Int J Clin Exp Med 2019;12(6):7271-7277
www.ijcem.com /ISSN:1940-5901/IJCEM0088206

Original Article

The role of the PI3K/Akt/GSK-3β pathway in postoperative cognitive dysfunction in elderly rats

Jinchao Shen[1*], Jinfen Ji[1*], Min Qian[1], Zhaodi Fan[1], Yan Yin[1], Yueqing Shi[1], Li Shen[2], Haiyan Sun[1]

[1]Department of Anesthesiology, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Zhangjiagang 215600, Jiangsu Province, China; [2]Department of Oncology, Zhangjiagang Ao Yang Hospital, Zhangjiagang 215600, Jiangsu Province, China. [*]Equal contributors.

Received November 10, 2018; Accepted April 8, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: The goal of this study was to investigate the effect of anesthesia and surgery on postoperative cognitive dysfunction (POCD). The role of the PI3K/Akt/GSK-3β pathway in POCD in elderly rats was also studied using 22-month-old female Sprague Dawley (SD) rats that were randomly assigned into a sham group, a splenectomy group and an anesthesia group, with 10 rats in each group. Morris water maze training was performed 6 days prior to the animal model construction. After establishment of anesthesia and splenectomy animal models for 7 days, the reverse Morris water maze test was performed to compare the behavioral differences among the three groups. Vital signs of each rat were detected. Expression levels of relative genes in the PI3K/Akt/GSK-3β pathway were detected by Western blot and quantitative real time-polymerase chain reaction (qRT-PCR), respectively. Compared with the control group, the swimming distance and latency period were increased ($P<0.05$), whereas the target quadrant retention time ratio was decreased in anesthesia group and splenectomy group. No significant difference in swimming speed was seen among the three groups. qRT-PCR results indicated that the mRNA levels of PI3K, AKT, and GSK-3β were decreased, whereas Cyto C and caspase-9 were increased in rat hippocampus of the anesthesia group and the splenectomy group ($P<0.05$). Downregulated p-PI3K, p-AKT, and GSK-3β, as well as upregulated Cyto-C and caspase-9 was found in the anesthesia group and splenectomy group by Western blot ($P<0.05$). Furthermore, no significant differences in the abovementioned gene expressions were found between the anesthesia group and splenectomy group ($P>0.05$). Anesthesia can cause cognitive dysfunction in elderly rats. There was no correlation between procedures and cognitive function decline. Inhibition of PI3K/Akt/GSK-3β pathway caused by anesthesia may lead to apoptosis of hippocampal tissue, which may be explained for the postoperative cognitive dysfunction.

Keywords: Postoperative cognitive dysfunction, PI3K/Akt/GSK-3β, elderly, apoptosis

Introduction

Postoperative cognitive dysfunction (POCD) after anesthesia is one of the common postoperative complications in elderly patients [1, 2]. It usually occurs within several weeks or months after surgery, mainly includes deterioration of learning and memory, impairment of consciousness and information processing and even severe dementia [3]. Studies have shown that about 25%-50% of surgery patients experienced POCD [4]. Additionally, nearly 40% patients still suffer from cognitive impairment 5 years after surgery [5]. People with mild cognitive impairment (MCI) have a high probability of transforming to AD (Alzheimer's disease) every year, while the transformation rate in healthy people is only 1-2% [6]. Recent studies have shown a certain relationship among anesthesia, surgery and dementia [7-9]. POCD prolongs the length of stay, increases the hospitalization expenses and affects the life quality of affected patients [10]. As the ageing of the population, POCD has become a significant medical problem that is needed to be concerned [11].

