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Catalpol Exerts an Anti-Epilepticus Effect, Possibly by Regulating the Nrf2-Keap1-ARE Signaling Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Status epilepticus (SE) is a refractory neurological disease with high mortality and morbidity rates. SE can be induced by numerous factors, including oxidative stress. Catalpol has several biological activities, including regulating the oxidative stress response. However, the role of catapol in SE has not been fully elucidated.

Material/Methods: Thirty Wistar rats were randomly and equally divided into 3 groups: a control group, an SE group established by LiCl-pilocarpine intraperitoneal injection, and an SE+catalpol group established administering catalpol to SE rats. Epileptic seizure level and after-discharge duration (ADD) were analyzed. Cognitive function was assessed by Morris water maze. Myeloperoxidase (MPO) and superoxide dismutase (SOD) activities were tested. Keap1 and ARE mRNA expressions were detected by real-time PCR. Nrf2 protein expression was determined by Western blot.

Results: Catalpol significantly decreased epileptic seizure level, extended ADD, and improved cognitive function compared with the SE group ($P<0.05$). MPO was increased, SOD was reduced, Keap1 mRNA was upregulated, and Nrf2 protein and ARE mRNA were reduced in the SE group compared with the control group ($P<0.05$). Catalpol markedly decreased MPO, enhanced SOD activity, decreased Keap1 mRNA level, and elevated Nrf2 protein and ARE mRNA expressions compared with the SE group ($P<0.05$).

Conclusions: Catalpol plays an anti-epileptic role and improves cognitive function by regulating the Nrf2-Keap1-ARE signaling pathway to inhibit oxidative stress response.

MeSH Keywords: Cross Protection • Oxidative Stress • Status Epilepticus

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Background

Status epilepticus (SE) refers to repeated epilepsy attacks, including 30 min unconscious in intermission or seizures sustained for more than 30 min [1]. Different types of epilepsy can occur in SE; the most common is tonic-clonic seizure [2]. SE is a serious, refractory, neurological disease [3]. Epileptic seizure is still difficult to control after treatment, thus developing to refractory SE, leading to high mortality and morbidity [4,5]. SE can be induced by numerous factors, including calcium overload, glutamate excitability toxicity, and oxidative stress damage. They can trigger epilepsy by inducing neurons paradoxical discharge, but the specific mechanism has not yet been fully elucidated [6, 7]. Among these, oxygen free radicals produced by oxidative stress can lead to the loss of neurons and oxidative stress, becoming the core pathological link of SE [8,9].

Catalpol, the main effective component extracted from the traditional Chinese medicine Scrophulariaceae plant Radix rehmanniae, contains iridoid glycoside compounds [10]. It was shown that catalpol has various biological activities, including antitumor, antifungal, antivirus, antidementia, inhibiting capillary permeability, and anti-inflammatory reaction [11]. Catalpol plays a protective role in the oxidative stress damage model caused by H_2O_2 [12], but the specific role of catalpol in SE is still unclear. The Nrf2-Keap1-ARE signaling pathway plays a crucial role in antioxidative signaling and maintaining a balance between peroxides and antioxidants [13]. It was reported that the Nrf2-Keap1-ARE signaling pathway is involved in SE [14–16]. There are no previous reports on whether catalpol regulates SE through the Nrf2-Keap1-ARE signaling pathway. The present study investigated the role and mechanism of catalpol in SE by establishing a rat SE model.

Material and Methods

Experimental animals

Healthy male Wistar rats in SPF grade at 2 months old and 250±20 g were provide by Harbin Medical University. The rearing conditions were constant temperature at 21±1°C, constant humidity 50–70%, and 12 h day/night cycle.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Daqing Oilfield General Hospital (Daqing, Heilongjiang, China).

Main materials and instruments

Pentobarbital sodium and lidocaine were purchased from Pharma (Shanghai, China). Catalpol was bought from the National Institutes for Food and Drug Control. LiCl, bromine methyl scopolamine, and pilocarpine were from Sigma (USA). MPO and SOD activity detection kits, rabbit anti-mouse Nrf2 monoclonal antibody, and goat anti-rabbit HRP-labeled IgG secondary antibody were from Cell Signaling (USA). PVDF membranes were obtained from Pall Life Science. Western blot-related reagents were purchased from Beyotime (Shanghai, China). ECL reagent was bought from Amersham Biosciences. RNA extraction and reverse transcription kits were from ABI company (USA). Microscopic surgery instruments were from Suzhou Medical Equipment Factory (China). The Multi-Parameter Monitor and YC-2 stimulator were obtained from Yunani (Shanghai, China) and the DNA amplifier was from PE Gene Amp PCR System 2400. The Spectra Max Paradigm was obtained from Molecular devices (USA). Other reagents were purchased from Sangon (Shanghai, China).

