


Lithium chloride ameliorates cognition dysfunction induced by sevoflurane anesthesia in rats

Yilong Wang^{1,2,3}, Xiaohu An^{1,2,3}, Xiaoqing Zhang⁴, Jianhui Liu⁴, Jianwei Wang^{1,2,3} and Zeyong Yang^{1,2,3} 

1 Department of Anesthesiology, International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, China

2 Shanghai Key Laboratory of Embryo Original Diseases, China

3 Shanghai Municipal Key Clinical Specialty, China

4 Department of Anesthesiology, Tongji Hospital, Tongji University, Shanghai, China

Keywords

anesthesia; apoptosis; cognitive deficits; glycogen synthase kinase-3 β ; lithium chloride; sevoflurane

Correspondence

Z. Yang, Department of Anesthesiology, International Peace Maternity and Child Health Hospital, Shanghai Jiao Tong University School of Medicine, No. 910 Hengshan Road, Xuhui District, Shanghai 200030, China
Tel: 86 021 69479904
E-mail: yangzeyo@163.com

(Received 10 June 2019, revised 5 November 2019, accepted 17 December 2019)

doi:10.1002/2211-5463.12779

Postoperative cognitive dysfunction is a common complication in elderly patients after surgeries involving anesthesia, but the underlying mechanisms are poorly understood. Lithium is a conventional treatment for bipolar disorder, which exerts a neuroprotective role in various diseases by inhibiting glycogen synthase kinase-3 β (GSK-3 β) in the brain and spinal cord. However, it is not known whether lithium chloride (LiCl) can protect against cognitive dysfunction induced by sevoflurane (SEV) anesthesia. Here, we examined the effects of LiCl on SEV-induced cognitive dysfunction in rats and on SEV-induced neuron apoptosis. We report that anesthesia with SEV significantly impaired memory performance, induced oxidative stress and hippocampal neuron apoptosis, and stimulated GSK-3 β activity. Treatment with LiCl ameliorated SEV-induced cognitive disorder in rats by inhibiting the GSK-3 β / β -catenin signaling pathway. In addition, LiCl reduced hippocampal neuron apoptosis and oxidative stress induced by SEV anesthesia. These results suggest that LiCl may have potential for development into a therapeutic agent for treatment of SEV anesthesia-induced cognitive dysfunction.

Anesthesia and surgery have relations with brief or long-time deterioration in cognition condition, which was named postoperative cognitive dysfunction (POCD) [1,2]. POCD occurs more commonly in elderly people, because aging has been considered the most important risk factor in its course [3,4].

However, not much is known about the etiology of POCD [5–7]. One study showed that anesthesia with sevoflurane (SEV) might reduce neuronal survival and neurogenesis rate in the hippocampus, resulting in cognitive dysfunction and neurotoxicity in elder rats [8]. In addition, an *in vitro* study demonstrated that

inhaling an anesthetic might result in the activation of caspases and cell apoptosis, and also increase the production of A β , which can finally influence the course of Alzheimer's disease [9]. POCD is considered a severe disease, particularly in elderly people, because this condition significantly lengthens the time for rehabilitation [10,11]. POCD can prolong hospital course, hinder disease recovery, raise postoperative complications and lower the quality of life after discharge [12,13]. Yet, the current approaches to prevention and treatment for POCD are quite rare, and the treatment effect is still unsatisfactory [14,15]. Therefore, it is

Abbreviations

CAT, catalase; GSK-3 β , glycogen synthase kinase-3 β ; LiCl, lithium chloride; MWM, Morris water maze; POCD, postoperative cognitive dysfunction; ROS, reactive oxygen species; SOD1, superoxide dismutase 1.

important to find more effective approaches to treat POCD.

Evidence suggests that cognitive impairments caused by brain trauma or surgical anesthesia are related to dysregulated signaling pathways. For example, the phosphoinositide 3-kinase/Akt and extracellular signal-regulated kinase 1/2 signaling pathways are inactivated, whereas glycogen synthase kinase-3 β (GSK-3 β) and neuroapoptosis or neurotoxicity are enhanced [16–18]. Emerging evidence shows that SEV impairs memory consolidation in rats, possibly through inhibiting phosphorylation of GSK-3 β in the hippocampus [19]. Also, our previous study already showed that SEV anesthesia caused alterations in apoptosis-related proteins and GSK-3 β phosphorylation, and induced cognitive dysfunction in mice [20]. In addition, it is reported that lithium treatment prevents apoptosis in neonatal rat hippocampus resulting from SEV exposure [21]. Over 60 years, lithium chloride (LiCl), the mood stabilizer, has been used for the treatment of mental diseases, in part by directly inhibiting GSK-3 β [22–24]. Although the theory of LiCl treatment is known, genetic and pharmacological research suggest that activating GSK-3 β is among the major mechanisms of LiCl [25]. A recent study has suggested that LiCl probably plays a neuroprotective role in motor dysfunctions by inhibiting GSK-3 β in rats who suffered from intracerebral hemorrhage [26].

