

Original Article

Puerarin alleviates cognitive dysfunction in rats after anesthesia with propofol

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Received November 13, 2018; Accepted January 8, 2019; Epub March 15, 2019; Published March 30, 2019

Abstract: *Backgrounds:* Cognitive dysfunction is a complication of anesthesia in surgery. It not only affects the outcome of the surgery, but also increases the patient's mortality rate. This study aimed to investigate the effects of puerarin on cognitive dysfunction in rats after anesthesia with propofol. *Methods:* Sixty rats were randomly divided into control, model, and low-, middle- and high-dose puerarin groups. The model of cognitive dysfunction after anesthesia was established by intraperitoneal injection of propofol. The low-, medium- and high-dose puerarin groups were intraperitoneally injected with 10, 20 and 40 mg/kg puerarin, respectively. After treatment, animal behaviors were tested. Serum amyloid β -protein (A β) and neuron-specific enolase (NSE) levels, hippocampal nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) levels, hippocampal cAMP-response element binding protein (CREB) and extracellular signal-regulated kinase (ERK) proteins and their phosphorylated forms (p-ERK and p-CREB) were determined. *Results:* Compared with the model group, in the high-dose puerarin group the escape latency was significantly decreased ($P < 0.05$), the platform crossing times were significantly increased ($P < 0.05$), serum A β and NSE levels were significantly decreased ($P < 0.05$), hippocampal NGF and BDNF levels were significantly increased ($P < 0.05$), and p-CREB and p-ERK protein expression levels were significantly increased ($P < 0.05$). *Conclusion:* Puerarin can alleviate the cognitive dysfunction after anesthesia in rats, which may be related to its decrease of the A β and NSE levels, increase of hippocampal NGF and BDNF levels, and increase of hippocampal p-CREB and p-ERK protein expression.

Keywords: Puerarin, cognitive dysfunction, anesthesia, propofol

Introduction

With the continuous progress of science and technology and improvement of medical level, more and more diseases can be well treated through surgery. Anesthesia is a very important link during surgery. The selection and operation skills of anesthetics directly affect the success or failure of the surgery. Anesthesia is also accompanied by complications, in which cognitive dysfunction is more common. It not only affects the outcome of the surgery, but also increases the patient's mortality rate [1, 2]. Currently, many medical workers are working hard to solve this problem. Propofol is one of the most commonly used anesthetics. It exerts the sedative and hypnotic effect by activating the gamma-aminobutyric acid receptor-chloride chan-

nel complex. Propofol is widely used in anesthesia induction, anesthesia maintenance, and sedation for critical patients in the intensive care unit [3-5]. Propofol has the advantages including rapid effectiveness, smooth induction, rapid recovery, complete functional recovery, and low incidence of postoperative nausea and vomiting. However, cognitive dysfunction after anesthesia also often happens with propofol [6, 7]. Puerarin is a common natural medicine extracted from leguminous plants *Pueraria lobata*. Previous studies have shown that, puerarin has obvious protective effect on cardiac and cerebral vessels [8], nervous system [9] and cognitive function [10]. However, the application of puerarin to alleviate the cognitive dysfunction after anesthesia is seldom reported. This study investigated the effects of puerarin

on the recovery of cognitive function in rats after anesthesia and the possible mechanisms.

Material and methods

Animal grouping and treatment

Sixty clean-grade and healthy Sprague-Dawley rats (20 months old, either male or female, weighing 380-400 g; Beijing Experimental Animal Research Center, Beijing, China) were randomly divided into control, model, and low-, medium- and high-dose puerarin groups, with 12 rats in each group. On the first day, the control group was given by intraperitoneal injection of normal saline (5 ml/day). The model and puerarin groups were intraperitoneally injected with 60 mg/kg of propofol (Guangdong Jiabo Pharmaceutical Co., Ltd., Qingyuan, China), followed by propofol injection once per hour with dose increased by half to the first dose. The anesthesia was maintained for 6 h. After each anesthesia, the rats were lying on the self-made oxygen inhalation box immediately for continuous oxygenation with flow of 5 L/min. After the righting reflex was restored, the rats were removed from the oxygen absorption box. The rats were free to eat and water excepting the experiment time. From day 2, the control and model groups were intraperitoneally injected with normal saline (5 ml/day), once per day. The low-, medium- and high-dose puerarin groups were intraperitoneally injected with puerarin (dissolved in 50% propanediol; Shaanxi Tianyi Biological Technology Co., Ltd., Xi'an, China), with doses of 10, 20 and 40 mg/kg respectively, once per day. The treatment in each group was ended on the day 7. This study was performed 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 of Beijing An Zhen Hospital.