The hippocampus is the earliest confirmed central structure that exerts a key role in learning and memory processes. In addition to a regular, complex lamellar cell structure of hippocampus, it has a complete synaptic pathway. Eichenbaum et al. [12] confirmed that hippocampus damage can impair learning and memory of space navigation. It has been reported that

general anesthetics, such as isoflurane, halothane, nitrous oxide and ketamine, may result in the loss of mental activity. It is suggested that pathological injury after surgery may occur in functional areas that are related to the central nervous system. For example, Bedford et al. [13] reported there were 112 POCD cases were observed in 1193 cases of elderly surgery patients, and the anesthetic is considered as the main predisposing factor. Lewis MC believed that anesthetic drugs have a significant and long-term effect on the occurrence and development of POCD. It is generally believed that inflammation, oxidative stress, and apoptosis are involved in the occurrence and development of cognitive impairment in diabetes [14]. The PI3K/Akt pathway is a classical anti-apoptosis and pro-survival pathway that exerts an important role in ischemic brain protection, neovascularization, and anti-apoptosis [15]. The PI3K/Akt pathway has been proven to be associated with apoptosis after cerebral hypoxia-ischemia.

In this study, inhalation anesthesia and splenectomy model of Sprague Dawley (SD) rats were established. Behavioral changes in different groups were observed and analyzed. Furthermore, the effect of PI3K/AKT/GSK-3β pathway on the development of POCD was explored by detecting expressions of caspase-9 and Cyto C in rats.

Materials and methods

Experimental rats

These studies used 22-month-old female Sprague Dawley (SD) rats. Inclusion criteria for SD rats were as follows: An average arterial pressure of 65-105 mmHg; Body weight of 450-550 g; No surface lesions and lumps; No disease of skin. The enrolled 30 SD rats were randomly assigned into sham group, splenectomy group and anesthesia group, with 10 rats in each group. This study was approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine Animal Center.

Establishment of anesthesia and splenectomy animal models

Inhalation anesthesia animal model: The anesthesia box was filled with oxygen and isoflurane, and the concentrations of which in the anesthesia box were measured using a multi-functional anesthesia detector. When the oxygen concentration in the anesthesia box reached 80% and the isoflurane concentration reached 3%, rats were placed in the box for anesthesia induction. Rats were removed from the box 5 minutes later and received orotracheal intubation under visual light. During the procedure, rats were inhaled in 2% isoflurane and 80% oxygen for 2 hours and maintained spontaneous breathing. After anesthesia terminated, the oxygen output was maintained until rats were naturally resuscitated. Each rat was observed for 10 minutes and kept in an individual cage after anesthesia.

Splenectomy animal model: The anesthesia induction was previously described. 0.5 cm of incision at the lower margin of the left rib was cut to explore the abdomen. Splenic artery branches and venae comitans were ligated and resected. Postoperative penicillin was administrated to prevent infection.

Rats in the sham group were placed in the anesthesia box with 80% oxygen for 2 hours without any procedure.

Detection of vital signs in rats

Breathing rate (BR), rat tail oxygen saturation (SPO_2) and rectal temperature (RT) were monitored during the anesthesia induction. Blood pressure (BP) and heart rate (HR) in rats of anesthesia group and splenectomy group before anesthesia, 0, 1, 2 hours after anesthesia were recorded, respectively. Rats in sham group were only recorded for BR, SPO_2 and oxygen concentration in the anesthesia box.

Morris water maze tests

Spatial memory training: The surrounding environment should be quiet during testing, and the water temperature was maintained at 26 ± 2°C. The water surface was divided into 4 quadrants, with the mobile platform fixed in the southwest quadrant. SD rat was first placed on the platform for 30 seconds, and then randomly placed into buckets from the other three quadrants. If the rat could not find the platform within 60 seconds, it was guided to the platform and the second training was performed after 45 seconds rest. Otherwise, the spatial memory training was considered to be success-

