Effect of total flavonoids of *Scutellaria barbata* on cognitive function and nogo-A expression in the hippocampus in cerebral ischemia model in gerbils

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Abstract: To explore the effect of total flavonoids of *Scutellaria barbata* on cognitive function and nogo-A expression in the hippocampus region in cerebral ischemia model in gerbils. 30 gerbils were randomly divided into model group, sham operation group, large dose of total flavonoids of *Scutellaria barbata* group (large dose group), middle dose of total flavonoids of *Scutellaria barbata* group (middle dose group) and small dose of total flavonoids of *Scutellaria barbata* group (small dose group), with 6 cases in each group. All the groups except the sham operation group were received bilateral common carotid artery ligation to established the cerebral ischemia model in gerbils. After that, the large, middle and small doses groups were given 400mg/kg, 200mg/kg and 100mg/kg of total flavonoids of *Scutellaria barbata* respectively, while the other two groups were injected with sodium chloride for 4 continuous weeks. At the 5^{th} and 8^{th} week after modeling, the cognitive function (e.g. escape latency period and original platform crossing times) of the gerbils in the three groups were detected by Morris water maze test. Moreover, the nogo-A expressions in the hippocampus region were detected by immunohistochemical staining method at the 8^{th} week. The escape latency period and platform crossing times at the 5^{th} and 8^{th} week after modeling in the large dose group were significantly higher than the rest groups (except slam operation group) ($p<0.05$), while the difference was not significant when compared with slam operation group ($p>0.05$). The difference of the gray value of nogo-A positive cells in hippocampus in the large dose group was not significant compared with middle dose group and sham operation group ($p>0.05$), while it was significant compared with model group and small dose group ($p<0.05$). Large dose of total flavonoids of *Scutellaria barbata* can obviously improve the cognitive function in cerebral ischemia model in gerbils by reducing nogo-A expression in the hippocampus region.

Keywords: Total flavonoids of *Scutellaria barbata,* cerebral ischemia, cognitive function, nogo-A expression.

INTRODUCTION

Cerebral ischemia is caused by various factors, like vessel occlusion, stenosis and convulsion of the vertebro-basilar artery or internal carotid artery system leading to cerebral insufficient blood supply, thus resulting in cerebral hypoxia ischemia and brain dysfunction disease (Song *et al*., 2012). The earliest manifestations of cerebral ischemia is cognitive impairment. The cognitive impairment and post-onset movement disorders are all showing a progressive trend, which seriously affect the quality of life of the patients and their families (Hu *et al*., 2010). Nogo-A in Hippocampus region can inhabit axon growth, cell migration and adhesion during the central nerve damaging and repairing process, which is now regarded as the most powerful axon anti-regeneration protein and also closely related to the cognitive function. *Scutellaria barbata* belongs to the labiatae herbs, with the function of anti-cancer, diuresis detumescence, eliminating stagnant blood and stopping bleeding, clearing heat and removing toxicity etc. The contained flavonoids are effective pharmaceutical ingredients in the treatment of cardiovascular diseases, which can eliminate free radicals, inhibit platelet aggregation and cell apoptosis, and meanwhile have a significant role in regulating blood lipid and anti oxidative damage (Li *et al*., 2010). The aim of this study is to establish the model of gerbil, to observe the changes of cognitive function and nogo-A expression of total flavonoids of *Scutellaria barbata* and to explore the mechanism of total flavonoids of *Scutellaria barbata* to recover the cognition function in gerbils.

MATERIAL AND METHODS

General information

30 male gerbils at VAF (Virus Antibody Free) level were selected, with weight 250-320g and 13-15 weeks. They were put into the Laboratory Animal Room with 45-50% humidity, 20-25□ for 12 h alternate illumination. All the animals were provided by Laboratory Animal Center of Henan University of TCM. Animal permit number: SYXK (Sichuan) 2013-2181.

Reagents and instruments

The alcohol extraction methods were used to extract the total flavonoids from the *Scutellaria barbata*, with content 35.24%. Sodium Chloride Injection (specification: 100mg, batch number: H20056626, manufacturer:

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Sichuan Koren Pharmaceutical Co., Ltd.); primary nogo-A goat anti-rabbit polyclonal antibody (Wuhan Aobosen Medical Technology Co., Ltd.); DAB chromogenic reagent kit (Fuzhou Maixin Biological Technology Co., Ltd.); SP staining kits (Beijing Jinqiao Co., Ltd.); Morris water maze (Changsha Changjin Technology Co., Ltd); thermostatic drum wind drying oven (ZKX250, Shanghai Yuejin Medical Instrument Co., Ltd.); paraffin slicing machine (RM2245, Germany LEICA Co., Ltd.); binocular microscope (DM1000, Germany LEICA Co., Ltd.).

