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Original Article

Senkyunolide A Affects the Development of Cerebral Ischemia Injury by Regulating Autophagy of Neuronal Cells

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ABSTRACT

Objectives: Cerebral ischemia is one cardio-cerebrovascular disease with the highest incidence, which will result in irreversible damage to brain function and even death. Rhizoma chuanxiong is a common Chinese medicine, and Senkyunolide A is one of its components, with a relatively high content in it. This study was for exploring the mechanism underlying the impact of Senkyunolide A on neuronal cells during Cerebral Ischemia Injury (CII). **Materials and Methods:** Through animal experiments, we found that after the treatment of Senkyunolide A, the neurological function of CII rats was significantly improved, and the apoptosis of neuronal apoptosis and autophagy were significantly inhibited. **Results:** These results suggest that Senkyunolide A can promote the recovery of neurological function after CII by inhibiting the autophagy of neuronal cells and improving their apoptosis. **Conclusion:** So, it may provide a new choice for clinical treatment of cerebral ischemia injury in the future.

Keywords: Cerebral Ischemic Injury, Senkyunolide A, Autophagy of Neuronal Cells, Neurological Dysfunction, Oxidative Stress.

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INTRODUCTION

Cardiovascular diseases are very common among middle-aged and elderly people and are characterized by high mortality, recurrence and disability rates.[1] Cerebral ischemia is one cardio-cerebrovascular disease with the highest incidence, which will result in irreversible damage to brain function and even death.[2] There are many inducing factors of cerebral ischemia, including age, hypertension, thrombosis, smoking, diabetes, emotional excitement, and fatigue.[3] Its symptoms primarily include dizziness, headache, unresponsiveness, neurological dysfunction and abnormal visual function.[4] At the current stage, conservative treatment is recommended for cerebral ischemia, that is, increasing perfusion, controlling the primary disease, and using drugs to yield higher life quality.[5] However, the prognosis of cerebral ischemia that is complex with many inducements is not highly satisfactory.[6] Thus, researchers are committed to searching an efficient and effective way against the disease.[7]

Neuronal cells are the most basic structural and functional units of the nervous system, which are capable of connecting and integrating input information and transmitting information.[8] One study by Luo *et al.*,[9] has revealed the association of biological behavior of neuronal cells with cerebral ischemia. Emerging evidence suggests that autophagy plays a dual role in cerebral ischemia. Moderate autophagy promotes cellular homeostasis, whereas excessive activation exacerbates neuronal injury by degrading essential proteins and organelles.[10] In cases with cerebral ischemia, autophagy of neuronal cells can accelerate their own apoptosis and induce Cerebral Ischemia Injury (CII).[11]

In recent years, the application of traditional Chinese medicine in the treatment of cerebral ischemia has received extensive attention. Rhizoma chuanxiong is a common Chinese medicine and Senkyunolide A is one of its components, with a relatively high content in it.[12] Previous studies have confirmed the protective effect of Senkyunolide A against cerebral ischemia in rats,[13] but the specific mechanism is not clear. Based on the above data, we inferred that Senkyunolide A may exert beneficial influences on alleviation of cerebral ischemic injury by regulating the autophagy of neuronal cells. Therefore, we carried out this detailed study, with the aim of offering new reference ideas for future clinical practice.

MATERIALS AND METHODS

Data about animals

Totally 30 purchased Sprague-Dawley (SD) rats (Shanghai Medicilon Biopharmaceutical Co., Ltd., animal experimentation

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license: SYXK (Hu) 2020-0038) at 8 weeks old (230 g-250 g) were raised adaptively for 2-3 days at (23±0.5°C) and (50±0.5%) humidity, with free access to light and drinking water.

Modeling methods

Rats were randomly divided into model (n=20) and control groups (n=10). Rats in the control group were raised normally. Rats in the model group were intraperitoneally injected with 10% chloral hydrate after being anesthetized. Then the hair on the top of the head was removed, and the site where the hair was removed was disinfected. Following anesthesia, the vertebral arteries within the bilateral transverse foramina of the first Cervical vertebra (C1) in rats were occluded using a high-frequency electrosurgical device. Middle Cerebral Artery Occlusion (MCAO) was induced using a 4-0 monofilament nylon suture (diameter 0.26 mm, Doccol Corp.) inserted 18np.) Inartery occlusion (MCAO) was induced using a 4-0 monofilament nyl4 hours of occlusion (total ischemia-reperfusion duration: 24 hr), bilateral common carotid arteries were transiently clamped with vascular forceps for 5 min. Animals exhibiting impaired consciousness, mydriasis, respiratory distress, reduced nociceptive responses, and absence of pupillary light reflexes post-procedure were considered successful models.