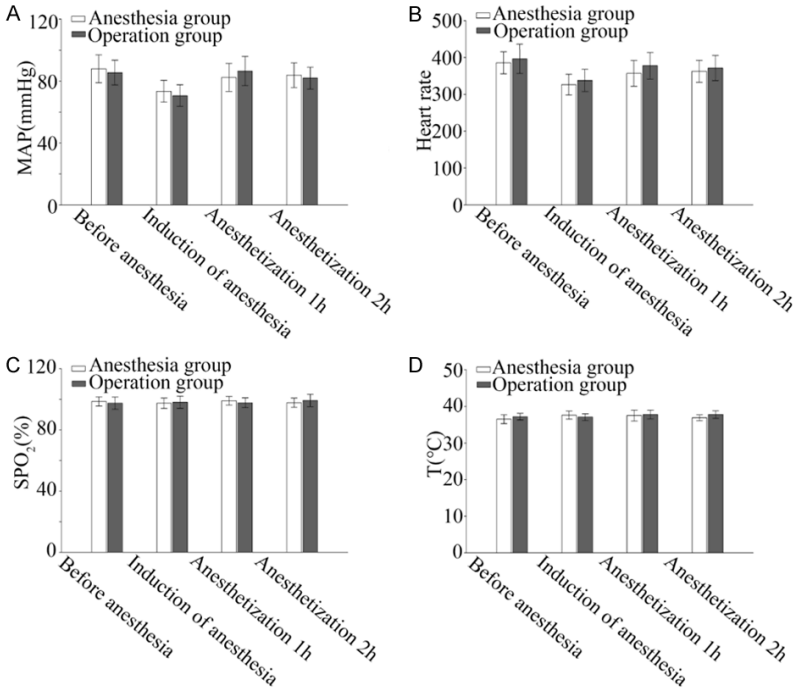

Figure 1. Vital sings comparison. Levels of MAP (A), HR (B), SPO_2 (C) and RT (D) in sham group, anesthesia group and splenectomy group. Note: MAP: Mean arterial pressure; HR: Heart rate; SPO_2: Rat tail oxygen saturation; RT: Rectal temperature.

ful to stay on the platform for 15 seconds. The next training was conducted after 30 seconds rest. Each rat was trained 3 times a day at a fixed time. The quadrant where the rat was placed each time should be different. After 6 days of training, the rats that learned to find the platform were selected for animal model construction.

Reverse test: After model construction, rats without wound infection were selected for reverse test. The platform was moved symmetrically to the northeastern corner of the contralateral side. Rats were then placed in buckets from the other three quadrants for testing.

Sample collection

Blood sample and hippocampal tissue of each rat were collected after sacrifice. All tissue samples were preserved in -80°C.

RNA extraction and quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNA in treated cells was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Tokyo, Japan). RNA concentration was detected using spectrometer. qRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq TM (TaKaRa, Tokyo, Japan). Relative gene expression was calculated using $2^{-\Delta Ct}$ method. Primers used in the experiment were: PI3K, F: 5'-CATCACTTCCTCCTGCTCTAT-3', R: 5'-CAGTTGTTGGCAATCTTCTTC-3'; AKT, F: 5'-GGACAACCGCCATCCAGACT-3', R: 5'-GCCAGGGACACCTCCATCTC-3'; GSK-3β, F: 5'-ATGCAGAGTCCCAAAATGAATGTCC-3', R: 5'-TCAGTCCACCTTTTCCACCTTGCCG-3'; Cyto C, F: 5'-ATGCCAAGTCAAAGAATC-3', R: 5'-GAGGGCAGTAAGCATAA-3'; Caspase-9, F: 5'-CTGAGCAGAATGCTGTCCCATA-3', R: 5'-GACACCATCCAAGGTCTGGATGTA-3'; β-actin, F: 5'-TAAAGACCTCTATGCCAACACAGT-3', R: 5'-CACGATGGAGGGGCCGGACTCATC-3'.

Western blot

Protein was extracted from hippocampal tissues and quantified using the BCA (bicinchoninic acid) protein assay kit (Pierce, Rockford, IL, USA). An equal amount of protein sample was loaded onto a 12% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) gel and then transferred to a PVDF (polyvinylidene fluoride) membrane (Millipore, Billerica, MA, USA) after being separated. After blocked with skim milk, membranes were incubated with primary antibody (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C and then incubated with HRP (horseradish peroxidase) conjugated secondary antibody. Finally, protein bands were captured by the Tanon detection system using electrochemiluminescence (ECL) reagent (Thermo, Waltham, MA, USA).