Method of extraction of flavanoid

Scutellaria barbata powder 2.0g was accurately placed in the conical flask To proceed to ultrasonic extraction under the conditions of ultrasonic generator fixed power 250W. The filtrate were degreased and decolorized after treatment by petroleum-diethyl ether, and diluted with water to 100 ml, which was the extracting solution.

Animals grouping and dosing methods

The animals were randomly divided into the model group, slam operation group, large dose group, middle dose group and small dose group, with 6 cases in each group. After 24 h modeling, the large, middle and small groups were injected into the abdominal cavity of total flavonoids of *Scutellaria Barbata* with 400 mg/kg, 200 mg/kg and 100 mg/kg respectively for once a day and 4 successive weeks. Meanwhile model group and slam operation group were injected with sodium chloridefor once a day and 4 successive weeks.

Preparing cerebral ischemia model

Gerbils were not allowed to drink water before operating. They were cut up in the middle of the neck after inducing anesthesia, and dissociated the bilateral common carotid artery, followed with double ligated (proximal end and distant end), and then cut off in the middle. Then were sutured layer by layer after ensuring no blood flow. While the slam operation group was only received the bilateral carotid arterio-dialysis without ligation.

Behavioral trail

Orientation navigation experiment: Gerbils were placed into water for 4 consecutive days at different time points, and the of finding the platform within 1 minute was recorded, which was the escape latency period. If they did not find the platform within the specified time, they would be guided into the platform, which the escape latency period was record as 1 min. Spatial probe test: The platform was removed on the 5^{th} day, and the platform crossing times were recorded. The two trials were conducted in the 5^{th} week and 8^{th} week once a time respectively.

Immunohistochemical analysis

After finishing the Morris water maze trial, the gerbils were induced abdominal cavity anesthesia with 7% chloral hydrate, with the heart exposed, the left ventricle punctured, and the right auricle cut. 200 ml normal saline was poured into the left ventricular untill clear liquid flowed out. 200ml 4% paraformaldehyde was used to fix the brain tissue, and then cut off the head and take off the brain. The brain tissue of gerbils was fixed into 4% paraformaldehyde for 1 day and preserved at -80^{o} refrigerator and embedded conventionally with paraffin. Then the prepared brain samples were cut into 4 μm serial sections by immunohistochemical method, with the nogo-A expression in one section detected. All the processes (including gradient hydrating, antigen retrieval, incubating and PBS flushing etc.) were based on the Experimental Manuals of Modern Molecular Biology (Zhang, 2007). All the sections were conducted in the same condition and processes. The detection process was strictly accordance with the specifications in the kits. At least 5 fields of vision were selected for each slice under a microscope, and then the image analysis system was used for optical density analysis.

Observation indexes

In this study, the escape latent periods and platform crossing times were observed to evaluate the cognitive function and the positive cells of nogo-A in the hippocampus region of the gerbils was detected by immunohistochemical method.

STATISTICAL ANALYSIS

Statistical software SPSS20.0 was used for data analysis. The measurement data was checked by *t*-test and expressed by ($\bar{x}\pm s$), and $p<0.05$ meant that the difference was statistically significant.

RESULTS

Morris water maze test outcomes

After 8 weeks treatment, there were significant difference in the escape latency periods and platform crossing times in large dose group, compared with the rest groups ($p<0.05$), except sham operation group ($p>0.05$). Seen in table 1.

Gray value of the Nogo-A positive cells in hippocampus region in gerbils

It had no significant difference in the gray value of the Nogo-A positive cells in the hippocampus region in Large group, compared with the sham operation group and Middle group ($p>0.05$), but with significant difference comparison with the model group and Small group ($p < 0.05$) (table 2).

DISCUSSION

Focal cerebral ischemia will lead to hypoxia and ischemia in brain tissue. Once the cerebral blood flow is completely blocked, it's just a few seconds that will involve the brain

Table 1: The outcomes of Morris water maze test

Groups	N	Escape latent period (s)		*t*	*p*	Original platform crossing times		*t*	*p*
		5th week	8th week			5th week	8th week		
Model group	6	30.31±5.84	38.42±4.27	4.100	0.000	4.06±1.47	2.48±1.25	3.275	0.001
Sham operation group	6	16.51±3.23^{abC}	13.54±3.42^{abC}	1.547	0.122	6.23±1.93^{abC}	7.46±1.85^{abC}	1.127	0.260
Large dose group	6	19.17±5.81^{abC}	17.59±5.60^{abC}	0.783	0.434	5.87±2.56^{abC}	7.26±1.72^{abC}	1.104	0.270
Middle dose group	6	22.66±5.70^{ab}	19.16±5.43^{ab}	1.711	0.087	4.85±2.71^{ab}	6.49±1.83^{ab}	1.229	0.219
Small dose group	6	28.61±4.38	25.45±5.27	3.989	0.000	3.07±1.69	4.32±1.67	2.320	0.020

