may be a potential drug for the treatment of neurodegenerative diseases through improving limb

motor function and protecting ultrastructure of hippocampal neurons in rats with cerebral ischemia.

In conclusion, our findings revealed that catalpol not only has protective effects on ultrastructure of hippocampal neurons but also it can significantly improve the limb function and tactile sensitivity in rats with ischemic injury. The mechanism may be related to reducing the number of hippocampal neurons; protecting the integrity of capillary endothelial cells; promoting the survival, repair, and regeneration of neurons in hippocampal lesion areas of rats with ischemic injury. Thus, catalpol may be a promising candidate for clinical trials.

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Author Contributions SX, YZ, and BZ conceived and designed the experiments, performed the experiments and analyzed the data, and drafted the manuscript. The authors read and approved the final manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval All experiments were performed according to standard protocols, incompliance with the Guide of the Animal Ethics Committee of Xinjiang Medical University.

Consent for Publication Not applicable.

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