Statistical analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was

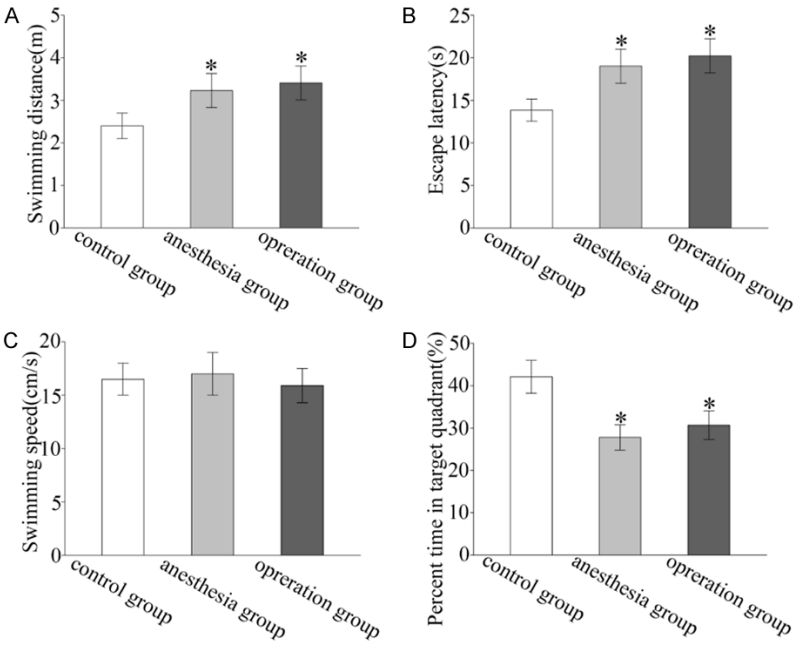

Figure 2. Behavioral test results. Results of the swimming distance (A), latency period (B), swimming speed (C) and target quadrant retention time ratio (D) in sham group, anesthesia group and splenectomy group.