Learning and memory function test

At the end of treatment, the learning and memory function was tested by Morris water maze. Before the experiment, the platform was placed in the center of the northeastern quadrant of water maze, with altitude 2 cm under the water surface. A proper amount of ink was added to the pool to make the animal not to distinguish the platform. Several objects were suspended on the wall of the pool to be the reference. The

water temperature was heated to 24-26°C. The positioning navigation experiment was performed for 5 days. Each day was divided into two time sessions, with 4 times of training in each time session. In the 4 times of training, the rats were put into the water from different quadrants, respectively. The duration before rat finding the platform (escape latency) was recorded by stopwatch. The interval of two times of training was 1 minute. In the afternoon of the fifth day, the space exploration experiment was carried out. The platform was removed, and the animals were put in the water from the southwest quadrant. The swimming path and crossing platform times of rats in the maze within 2 minutes were recorded by the camera.

Detection of serum amyloid β -protein and neuron-specific enolase (NSE) levels

At the end of behavior test, 3 ml of orbital blood was taken, followed by centrifugation at 3000 r/min, for 5 minutes. The serum was kept at -30°C for measurement. The serum amyloid β -protein (A β) and neuron-specific enolase (NSE) levels were detected using enzyme linked immunosorbent assay (ELISA). The process was according to the instructions in the kits (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China).

Detection of neurotrophic factors in hippocampus

The brain of rats was taken after craniotomy. The hippocampus tissue was taken, and its homogenate with normal saline (1:10) was quickly made, followed by centrifugation at 3000 r/min for 10 minutes. The supernatant was obtained. The content of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) were detected using ELISA. The operation was carried out in accordance with the instructions of the kits (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China).

Detection of cAMP-response element binding protein and extracellular signal-regulated kinase protein expression in hippocampus

The hippocampal tissue was grinded into dispersed cells in a glass grinder. One milliliter of cell lysate was added for lysis for 30 minutes, followed by centrifugation at 2000 r/min for 5 minutes. The supernatant was obtained. The expression levels of cAMP-response element

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Table 1. Escape latency and platform crossing times in rats

Group	Escape latency (s)	Platform crossing times (n)
Control	16.02±3.02	5.35±1.54
Model	30.17±4.21 ^a	2.48±1.11 ^a
Low-dose puerarin	28.44±3.05 ^a	3.16±1.03 ^a
Middle-dose puerarin	23.32±4.27 ^{a,b,c}	3.96±1.05 ^{a,b,c}
High-dose puerarin	17.38±4.01 ^{b,c,d}	4.76±1.87 ^{b,c}

^aP < 0.01 compared with control group; ^bP < 0.05 compared with model group; ^cP < 0.05 compared with low-dose puerarin group; ^dP < 0.05 compared with middle-dose puerarin group.

Table 2. Serum A β and NSE levels in rats

Group	A β (pg/ml)	NSE (ng/ml)
Control	36.25±10.37	2.01±0.43
Model	182.46±56.45 ^a	8.43±2.11 ^a
Low-dose puerarin	126.32±39.18 ^{a,b}	7.37±2.34 ^a
Middle-dose puerarin	83.15±33.81 ^{a,b,c}	5.03±1.95 ^{a,b,c}
High-dose puerarin	53.44±26.82 ^{b,c,d}	3.13±1.37 ^{a,b,c,d}

^aP < 0.01 compared with control group; ^bP < 0.05 compared with model group; ^cP < 0.05 compared with low-dose puerarin group; ^dP < 0.05 compared with middle-dose puerarin group. A β , amyloid β -protein; NSE, neuron-specific enolase.