Intervention treatment

Rats in the model group were randomized into two groups (10 rats in each group). One group was injected with Senkyunolide A (40 mg/kg) as the treatment group, and the other group was injected with the same amount of normal saline as the model group.

Neurological function evaluation

The Neurological Function Deficit (NFD) score was adopted to evaluate the NFD of each rat (no nervous lesion and loss of consciousness were excluded). A higher NFD score indicates more severe behavioral disorder of animals).

Morris water maze test

A Morris water maze test model was established (diameter 120 cm, height 60 cm). The rats were let to adapt to the water environment at 24±2°C one day in advance. The navigation experiment lasted for 5 days, and each rat was trained 4 times a day with a training interval of 20 min. During the training phase, the platform location remained fixed in the target quadrant, and visual cues were placed around the maze to facilitate spatial learning. The water maze test was carried out on the 6th day, and the escape latency, platform crossing times, and quadrant residence time of each rat were recorded. Animals underwent four trials/day with a 20-min inter-trial interval, and platform location was randomized across trials.

Blood sample collection

Blood from rat tail vein was subjected to centrifugation for serum collection, followed by determination of serum Neuron-Specific Enolase (NES) and S-100 calcium binding protein β (S-100β) via ELISA.

Detection of Water Content (WC) in brain tissue

After anesthesia, rats were humanely euthanized via cervical dislocation. Brains were rapidly harvested and sectioned coronally at the pinhole level. Three-millimeter-thick slices were prepared by aspirating cerebrospinal fluid and blood from the surfaces, followed by immediate wet weight measurement. Tissue samples underwent natural desiccation for 24 hr prior to dry weight determination. WC was calculated using the formula:

WC (%) = [(Wet Weight - Dry Weight)/Wet Weight]×100

Determination of neuronal apoptosis

Brain tissue (30-50 mg) of each rat was taken, and lysed to prepare tissue homogenate. The cell concentration of the homogenate was adjusted to 1×10^6 cell/mL, followed by addition of 1 μL Annexin-V-FITC and 5 μL PI. Then the cells were subjected to 10 min incubation with dark surroundings, followed by addition of 400 μL buffer. Finally, a flow cytometer was used for apoptosis determination.

Autophagy determination

Total protein of brain tissue homogenate was obtained, followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transfer to a membrane. The membrane was treated with overnight incubation (4°C) after being diluted with Tris-Buffered Saline with Tween (TBST) to 1:1000 on a shaking table. On the next day, the membrane was cleaned via TBST, followed by addition of II antibody and development. Finally, the relative expression of protein was calculated.

Statistical analyses

In this study, all experiments were repeated three times. Statistical analyses were carried out using SPSS 22.0. Data were confirmed to conform to normal distribution by Shapiro-wilk test. The results were presented via (±s), and compared among multiple group via the One-Way Analysis of Variance and Bonferroni method. p<0.05 suggests a notable difference.

RESULTS

Neurological function evaluation of rats

According to evaluation results of NFD scores, the score of the model group (2.88±0.65) points was notably higher than that of the treatment group (1.03±0.47) points and the control group (1.01±0.46) points (both p<0.05), but the score of treatment group and the control group was not greatly different (p>0.05).

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Morris water maze test

As shown in Figure 1, the escape latency, quadrant residence time, and platform crossing times of the three groups of rats were recorded. The treatment group experienced notably shorter escape latency than the model group and longer escape latency than the control group (p<0.05), and the quadrant residence time and platform crossing times of the treatment group were both higher than those of the model group and lower than those of the control group (p<0.05).

Changes of NES and S-100β levels in rats

In Figure 2, compared to the model group, the treatment group exhibited reduced NES and S-100β levels (p<0.05), though these values remained elevated relative to the control group (p<0.05).

Detection of WC in brain tissue of rats

In Figure 3, the WC of the brain tissue in the control, model, and treatment groups was (65.15±4.02%), (85.87±5.31%), and (74.19±2.84%), respectively. According to comparison of WC in brain tissue among the three groups, the treatment group showed lower WC in brain tissue than the model group, but higher WC in brain tissue than the control group (p<0.05).

Apoptotic activity in the treatment group was attenuated compared to the model group (p<0.05) but exceeded that of the control group. Additionally, pro-apoptotic protein expression was downregulated, while anti-apoptotic proteins were upregulated in the treatment group versus the model group (p<0.05), with no significant differences observed between the treatment and control groups (p>0.05).

Influence of Senkyunolide A on autophagy of neuronal cells

Finally, as shown in Figure 4, the Beclin-1 and LC3 levels in the treatment group were significantly lower than those in the model group (p<0.05) and comparable to the control group (p>0.05).