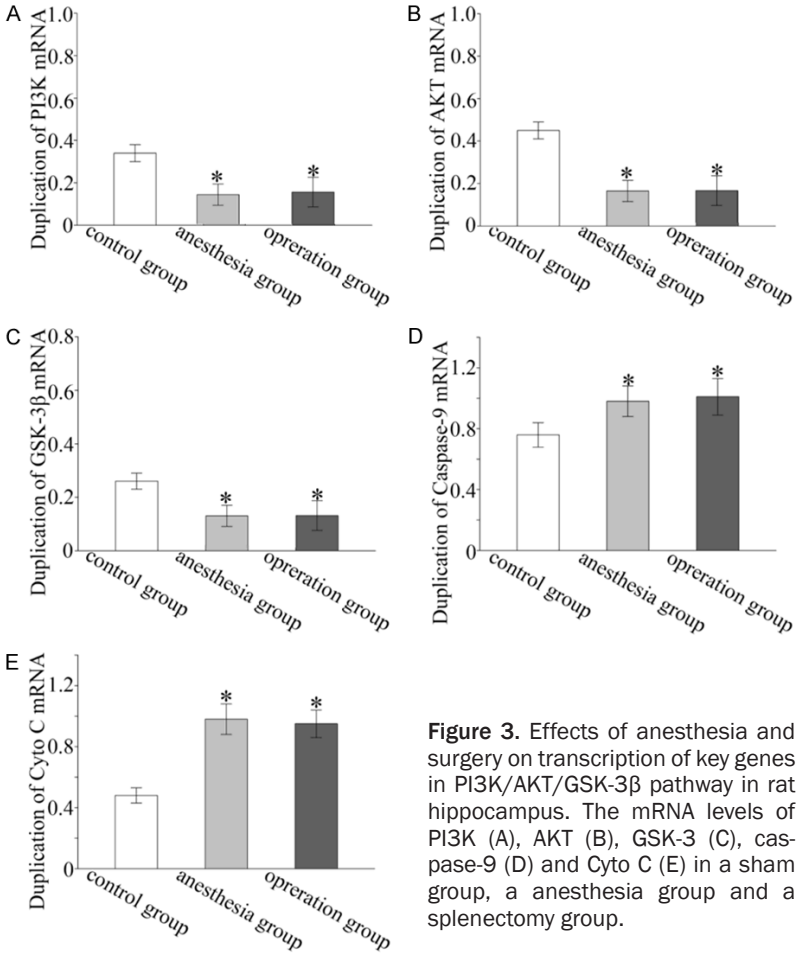

Figure 3. Effects of anesthesia and surgery on transcription of key genes in PI3K/AKT/GSK-3β pathway in rat hippocampus. The mRNA levels of PI3K (A), AKT (B), GSK-3 (C), caspase-9 (D) and Cyto C (E) in a sham group, a anesthesia group and a splenectomy group.

utilized for statistical analyses. The experimental data are expressed as mean ± SD ($\bar{x}$ ± s). The experimental results were analyzed with standard t-test analysis. $P<0.05$ was considered statistically significant.

Results

Comparison of MAP, HR, SPO_2 and RT

Mean arterial pressure (MAP) was calculated and compared among the three groups. No significant differences in MAP, HR, SPO_2 and RT were found in anesthesia group before anesthesia and 0, 1 and 2 hours after anesthesia induction ($P>0.05$). Additionally, no significant differences in MAP, HR SPO_2 and RT were found in splenectomy group before anesthesia and 0 and 2 hours after anesthesia induction ($P>0.05$, **Figure 1A-D**).

Comparison of Morris water maze tests

Compared with the sham group, the swimming distance and latency period were increased, whereas the target quadrant retention time ratio was decreased in anesthesia group and splenectomy group (**Figure 2A**, **2B** and **2D**). However, no remarkably changes were found in swimming speed among the three groups (**Figure 2C**). Moreover, no significant differences in behavioral test results were seen between the anesthesia group and the surgery group ($P>0.05$).

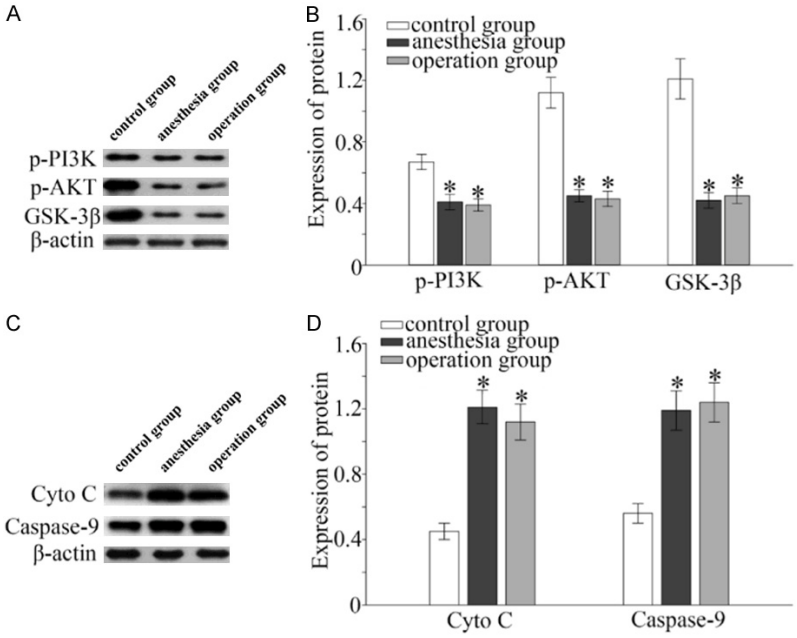

Figure 4. Effects of anesthesia and surgery on protein expressions of key genes in PI3K/AKT/GSK-3β pathway in rat hippocampus. A. Protein levels of p-PI3K, p-AKT, and GSK-3β in a sham group, a anesthesia group and a splenectomy group. B. Comparison of protein levels of p-PI3K, p-AKT, and GSK-3β in a sham group, a anesthesia group and a splenectomy group. C. Protein levels of Cyto-C and caspase-9 in a sham group, a anesthesia group and a splenectomy group. D. Comparison of protein levels of Cyto-C and caspase-9 in a sham group, a anesthesia group and a splenectomy group.