Table 3. Hippocampal NGF and BDNF levels in rats

Group	NGF (pg/ml)	BDNF (pg/ml)
Control	5.56±1.01	9.12±2.56
Model	2.18±0.67 ^a	4.24±1.02 ^a
Low-dose puerarin	2.84±0.52 ^{a,b}	5.19±1.34 ^a
Middle-dose puerarin	3.45±0.67 ^{a,b,c}	7.03±1.84 ^{a,b,c}
High-dose puerarin	4.96±0.98 ^{b,c,d}	8.34±2.78 ^{b,c}

^aP < 0.01 compared with control group; ^bP < 0.05 compared with model group; ^cP < 0.05 compared with low-dose puerarin group; ^dP < 0.05 compared with middle-dose puerarin group. NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor.

binding protein (CREB) and extracellular signal-regulated kinase (ERK) and their phosphorylated forms (p-ERK and p-CREB) were detected by western blot assay. β -actin was used as the internal reference. The CREB, p-CREB, ERK and p-ERK primary antibodies and secondary antibodies were provided by Shanghai Sangon Biological Engineering Technology And Service Co., Ltd., (Shanghai, China).

Statistical analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are

presented as mean \pm standard deviation, and were compared using single factor analysis of variance with LSD-t test. P < 0.05 was considered as statistically significant.

Results

Effects of puerarin on escape latency and platform crossing times in rats after anaesthesia

Compared with the control group, the escape latency in model group was significantly increased (P < 0.05), and the platform crossing times in model group was significantly decreased (P < 0.05). Compared with the model group, the escape latency in middle- and high-dose puerarin group were significantly decreased, respectively (P < 0.05), and the platform crossing times in these two groups were significantly increased, respectively (P < 0.05) (**Table 1**).

Effects of puerarin on serum A β and NSE levels in rats after anaesthesia

As shown in **Table 2**, compared with the control group, the serum A β and NSE levels in model group were significantly increased, respectively (P < 0.05). Compared with the model group, serum A β levels in low-, middle- and high-dose puerarin groups and the NSE levels in middle- and high-dose puerarin groups were significantly decreased, respectively (P < 0.05).

Effects of puerarin on hippocampal NGF and BDNF levels in rats after anaesthesia

Table 3 shows that, compared with the control group, the hippocampal NGF and BDNF levels in model group were significantly decreased, respectively (P < 0.05). Compared with the model group, the hippocampal NGF levels in low-, middle- and high-dose puerarin groups and the hippocampal BDNF level in middle- and high-dose puerarin groups were significantly increased, respectively (P < 0.05).

Effects of puerarin on hippocampal CREB and p-CREB protein expression in rats after anaesthesia

Figure 1 shows that, there was no significant difference in hippocampal CREB protein expression among 5 groups. Compared with the con-

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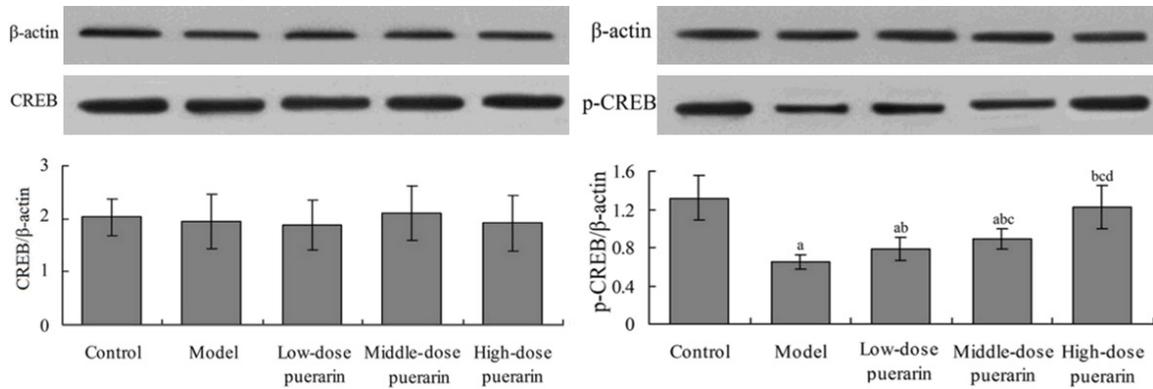