DISCUSSION

Cardio-cerebrovascular diseases are pervasive and frequently-occurring maladies that endanger human life and health and also the main diseases that kill and maim the middle-aged and the elderly.[14] In China, an annually increasing number of patients suffer cardio-cerebrovascular diseases and increasingly younger people suffer them.[15] Senkyunolide A has been verified to be able to greatly increase cerebral blood flow and reduce vascular resistance,[16] but there are few experimental research data about it, so its application value in cerebrovascular diseases cannot be fully reflected. Therefore, this study probed into the influence of Senkyunolide A on the development of CII by regulating the autophagy of neuronal cells.In this study, SD rats were first purchased for modeling. Rats in the control group were fed normally, and modeled rats were let to develop into cerebral ischemia rats and then assigned to a model group or a treatment group. The treatment group was injected intravenously with Senkyunolide A. The NFD scores of the three groups were evaluated. As a result, the model group got notably higher scores than the treatment group and the control group, and the score of treatment group and the control group was not greatly different. The results showed that the rats were modeled successfully, and the neurological deficit of rats with cerebral ischemia was alleviated greatly. In addition, the results also preliminarily confirmed a definite effect of Senkyunolide A on cerebral ischemia. An earlier study also found that Senkyunolide A reversed interstitial renal fibrosis in unilateral ureteral obstruction rats, and exert a therapeutic effect on migraine.[17] This may be attributed to the efficacy of Rhizoma chuanxiong in promoting blood circulation, dispelling wind and relieving pain.[18] In addition, we detected the nerve injury markers of the three groups of rats, and found decreases in NES and S-100β levels in the rats intervened with Senkyunolide A, which also fully suggested the strong effect of Senkyunolide A in alleviating the NFD under cerebral ischemia. NES and S-100β are specific markers for cerebrovascular damage.[19,20] NES and S-100β are extremely sensitive for nerve injury.[21] However, the mechanism underlying the down regulation of Senkyunolide A on NES and S-100β is still unclear. Thus, we further tested the WC in brain tissue of rats, and found that the WC of the treatment group decreased greatly. In one

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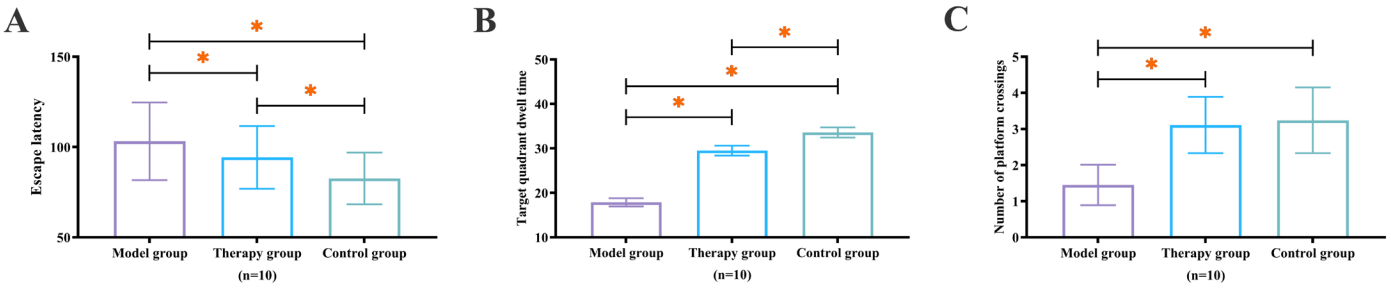


Figure 1: Morris water maze test. A) Escape latency of the three groups. B) Targeted quadrant residence time of the three groups. C) Platform crossing times of the three groups. Note: * p<0.05.