Table 2: Gray value of Nogo-A positive cells in the hippocampus region in gerbils

Groups	N	Nogo-A
Model group	6	23.81±6.08
Sham operation group	6	10.11±3.16^{abC}
Large dose group	6	11.75±3.08^{ab}
Middle dose group	6	12.08±4.76^{ab}
Small dose group	6	21.66±4.15

Note: compared with the model group, $^{a}p < 0.05$; compared with the small dose group, $^{b}p < 0.05$; and compared with middle dose group, $^{C}p<0.05$.

neurons. Numerous clinical studies have shown that brain energy metabolism and brain function are closely related, and the former can be a direct response to the latter (Li *et al*., 2011; Dehghani *et al*., 2016; Jiang *et al.*, 2013). Some scholars have made the cerebral ischemia model of bilateral carotid artery occlusion and found that sever cerebral ischemia will lead to the activity of acetylcholine esterase (AchE) and lactate dehydrogenase (LDH) decreased, while lactic acid (LD) activity increased and Adenosine triphosphate (ATP) used up, eventually resulting in brain cells energy metabolism disorder (Tutino *et al.*, 2016). Usually, the electrical activity of brain will stop after 2 min complete block of cerebral blood flow (CBF), and ionic equilibrium and cerebral energy metabolism will be broken after 6 min. As time goes on, brain cell membrane ion pump will appear dysfunction, large depletion of ATP, external flow of K^+, inflow of large amount of water, CI^- and Na^+. General cerebral ischemia generally last about 10 minutes, and it will cause irreversible damage to brain neurons (Zhu *et al*., 2010).

Bilateral carotid artery block method is often used in animal models of cerebral ischemia, and Morris water maze mainly study the learning and memory ability of gerbils, both which belong to the advanced cognition activities (Chen *et al*., 2013). The results of Morris water maze test showed that the learning and memory ability of gerbils were increased significantly with the prolongation of ischemic brain compared with the sham operation group, indicating there was severe cognitive impairment. The necrosis degree of nogo-A positive cells in the hippocampus of the model group was significantly higher than that in the sham operation group, large, middle and small dose group. Such changes in gerbils were consistent with their behavior in Morris water maze as well as the findings results of Zhenhong Wang and other scholars (Wang and Wang, 2013). These findings showed the accuracy of the results of this study on one hand, also showed the severity of brain injury caused by cerebral ischemia on the another hand. The hippocampus region in the brain mainly plays a narrative memory role and stores the short-term memory. The synaptic plasticity will be affected when hypoxic-ischemic injury occurs, causing progressive cognitive impairment (Caltagirone *et al*., 2016). Axonal regeneration in the nerve is an important part of nerve function recovery, and axonal nerve regenerated and re-linked with the surrounding tissue can induce nerve remodeling. The key factor of nerve regeneration is the nogo-A protein in hippocampus,, which can combine with the receptor specificity, inhibit cytoskeleton remodeling, induce growth cone collapse, block axon growth, and prevent nerve regeneration. In clinical practice, Nogo-A neutralizing antibody is usually used to treat nerve cell damage, which can decrease its expression and induce the nerve remodeling.

The results of this study showed that the nogo-A positive cells in the model group was higher than that in the sham operation group, indicating that nogo-A prolonged blockade of nerve fiber growth is an important cause of cognitive disorders, which is consistent with the results reported by Min Zhang *et al* (Zhang *et al*., 2015). The number of nogo-A positive cells in the large, middle and small dose group were all lower than that in the model group, with the large dose group as lowest which was consistent with the results of platform crossing times of Morris mazz water test after 8 weeks modeling. In addition, the number of nogo-A positive cells in the large dose group was not significantly different from that in the sham operation group, indicating that 400 mg/kg total flavonoids could significantly reduce the expression of nogo-A, improve cognitive function and reduce brain damage. History Medical literature recorded that

Scutellaria barbata were mainly used for the injury by snakes. While modern pharmacological studies showed that *Scutellaria barbata* was mainly composed of total flavonoids, which had a good oppose effect on expectoration and smooth muscle contraction. Moreover, it could reduce blood sugar level as well as the incidence of urinary infection. Furthermore, the promoting role of this drug on smooth muscle could also promote the cell immunity (Zheng *et al*., 2010; Liao *et al*., 2012). The results of this study confirmed that total flavonoids of *Scutellaria barbata* could reduce cerebral ischemia damage by lowering the expression of nogo - A, so as to play a protective role in brain, with 400 mg/kg dose as the best.

CONCLUSION

In summary, total flavonoids of *Scutellaria barbata* can somehow reduce nogo-A expression, and improve the cognitive function of gerbils with cerebral ischemia, but its specific mechanism and action pathway still need to be further studied.

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