Effects of anesthesia and surgery on transcription of key genes in PI3K/AKT/GSK-3β pathway in rat hippocampus

qRT-PCR results indicated that the mRNA levels of PI3K, AKT, and GSK-3β were decreased, whereas expression levels of Cyto C and caspase-9 were increased in rat hippocampus of the anesthesia group and the splenectomy group after the procedure (**Figure 3**, $P<0.05$). Additionally, no significant differences in the abovementioned gene expressions were found between the anesthesia group and splenectomy group ($P>0.05$).

Effects of anesthesia and surgery on protein expressions of key genes in PI3K/AKT/GSK-3β pathway in rat hippocampus

Downregulated p-PI3K, p-AKT, and GSK-3β, as well as upregulated Cyto-C and caspase-9 were found in the anesthesia group and splenectomy group after the procedure by Western blot (**Figure 4**, $P<0.05$). Additionally, no significant differences in the abovementioned gene expressions were found between the anesthesia group and splenectomy group ($P>0.05$).

Discussion

POCD is characterized by changes in perception, cognition, thinking, and memory, with an incidence as high as 20-83% [16]. POCD not only affects life quality of patients, prolongs length of stay, but also severely increases mortality. With the aging of population, POCD has been well recognized in recent years [17-19].

In elderly, reduced tissue volume and weight of brain are observed. The weight of brain tissue is reduced by 18% in elderly compared with those in 30 years old. In particularly, the brain tissue volume remarkably decreases after 60 years of age. At the cellular level, expression levels of neurotransmitters are remarkably downregulated, including cetylcholine (Ach), dopamine, norepinephrine, tyrosine and 5-serotonin. In contrast, activities of neurotransmitter decomposing enzymes such as monoamine oxidase and catechol-O-methyltransferase are enhanced, further downregulating the intracranial neurotransmitters. In addition, slower compensatory production of receptor speed and reduction of receptor affinity for neurotransmitter molecules are accompanied. AD is the most severe manifestation of senile mental disorders. POCD is very similar to that of the early stage of AD. Some studies considered that POCD may be the manifestation of early stages of AD [20], or POCD triggers pathophysiological process of AD [8]. Our Morris water maze experiments suggested that the learning and spatial abilities in rats of anesthesia group and splenectomy group were worse than those of sham group. The data indicated that anesthesia severely affects the cognitive and memory abilities. Abundant research has proven that hippocampus dysfunction could lead to learning and

memory impairments, as well as decreased spatial orientation [21, 22].

PI3K/Akt pathway is widely present in cells and is important for membrane receptor transduction. PI3K/Akt pathway is capable of promoting cell proliferation, differentiation, and survival by regulating cell cycle and apoptosis-related proteins.

Current studies have shown that PI3K/AKT pathway is widely expressed in the central nervous system and its neuroprotection role has been well studied. Dudek et al. [23] found that PI3K/Akt pathway can inhibit neuronal apoptosis [24]. Other experiments have demonstrated that Akt can inhibit neuronal apoptosis by promoting CREB activation and up-regulating Bcl-2 expression [25, 26]. In addition to its neuroprotective effects in acute cerebral ischemia, PI3K/Akt pathway also exerts an essential role in brain trauma and chronic neurodegenerative diseases, such as AD. Traditional Chinese medicine Herba Rhodiolae relieves cerebral edema and inhibits the occurrence of cortical neuron apoptosis through activating PI3K/Akt pathway [27, 28]. In animal models of AD, activation of PI3K/Akt pathway can antagonize the neurotoxic effects of Aβ and improve cognitive function in rats [29-31]. Akt is activated by PI3K and regulates cell apoptosis through multiple pathways. Among them, Akt could directly inhibits GSK-3 activity, thereafter inhibiting cell apoptosis [32, 33]. In the present study, protein levels of p-PI3K, p-Akt, and GSK-3β in rat hippocampus were remarkably downregulated in anesthesia group and splenectomy group compared with those of sham group. However, expression levels of Cyto C and caspase-9 were upregulated, indicating that the inhibition of PI3K/AKT/GSK-3β pathway exerts a crucial role in POCD development.