To explore the role of LiCl on the impairment of learning and memory induced by SEV, we established the rat model with cognitive impairment using SEV. Neuroapoptosis, the relevant proteins expression, memory and learning ability of rat, and the cognitive function were tested to confirm the theory of cognitive dysfunction caused by SEV anesthesia and LiCl as a potential therapeutic strategy.

Materials and methods

In vivo model

All of the *in vivo* experiments involving animal protocols were reviewed and approved by International Peace Maternity and Child Health Hospital. Prior to the experiments, Sprague Dawley rats (250 \pm 10 g, 7 weeks old, male; Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) were kept in a monitored 12/12 dark/light cycle lasting for 7 days and having free access to water and food. Then the rats were randomly split into three groups: the control group inhaling normal air for 6 h, the SEV group in identical conditions of 100% O₂ with 2.5% SEV at 600 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 6 h and the LiCl + SEV group receiving 60 $\text{mg}\cdot\text{kg}^{-1}$ LiCl (L4408; Sigma, St Louis, MO, USA) by intraperitoneal

injection twice a day before SEV exposure. After anesthesia, the rats were in recovery lasting for 7 days. The dose of LiCl at 60 $\text{mg}\cdot\text{kg}^{-1}$ is most effective in our pre-experiments (Fig. S1).

Morris water maze test

Certain investigators blinded to the experimental groups were assigned to carry out the Morris water maze (MWM) test using ANYMAZE software (Clever Sys Inc., VA, USA). The test was set in a pool with water at a temperature of 21 \pm 1 $^{\circ}\text{C}$ in which the nonpoisonous white powder was put to visualize the shape of the rats. Before the hidden platform training, rats were constrained to swim and locate the hidden platform, a rectangular channel. If the rats failed, they were gently put on the platform for 10 s to be familiar with their location. After 1 day, a circular water maze replaced the rectangular maze, and the platform was hidden 1.0 cm under the water. Rats were put into the water in different locations among the trials. All rats went through four trials for 60 s at most, during which the rats that failed to get to the platform were put on it to be familiar with the surroundings. Then we carried out the probe trial where the platform was taken out. The rats were put in the quadrant that was opposite from the target quadrant and swam for 60 s. Thereafter, the rats were trained to find a visible platform providing a black pole as a mark; rats went through four trials each day; meanwhile, the platform and start location were changed during each trial.

Barnes maze test

The impairment of spatial learning was analyzed with the Barnes maze. The settings were composed of a white round disk (diameter: 100 cm, height: 75.5 cm from the ground) with 12 holes (diameter: 4 cm) equally placed in a circle. One escape hole was linked to the opening on the black acrylic box (13 cm \times 17 cm \times 7 cm) just under the disk, with the remaining holes kept open. After about 10 s, we sank the cylinder below the water surface, and the investigator controlled the experiment remotely to allow rats starting from a random location. If the rats could not go into the escape hole in 300 s, the investigator gently guided them to the hole. Before the rats returned to their cage, they were placed in the escape box for 60 s. The rats underwent four trials each day and repeated them for 5 days. The location of the hole stayed the same for all rats during the training course. The rats could memorize the location of the hole by using the materials from the room as visual marks. The rats that failed to find the hole for eight consecutive trials were kept out from this experiment. On the sixth day, the rats went through a probe test for 300 s in the maze to test whether they could memorize the position of the hole, while the box was removed from its original place. During all trials, including the probe test, we used a charge-coupled device camera to record the whole

view of the maze, and the camera was connected to the computer. The data were analyzed by MATLAB software (MATLAB 7.0, MathWorks, St. Louis, MO, USA), which could automatically track and analyze the escape routes.

Measurement of intracellular reactive oxygen species

Tissues were incubated in 25 μM 2',7'-Dichlorodihydrofluorescein diacetate for about 30 min and rinsed twice using PBS. The excitation and emission wavelengths were 515 and 585 nm, respectively, and the fluorescence intensity was measured with a luminometer.