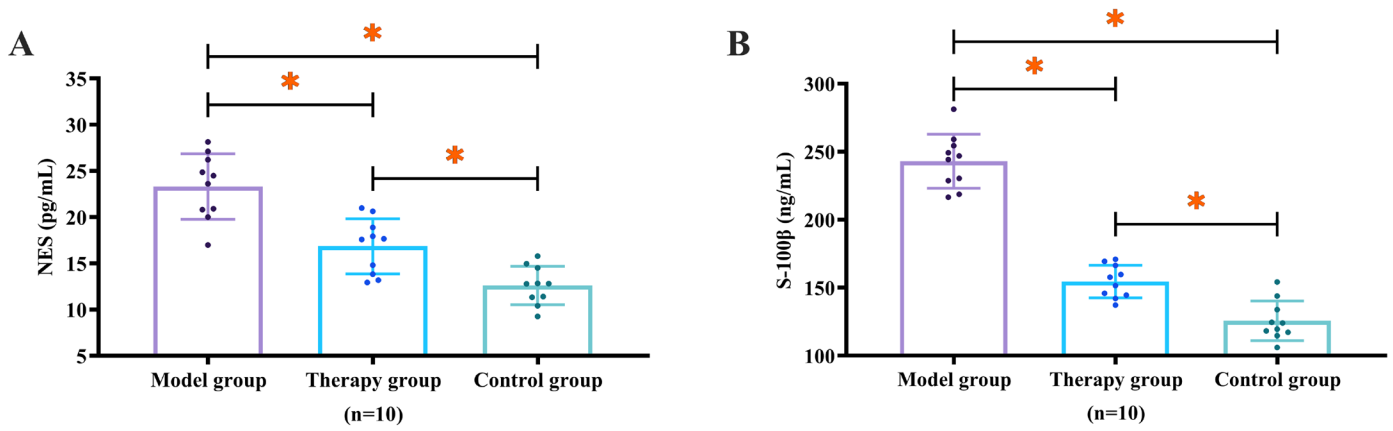


Figure 2: Changes of NES and S-100β levels in rats. A) NES level in the three groups. B) S-100β level in the three groups. Notes: * $p<0.05$.

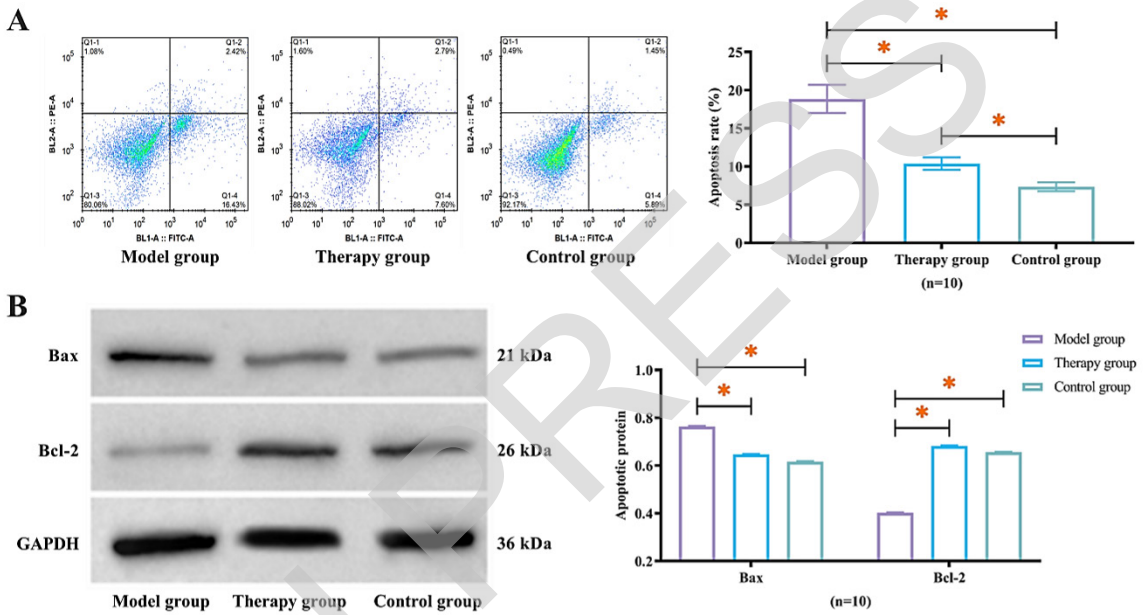


Figure 3: Influence of Senkyunolide A on neuronal apoptosis. A) Apoptosis rate of neuronal cells. B) Apoptosis-associated proteins. Notes: * $p<0.05$.

previous study, Senkyunolide A exerted a certain inhibitory and inductive effect on erythrocyte deformation index and orientation index.[22] In some cases, the ability of red blood cells to deform is greatly reduced and they are unable to complete normal blood circulation.[23] Then a large number of erythrocytes gather and accumulate at the Blood-Brain Barrier (BBB) and then form thrombus, which is also the initial pathological manifestation of brain tissue embolism.[24] At this time, the brain tissue of patients will have obvious damage due to ischemia and hypoxia, and the permeability of BBB is thus significantly enhanced.[25] Due to the increase of the permeability of the BBB, the hypoxia and edema of capillaries caused the increase in WC of brain tissue.[26] The decrease in WC of brain tissue in the treatment group also fully indicated that Senkyunolide A had a remarkable effect on the improvement of brain tissue microenvironment in cases with brain defect. Finally, we determined the neuronal cells of rats, finding that Senkyunolide A could strongly inhibit neuronal apoptosis and expression of autophagy-associated proteins. As we all know, autophagy is a crucial process of evolutionarily conservative turnover of intracellular substances in eukaryotes, and cells complete self-differentiation and apoptosis through it.[27] Beclin-1 and LC3, as the most classical autophagy-related markers, can accurately reflect the autophagy in cells.[28] Western blot analysis revealed that Senkyunolide A reduced the LC3-II/I ratio in model group and Beclin-1 expression, indicating suppression of autophagosome formation. In previous studies, Senkyunolide A has also been verified to have certain influence on the autophagy of myocardial ischemic cells, which once again supports our experimental results.