Methods

Experimental animal grouping and treatment

The Wistar rats were randomly divided into 3 groups: a control group, an SE group established by LiCl-pilocarpine intraperitoneal injection, and an SE+catalpol group constructed by intraperitoneal injection of 5 mg/kg catalpol to SE rats at 30 min before modeling.

Rat SE establishment

LiCl-pilocarpine intraperitoneal injection was used to construct the rat SE model [17]. The rats were anesthetized by 30 mg/kg pentobarbital sodium intraperitoneal injection and fixed on a stereotaxic apparatus. The skull was exposed and opened by micro-drill. The electrode was placed into the hippocampus. After 2 weeks, the rats were treated with 1 mg/kg bromine methyl scopolamine and received 130 mg/kg LiCl after 30 min. Next, the rats were intraperitoneally injected with 50 mg/kg pilocarpine after 24 h to stimulate SE. The rats without convulsions were injected with 25 mg/kg pilocarpine again until SE appeared.

Epileptic seizure level ranking

Rat epileptic seizure was defined by Racine behavior grading [18]. No response was defined as grade 0. Rhythmic facial clonus, such as chewing, blinking, and moving whiskers was defined as grade 1. Rhythmic nodding based on grade 1 was defined as grade 2. Forelimb clonus based on grade 2 was defined as grade 3. Hind-limb standing based on grade 3 was defined as grade 4. Falling down and loss of balance based on grade 4 was defined as grade 5.

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Table 1. Primer sequences.

Gene	Forward 5'-3'	Reverse 5'-3'
GAPDH	AGTGCCAGCCTCGTCTCATAG	ACTTGCAACTTGCCGTGGGTAG
Keap1	TAAGAGGAACGGAATGTCATA	ACATCATCTATTCTCTCTATT
ARE	TCATCATCTAGCCTCTAGCT	ACTTGCTTGCACGGGCTTGCA

After discharge duration (ADD) measurement

ADD value was monitored for a period of 180 s as the total of monitoring time by connecting to a stimulator and electroencephalograph recorder.

Morris water maze detection

Place navigation and space probe tests in the Morris water maze were used for detection [19]. The escape latency was recorded for 6 continuous days (4 trials/day and 120 s/trial). The swimming time within the platform quadrant and the platform quadrant crossing times within 120 s were recorded to detect rat learning and memory abilities.

MPO and SOD activities detection

MPO and SOD activities in the hippocampus were detected according to the manual. Total protein was extracted and water-bathed at 95°C for 40 min. Then, the protein was washed and centrifuged at 4000 rpm for 10 min. The ethanol phase was extracted by ethanol-chloroform mixture (v/v, 5: 3) to test total SOD activity. The tissue was mixed with 30 mM H_2O_2 at pH 7.0 for 10 min. The reduction level of H_2O_2 was used to evaluate MPO activity changes by testing at 240 nm.

Real-time PCR

The hippocampus tissue was extracted and rinsed in liquid nitrogen. Total RNA was extracted by Trizol reagent and reverse-transcribed to cDNA (Table 1). Real-time PCR was used to test target gene expression. The reaction condition was composed of 52°C for 1 min, followed by 35 cycles of 90°C for 30 s, 58°C for 50 s, and 72°C for 35 s. GAPDH was used as an internal control. The relative expression was calculated by $2^{-\Delta Ct}$ method.

Western blot

The hippocampus tissue was rinsed in liquid nitrogen and lysed on ice for 15–30 min. Next, the cells were treated by ultrasonication at 5 s for 4 times. After centrifuging at 10 000 g and 4°C for 15 min, the supernatant was moved to a new Ep tube and quantified by Bradford method. The protein was separated by 10% SDS-PAGE and transferred to a PVDF membrane at 160 mA for 1.5 h. After blocking in 5% skim milk at room temperature for 2 h, the membrane was incubated in Nrf2 monoclonal antibody (1: 1000) at 4°C overnight. Then, the membrane was incubated in goat anti-rabbit secondary antibody (1: 2000) in the dark at room temperature for 30 min. Finally, the membrane was developed by ECL and analyzed by Quantity One software. Each test was repeated 4 times.