Conclusions

PI3K/Akt/GSK-3β pathway inhibits the postoperative cognitive dysfunction in elderly rats after anesthesia and splenectomy.

Disclosure of conflict of interest

None.

Address correspondence to: Li Shen, Department of Oncology, Zhangjiagang Ao Yang Hospital, Zhangjiagang 215600, Jiangsu Province, China. Tel: 86-013915710816; E-mail: vernon35@163.com; Haiyan Sun, Department of Anesthesiology, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Zhangjiagang 215600, Jiangsu Province, China. Tel: 86015895678696; E-mail: 1902039717@qq.com

References

[1] Feinkohl I, Winterer G, Spies CD and Pischon T. Cognitive reserve and the risk of postoperative cognitive dysfunction. Dtsch Arztebl Int 2017; 114: 110-117.

[2] He X, Wen LJ, Cui C, Li DR and Teng JF. The significance of S100beta protein on postoperative cognitive dysfunction in patients who underwent single valve replacement surgery under general anesthesia. Eur Rev Med Pharmacol Sci 2017; 21: 2192-2198.

[3] Chandrasekhar R, Ely EW and Patel MB. Challenges with postoperative cognitive impairment research. JAMA Surg 2019; [Epub ahead of print].

[4] Chi YL, Li ZS, Lin CS, Wang Q and Zhou YK. Evaluation of the postoperative cognitive dysfunction in elderly patients with general anesthesia. Eur Rev Med Pharmacol Sci 2017; 21: 1346-1354.

[5] Lyketsos CG, Toone L, Tschanz J, Corcoran C, Norton M, Zandi P, Munger R, Breitner JC and Welsh-Bohmer K. A population-based study of the association between coronary artery bypass graft surgery (CABG) and cognitive decline: the Cache County study. Int J Geriatr Psychiatry 2006; 21: 509-518.

[6] Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L and Winblad B. Current concepts in mild cognitive impairment. Arch Neurol 2001; 58: 1985-1992.

[7] Miller D, Lewis SR, Pritchard MW, Schofield-Robinson OJ, Shelton CL, Alderson P and Smith AF. Intravenous versus inhalational maintenance of anaesthesia for postoperative cognitive outcomes in elderly people undergoing non-cardiac surgery. Cochrane Database Syst Rev 2018; 8: D12317.

[8] Arora SS, Gooch JL and Garcia PS. Postoperative cognitive dysfunction, Alzheimer's disease, and anesthesia. Int J Neurosci 2014; 124: 236-242.

[9] Han Y, Han L, Dong MM, Sun QC, Zhang ZF, Ding K, Zhang YD, Mannan A, Xu YF, Ou-Yang CL, Li ZY, Gao C and Cao JL. Preoperative salivary cortisol AM/PM ratio predicts early postoperative cognitive dysfunction after noncardiac surgery in elderly patients. Anesth Analg 2019; 128: 349-357.