Figure 1. Hippocampal CREB and p-CREB expressions in rats. ^aP < 0.01 compared with control group; ^bP < 0.05 compared with model group; ^cP < 0.05 compared with low-dose puerarin group; ^dP < 0.05 compared with middle-dose puerarin group. CREB, cAMP-response element binding protein; p-CREB, phosphorylated cAMP-response element binding protein.

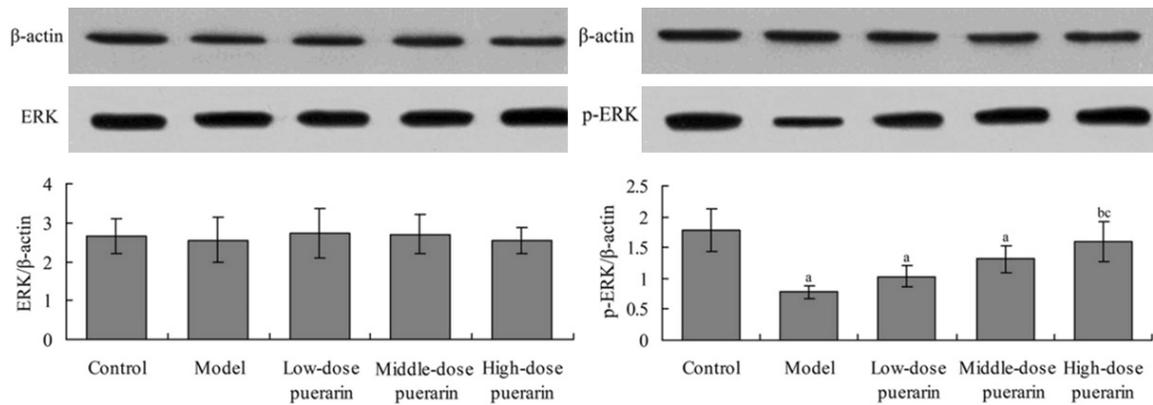


Figure 2. Hippocampal ERK and p-ERK expression in rats. ^aP < 0.01 compared with control group; ^bP < 0.05 compared with model group; ^cP < 0.05 compared with low-dose puerarin group. ERK, extracellular signal-regulated kinase; p-ERK, phosphorylated extracellular signal-regulated kinase.

control group, the hippocampal p-CREB protein level in model group was significantly decreased ($P < 0.05$). Compared with the model group, the p-CREB protein levels in 3 puerarin groups were significantly increased, respectively ($P < 0.05$).

Effects of puerarin on hippocampal ERK and p-ERK protein expression in rats after anesthesia

There was no significant difference in hippocampal ERK protein expression among 5 groups. Compared with the control group, the hippocampal p-ERK protein level in model group was significantly decreased ($P < 0.05$). Compared with the model group, the p-ERK protein level in the high-dose puerarin group was significantly increased ($P < 0.05$) (Figure 2).

Discussion

In this study, the rat model of cognitive dysfunction after anesthesia was established, and the effects of puerarin on this model were investigated. Results showed that, compared with the control group, the escape latency in model group was significantly increased ($P < 0.05$), and the platform crossing times in model group was significantly decreased ($P < 0.05$). This indicates that, the cognitive dysfunction after anesthesia model of rats was successfully established. Compared with the model group, the escape latency in middle- and high-dose puerarin group were significantly decreased, respectively ($P < 0.05$), and the platform crossing times in these two groups were significantly increased, respectively ($P < 0.05$). This indi-

cates that, puerarin can mitigate the cognitive dysfunction after anesthesia in rats.