Statistical analysis

The data were analyzed on SPSS 16.0 software. Measurement data are depicted as mean ± SD. The *t* test was performed for comparison of differences between 2 groups and one-way ANOVA was used for comparisons of differences among multiple groups. $P < 0.05$ was considered as statistical significance.

Results

The impact of catalpol on SE rat general condition and epileptic seizure degree

The rats in the control group exhibited normal hair color, active spirit and action, and normal eating and drinking. The rats in the SE group had dull fur, hair loss, and lethargy. They gradually showed SE phenomena, such as nodding, head convulsions, fore-limb lifting, and even tonic-clonic seizures. The rats in the SE+catalpol group presented significantly more lustrous fur, less fur loss, more activity, and lower Racine degree compared with the SE group ($P<0.05$) (Figure 1).

The impact of catalpol on ADD in SE rats

ADD changes were detected on the 15th day after modeling. ADD was obviously elevated in the SE group, while its progression was markedly reduced in the SE+catalpol group compared with the SE group ($P<0.05$) (Figure 2).

The impact of catalpol on the learning and memory abilities in SE rats

Place navigation and space probe tests in the Morris water maze were used to analyze the influence of catalpol on the learning and memory abilities in SE rats. The escape latency significantly extended, while the platform region crossing times were obviously decreased in rats from the SE group compared

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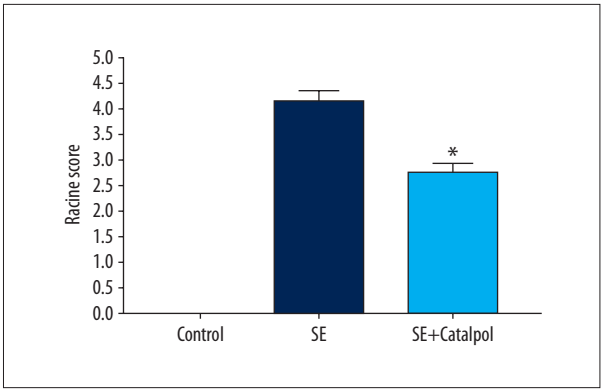

Figure 1. Effect of catalpol on Racine score in rats. * P<0.05, compared with SE group.

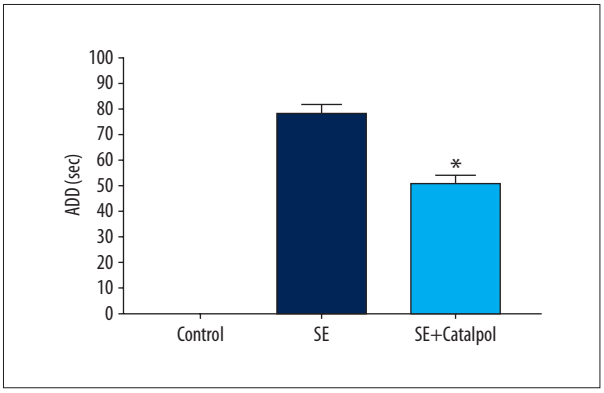

Figure 2. Effect of catalpol on ADD in SE rats. * P<0.05, compared with SE group.

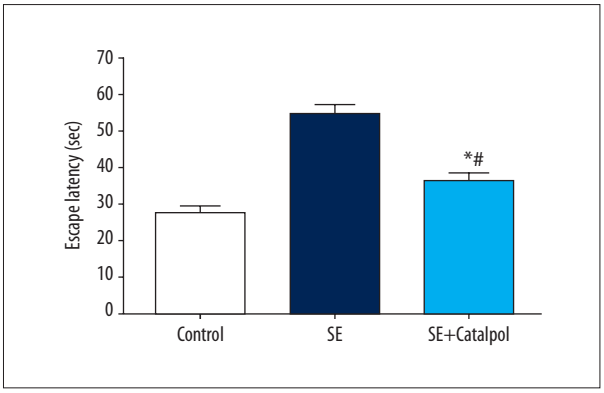

Figure 3. The changes of escape latency in Morris water maze. * P<0.05, compared with control. # P<0.05, compared with SE group.

with controls (P<0.05). Catalpol markedly decreased the escape latency and increased the platform region crossing times compared with the SE group (P<0.05) (Figures 3, 4).