Superoxide dismutase 1 activity assay

All reagents we used were purchased from Sigma-Aldrich Sp. z o.o. (Poznań, Poland), which included HCl, (–) epinephrine, EDTA- Na_2 , $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer (0.05 M, pH 10.2), ethanol and chloroform. They were used for extracting superoxide dismutase (SOD). EtOH (v/v; 3 : 5), chloroform, hemolysate and distilled water were mixed in a test tube. The mixture was then vortexed vigorously and centrifuged (3824 g; 4 °C; 5 min). After SOD was extracted, adrenaline and the $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer were added and incubated for 3 min at 37 °C. The UV/VIS Lambda 40 spectrophotometer was used to analyze SOD1 activity. Setting the wavelength at 320 nm (at 30 °C), the absorbance of the materials was analyzed for 5 min. The activity of SOD1 was measured per gram of hemoglobin in red blood cell. The variation coefficient and assay sensitivity were <4% and 97%, and the specificity was 0.1 U·mL⁻¹.

Catalase activity assay

Chemical reagents were purchased from Sigma-Aldrich. Phosphoric buffer (50 mM) was applied to dilute the hemolysate to 500-fold. A UV/VIS Lambda 40 spectrophotometer (Perkin-Elmer, St. Louis, MO, USA) was used to analyze catalase (CAT) activity. Setting the wavelength at 1240 nm (at 30 °C), absorbance detection of the experiment samples was conducted within 30 s. CAT activity was measured according to the calibration curve. The activity of CAT was determined per gram of hemoglobin in erythrocytes. The assay specificity and variation coefficient were 89% and < 2%, and the sensitivity was 1.71 U·mL⁻¹.

TUNEL assay

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was conducted to determine whether DNA fragmentation, a hallmark of apoptosis, occurred in the region of rat hippocampus. After the sections (6 μm) embedded with paraffin were prepared, the

TUNEL staining was performed with the *in situ* cell death detection kit (Thermo Fisher Scientific, St. Louis, MO, USA). A Nikon Labophot 2 microscope was used to count the total number of cells and TUNEL-positive cells in the dentate gyrus of hippocampus at 200 \times magnification. DAPI stains nucleus. Cells were quantified using a microscope, and six randomly selected visual fields were assessed for every assay.

Western blotting analysis

Cell homogenization was carried out using lysis buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet P-40 (FNN0021; Thermo)], 0.5% sodium deoxycholate (D6750; Sigma) and 0.1% SDS (74255; Sigma). A 40- μg protein aliquot of each sample was separated using SDS/PAGE (10–15%) and was electrophoretically transferred onto poly(vinylidene difluoride) membranes (IPVH00010; Millipore, Billerica, MA, USA). At room temperature, the membrane was blocked using 5% BSA for 1 h. The proteins were probed with anti-Bax Ig (1 : 1000, 2774; Cell Signaling Technology, St. Louis, MO, USA), anti- β -catenin Ig (1 : 1000, 8480; Cell Signaling Technology), anti-caspase-3 Ig (1 : 1000, 9664; Cell Signaling Technology), anti-JNK Ig (1 : 1000, 9257; Cell Signaling Technology), anti-Bcl2 Ig (1 : 1000, 3498; Cell Signaling Technology), anti-p-JNK Ig (1 : 1000, 4668; Cell Signaling Technology), anti-GSK-3 β Ig (1 : 1000, 12456; Cell Signaling Technology), anti- β -actin Ig (1 : 1000, 4970; Cell Signaling Technology) and anti-p-GSK-3 β Ig (1 : 1000, 5558; Cell Signaling Technology) for 12 h at 4 °C. The membranes were washed four times with TBST. A secondary antibody conjugated with HRP (1 : 10 000, 7074; Cell Signaling Technology) was used for immunoblot detection. The protein bands were visualized with a chemical luminescence reagent (Pierce, Pleasanton, CA, USA). Protein levels were determined by normalizing to the level of β -actin.

Statistics

Data are demonstrated as mean \pm SEM. Followed by Tukey's *post hoc* analysis, a one-way ANOVA was applied to compare data from multiple groups. Differences were considered statistically significant when the *P* value was <0.05.