[10] Monk TG and Price CC. Postoperative cognitive disorders. Curr Opin Crit Care 2011; 17: 376-381.
[11] Newman S, Stygall J, Hirani S, Shaefi S and Maze M. Postoperative cognitive dysfunction after noncardiac surgery: a systematic review. Anesthesiology 2007; 106: 572-590.
[12] Eichenbaum H. The hippocampus and declarative memory: cognitive mechanisms and neural codes. Behav Brain Res 2001; 127: 199-207.
[13] Bedford PD. Adverse cerebral effects of anaesthesia on old people. Lancet 1955; 269: 259-263.
[14] Lewis MC, Nevo I, Paniagua MA, Ben-Ari A, Pretto E, Eisdorfer S, Davidson E, Matot I and Eisdorfer C. Uncomplicated general anesthesia in the elderly results in cognitive decline: does cognitive decline predict morbidity and mortality? Med Hypotheses 2007; 68: 484-492.
[15] Jie P, Hong Z, Tian Y, Li Y, Lin L, Zhou L, Du Y, Chen L and Chen L. Activation of transient receptor potential vanilloid 4 induces apoptosis in hippocampus through downregulating PI3K/Akt and upregulating p38 MAPK signaling pathways. Cell Death Dis 2015; 6: e1775.
[16] Wagner S, Quente J, Staedtler S, Koch K, Richter-Schmidinger T, Kornhuber J, Ihmsen H and Schuettler J. A high risk of sleep apnea is associated with less postoperative cognitive dysfunction after intravenous anesthesia: results of an observational pilot study. BMC Anesthesiol 2018; 18: 139.
[17] Maitra S, Baidya DK, Pawar DK, Arora MK and Khanna P. Epidural anesthesia and analgesia in the neonate: a review of current evidences. J Anesth 2014; 28: 768-779.
[18] Gong GL, Liu B, Wu JX, Li JY, Shu BQ and You ZJ. Postoperative cognitive dysfunction induced by different surgical methods and its risk factors. Am Surg 2018; 84: 1531-1537.
[19] Alalawi R and Yasmeen N. Postoperative cognitive dysfunction in the elderly: a review comparing the effects of desflurane and sevflurane. J Perianesth Nurs 2018; 33: 732-740.
[20] Vanderweyde T, Bednar MM, Forman SA and Wolozin B. Iatrogenic risk factors for Alzheimer's disease: surgery and anesthesia. J Alzheimers Dis 2010; 22 Suppl 3: 91-104.
[21] Ribak CE, Nitsch R and Seress L. Proportion of parvalbumin-positive basket cells in the GABAergic innervation of pyramidal and granule cells of the rat hippocampal formation. J Comp Neurol 1990; 300: 449-461.
[22] Gao H, Han Z, Huang S, Bai R, Ge X, Chen F and Lei P. Intermittent hypoxia caused cognitive dysfunction relate to miRNAs dysregulation in hippocampus. Behav Brain Res 2017; 335: 80-87.
[23] Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR and Greenberg ME. Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 1997; 275: 661-665.
[24] Crowder RJ and Freeman RS. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. J Neurosci 1998; 18: 2933-2943.
[25] Du K and Montminy M. CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 1998; 273: 32377-32379.
[26] Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE and Reusch JE. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem 2000; 275: 10761-10766.
[27] Chen SF, Tsai HJ, Hung TH, Chen CC, Lee CY, Wu CH, Wang PY and Liao NC. Salidroside improves behavioral and histological outcomes and reduces apoptosis via PI3K/Akt signaling after experimental traumatic brain injury. PLoS One 2012; 7: e45763.
[28] Zhi WH, Zeng YY, Lu ZH, Qu WJ, Chen WX, Chen L and Chen L. Simvastatin exerts antiamnesic effect in Abeta25-35-injected mice. CNS Neurosci Ther 2014; 20: 218-226.
[29] Shang YC, Chong ZZ, Wang S and Maiese K. Prevention of beta-amyloid degeneration of microglia by erythropoietin depends on Wnt1, the PI 3-K/mTOR pathway, Bad, and Bcl-xL. Aging (Albany NY) 2012; 4: 187-201.
[30] Lee ST, Chu K, Park JE, Jung KH, Jeon D, Lim JY, Lee SK, Kim M and Roh JK. Erythropoietin improves memory function with reducing endothelial dysfunction and amyloid-beta burden in Alzheimer's disease models. J Neurochem 2012; 120: 115-124.
[31] Echeverria V, Zeitlin R, Burgess S, Patel S, Barman A, Thakur G, Mamcarz M, Wang L, Sattelle DB, Kirschner DA, Mori T, Leblanc RM, Prabhakar R and Arendash GW. Cotinine reduces amyloid-beta aggregation and improves memory in Alzheimer's disease mice. J Alzheimers Dis 2011; 24: 817-835.
[32] Chen Z, Yang L, Liu Y, Tang A, Li X, Zhang J and Yang Z. LY294002 and rapamycin promote coxsackievirus-induced cytopathic effect and apoptosis via inhibition of PI3K/AKT/mTOR signaling pathway. Mol Cell Biochem 2014; 385: 169-177.
[33] Song G, Ouyang G and Bao S. The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med 2005; 9: 59-71.