However, due to the limited experimental conditions, we are still unclear about the action pathway underlying the influence of Senkyunolide A on the autophagy ability of neuronal cells. In addition, due to the differences between animal models and humans, the effect of Senkyunolide A in the actual clinical

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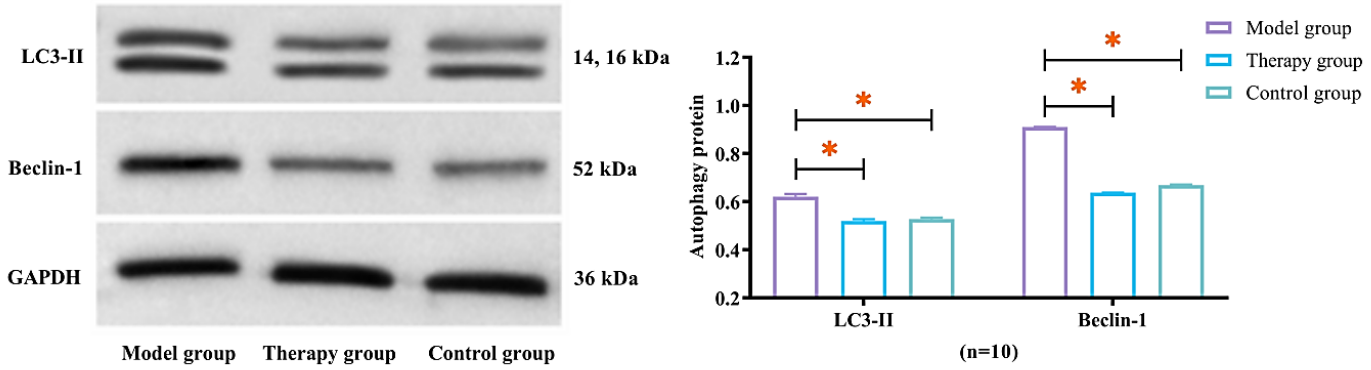


Figure 4: Influence of Senkyunolide A on autophagy of neuronal cells. A) Expression of Beclin-1. B) Expression of LC3. Notes: *p<0.05.

treatment has not been determined, and the dosage needs to be further studied. This study focused exclusively on male SD rats, which may limit the generalizability of findings to female populations due to sex-specific neuroprotective responses. While this study demonstrates the neuroprotective effects of Senkyunolide A in CII rats, its clinical translation hinges on critical pharmacokinetic parameters. Future studies should explore nanoformulations (e.g., lipid nanoparticles) to enhance brain accumulation and optimize dosing regimens. We will continue to carry out more in-depth and perfect experimental analysis on the application of Senkyunolide A to obtain the best experimental results for clinical reference.

CONCLUSION

To sum up, Senkyunolide A can promote the recovery of neurological function after CII by inhibiting the autophagy of neuronal cells and improving their apoptosis.

ABBREVIATIONS

CII: Cerebral Ischemia Injury; **SD:** Sprague-Dawley; **NFD:** Neurological Function Deficit; **NES:** Neuron-Specific Enolase; **S-100β:** S-100 calcium binding protein β; **TBST:** Tris-Buffered Saline with Tween.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING STATEMENT

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

CG.Z conceived and designed the study, YL.W wrote and revised the manuscript, FJ.L. collected and analyzed data, HM.C. supervised the study. All authors read and approved the final submitted manuscript.

ETHICAL APPROVAL

All the experimental procedures involving animals were conducted in accordance with ARRIVE guidelines and approved by the Animal Ethics Committee of The First People's Hospital of Chuzhou (No. 20240910).

SUMMARY

By establishing a rat model of Cerebral Ischemic Injury (CII) and treating it with Senkyunolide A intervention, we found that Senkyunolide A improved the neurological function of CII rats, while inhibiting both apoptosis and autophagy of their neuronal cells were suppressed. These results confirm the potential therapeutic value of Senkyunolide A for CII.

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