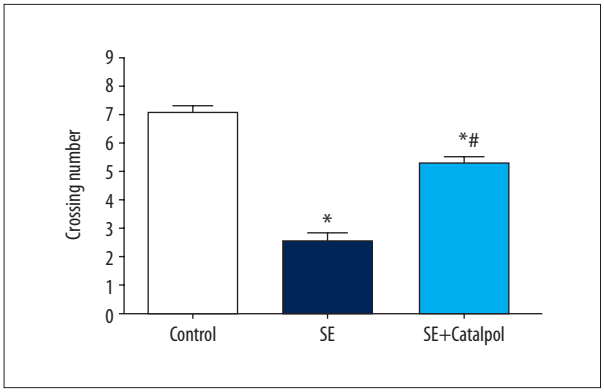

Figure 4. Crossing number in the space probe test. * P<0.05, compared with control. # P<0.05, compared with SE group.

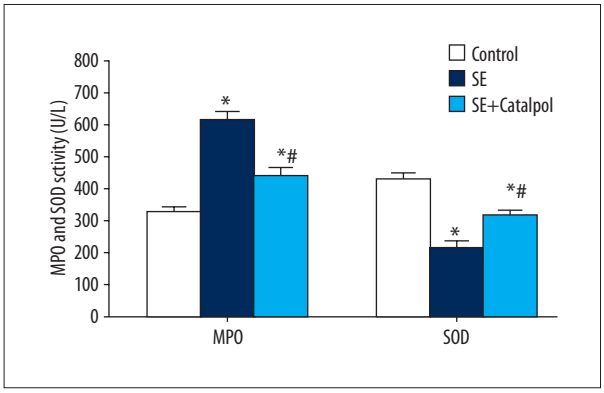

Figure 5. Effect of catalpol on MPO and SOD activities in hippocampus tissue. * P<0.05, compared with control. # P<0.05, compared with SE group.

The impact of catalpol on MPO and SOD activities in hippocampus tissue

MPO and SOD activities in hippocampus tissue were measured. MPO was significantly increased in the SE group compared with controls (P<0.05). Catalpol obviously decreased MPO activity in hippocampus tissue from the SE group (P<0.05). Contrary to MPO, SOD activity was reduced in the SE group, while catalpol markedly enhanced SOD activity in the SE group (P<0.05) (Figure 5).

The impact of catalpol on Keap1 and ARE mRNA levels in hippocampus tissue

Keap1 and ARE mRNA levels in hippocampus tissue were assessed. Keap1 expression was upregulated, while ARE level was markedly decreased in the SE group compared with controls (P<0.05). Catalpol significantly suppressed Keap1 expression and promoted ARE expression compared with the SE group (P<0.05) (Figure 6).

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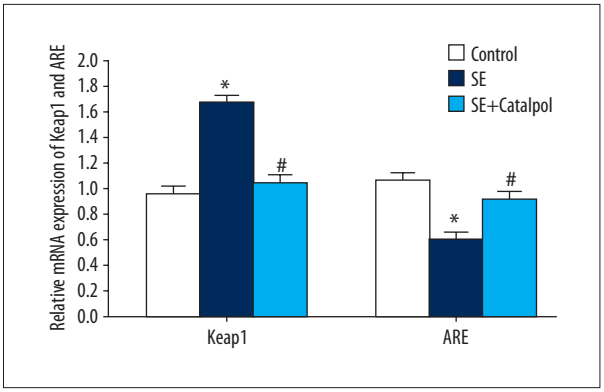

Figure 6. Effect of catalpol on Keap1 and ARE mRNA expressions in hippocampus tissue. * P<0.05, compared with control. # P<0.05, compared with SE group.