A β is one of the potential markers of nervous system damage and persistent inflammatory response [11]. Some scholars believe that, cognitive dysfunction is related to the anesthesia and expression of A β [12]. The change of A β concentration in plasma can indirectly reflect the aggregation of A β in brain tissue. NSE is an isoenzyme of enolase in glycolysis. It is specifically distributed in neurons and neuroendocrine cells, and is a specific marker of neurons [13]. Under normal circumstances, there is a small amount of NSE in cerebrospinal fluid and serum. When the cerebral ischemia, hypoxia, poisoning or trauma occurs, the integrity of the nerve cell membrane is damaged, and the permeability of the blood-brain barrier increases, so the NSE protein leaks into the serum or cerebrospinal fluid, leading to the increased content [14]. Results of this study showed that, compared with the control group, the serum A β and NSE levels in the model group were significantly increased, respectively ($P < 0.05$). Compared with the model group, serum A β level in the 3 puerarin groups and NSE level in middle- and high-dose puerarin groups were significantly decreased, respectively ($P < 0.05$). This indicates that, the mechanism of puerarin alleviating cognitive dysfunction after anesthesia may be related to its decreasing A β and NSE level in the body.

Neurotrophic factors are the small-molecular polypeptide factors that play a special role in neural tissue. They can promote the proliferation, growth, differentiation and survival of the nerve cells during the development of the central nervous system, and regulate the plasticity of synapses [15]. NGF and BDNF are the most important neurotrophic factors. NGF combines with the specific TrkA receptors to activate the cell metabolism, thus promoting the proliferation and differentiation of nerve cells, regulating the survival of central and peripheral nerve cells and the growth of axons. It plays a very important role in the repair of nerve cell damage [16]. BDNF plays the neurotrophic role by binding to its specific high affinity receptor TrkB [17]. It is found that NGF and BDNF play an important role in improving the cognitive function. They can improve learning and memory ability of rats with Alzheimer's disease, and

promote the neuron growth [18]. This study found that, compared with the control group, hippocampal NGF and BDNF levels in the model group were significantly decreased, respectively ($P < 0.05$). Compared with the model group, the hippocampal NGF level in the 3 puerarin groups and the BDNF level in the middle- and high-dose puerarin groups was significantly increased, respectively ($P < 0.05$). This indicates that, the role of puerarin in alleviating cognitive dysfunction after anesthesia may be related to its increasing of hippocampal NGF and BDNF levels.

CREB is an important nuclear transcription factor, which can regulate a wide range of biological functions including learning and memory. When the 133rd-bit serine of CREB is phosphorylated, it becomes the active state (p-CREB) [19]. p-CREB can regulate transcription of genes with cAMP response elements in their promoter. In the central nervous system, CREB participates in synaptic plasticity and long-term memory formation. The decrease of CREB expression or its activity leads to learning and memory impairment, while the high expression of CREB can improve learning and memory function [20]. The activity of CREB is regulated by multiple signal transduction pathways, such as the cAMP-PKA pathway, mitogen activated protein kinase pathway, and ERK pathway [21]. The learning and memory impairment is related to inhibition on the activity of CREB and ERK [22, 23]. Compared with the control group, the hippocampal p-CREB and p-ERK protein level in model group were significantly decreased ($P < 0.05$). Compared with the model group, the p-CREB protein levels in the 3 puerarin groups and p-ERK protein level in high-dose puerarin group were significantly increased, respectively ($P < 0.05$). This indicates that, puerarin can increase the hippocampal p-CREB and p-ERK protein expressions, thus exerting the protective effects on cognitive dysfunction after anesthesia.

In conclusion, puerarin can alleviate cognitive dysfunction after anesthesia in rats, which may be related to decreasing the brain A β and NSE levels, increasing NGF and BDNF levels and increasing p-CREB and p-ERK protein expressions. This study has provided a reference for clinical application of puerarin to prevention of cognitive dysfunction after anesthesia. How-

ever, this study still has some limitations. The sample size of this study is relatively small. Larger sample size will make the results more convincing. Whether there are other mechanisms in protective effects of puerarin on cognitive dysfunction after anesthesia needs to be further determined.

Acknowledgements

This work was supported by the Project of Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support (No. ZYLX201810).

Disclosure of conflict of interest

None.

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