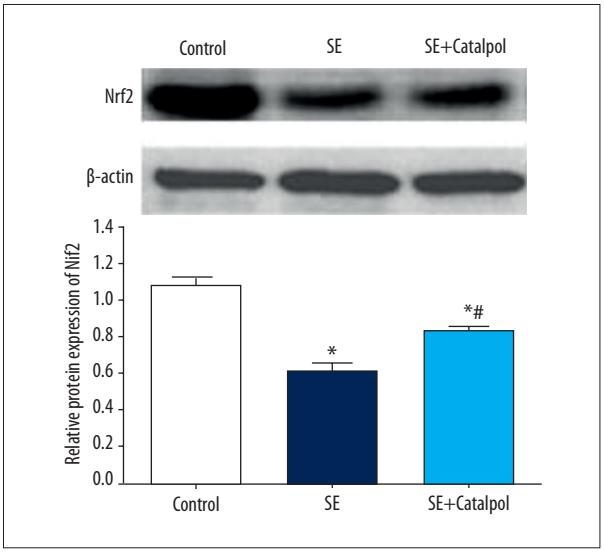

Figure 7. Effect of catalpol on Nrf2 protein expressions in hippocampus tissue. * P<0.05, compared with control. # P<0.05, compared with SE group.

The impact of catalpol on Nrf2 expression in hippocampus tissue

Nrf2 protein expression was tested in hippocampus tissue. Nrf2 expression was clearly downregulated in the SE group, whereas catalpol significantly facilitated Nrf2 expression compared with the SE group (P<0.05) (Figure 7).

Discussion

Status epilepticus (SE) is a single epileptic seizure lasting more than 5 min or 2 or more seizures within a 5-minute period without the person returning to normal between them. In recent years, it is believed that the initiation and propagation of paroxysmal discharges might be involved in the pathogenesis of SE [16]. Sustained neuronal electrical activity and seizures can lead to neuronal injury and death resulting from underlying biochemical mechanisms such as the formation of excessive ROS [20]. This leads to oxidative stress-induced abnormal structural alterations of cellular proteins, membrane lipids, DNA, and RNA, which can further damage brain tissues and lead to neuron death [21]. Therefore, antioxidants have been considered as therapeutic strategies for the treatment and modulation of epilepsy [22]. Nrf2 plays a key role in the antioxidation process [23]. Keap1, which is a chaperone of cytoplasmic protein, is a specific inhibitor of Nrf2. ARE in the nucleus is the promoter sequence of DNA that can be activated by Nrf2 to regulate antioxidant enzyme gene expression and participate in regulating oxidative stress level [24]. Under physiological conditions, Nrf2 closely combines with Keap1, which makes the Nrf2 in deactivation. Multiple factors can dissociate Nrf2 and Keap1 to induce binding between Nrf2 and ARE, such as electrophilic stress, oxidative stress, harmful substances, and metabolites [14]. The Nrf2-Keap1-ARE signaling pathway plays a protective role through regulating oxidative stress to protect against oxidation and harmful chemical substances, and it is one of the key endogenous antioxidation pathways [25]. Several previous studies demonstrated the close association of the abnormal Nrf2-Keap1-ARE signaling pathway with the development and pathogenesis of SE [14–16]. Consistent with this, in the present study, we found increased MPO level, decreased SOD activity, and enhanced Keap1 expression, as well as reduced Nrf2 and ARE expressions, in SE model rats, further supporting the role of oxidative stress in the pathogenesis of SE.

Catalpol, which is derived from traditional Chinese medicine, has various pharmacological activities. It plays a key role in anti-inflammation and redox equilibrium. It also protects neurons in nervous system diseases such as senile dementia and Parkinson's disease [26]. It is still unclear whether catalpol regulates SE through the Nrf2-Keap1-ARE signaling pathway. In this study, we found that catalpol markedly decreased epileptic seizure degree, extended ADD, improved rat learning and memory activities, reduced MPO level, enhanced SOD activity, decreased Keap1 mRNA levels, and elevated Nrf2 protein and ARE mRNA expression, indicating that catalpol can ameliorate SE, possibly through activating the Nrf2-Keap1-ARE signaling pathway, suggesting the neuroprotective effects of catalpol, which is consistent with a previous study demonstrating that catalpol facilitated neurological function recovery, reduced infarction volume, and increased cerebral blood flow, as well as decreasing the escape latency and increasing the numbers of platform crossings in stroke mice [27]. However, whether catalpol affects the transduction of other signaling pathway, such as ERK signaling [28] and astrocytic Ca2+ signaling [29], which have been shown to be involved in the pathogenesis of SE, was not investigated in the present study and require further research.

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Conclusions

Catalpol plays an anti-epileptic role and improves cognitive function, possibly through regulating the Nrf2-Keap1-ARE signaling pathway to inhibit the oxidative stress response. This study provides new treatment options and a theoretical basis for the treatment of SE in clinical practice.

Conflict of interest

None.

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