Results

LiCl improves SEV-induced memory impairment in rats

To evaluate the memory ability and spatial learning of rats between multiple groups, the investigators made them to go through the Barnes maze test and MWM test. As presented in Fig. 1A–E, rats exposed in SEV

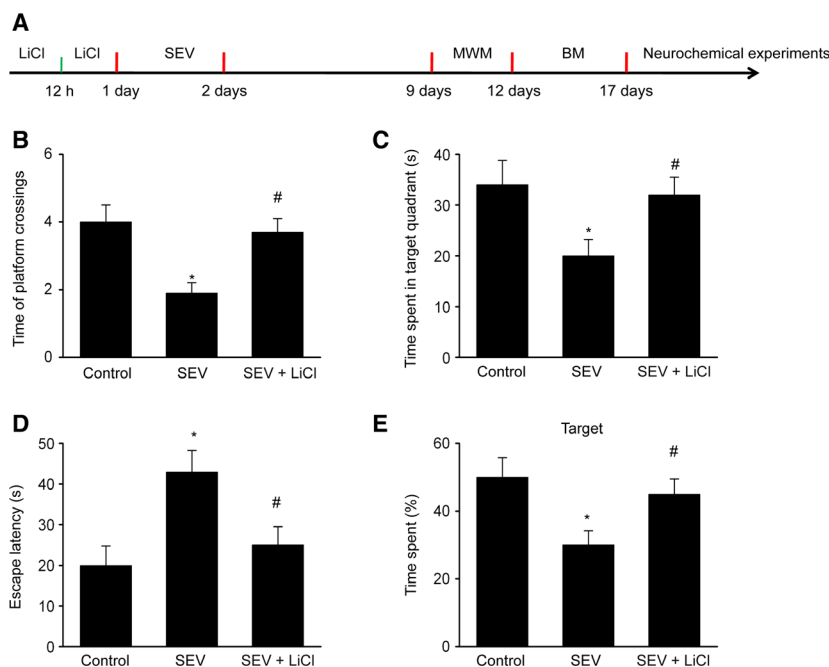


Fig. 1. LiCl improves SEV-induced memory impairment in rats. (A) Experimental time course. (B, C) After removing the platform on the sixth day, we counted the number of platform crossings (B) and the time spent in the target quadrant (C) within 90 s in the MWM test. (D) The latency for rat to enter a target hole in the training courses on the fifth day in BM test. (E) The percent of time spent in the target in the BM test. Data are represented as mean \pm SEM; $n = 12$; one-way ANOVA, $F(2, 33) = 58.72$; * $P < 0.05$ compared with control group; # $P < 0.05$ compared with SEV group.

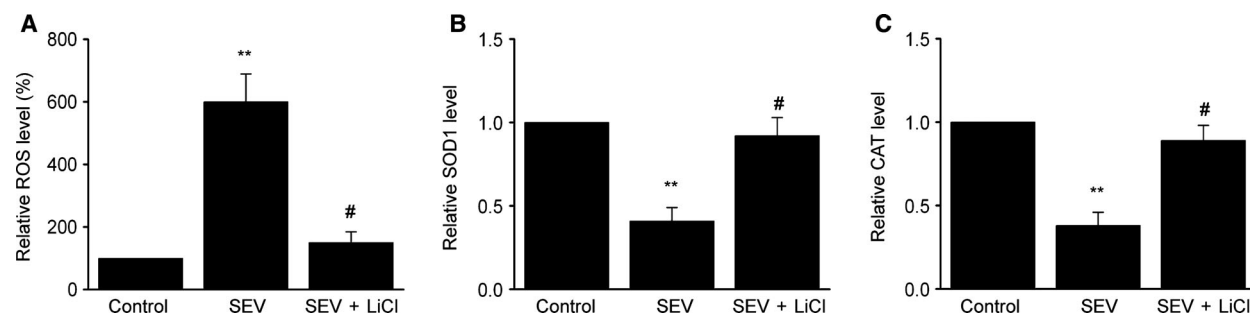


Fig. 2. LiCl inhibits oxidative stress induced by SEV in rat hippocampus. (A) Quantification data of ROS level in the hippocampus. (B) SOD1 activity was determined by SOD1 activity colorimetric assay kit. (C) CAT activity was determined by CAT-specific activity assay kit. Data are represented as mean \pm SEM; $n = 8$; one-way ANOVA, $F(2, 21) = 48.92$; ** $P < 0.01$ versus control group; # $P < 0.05$ versus SEV group.

presented cognitive impairment, which was suggested by decreased platform crossings, reduced time in the objective quadrant and prolonged escape latency. The SEV-induced cognitive impairment was ameliorated when treated with LiCl, as presented as less escape latency, increased time in the objective quadrant and raised platform crossings.

LiCl suppresses SEV-induced oxidative stress in the hippocampus

Oxidative stress is important in SEV-induced memory deficits [26]. As shown in Fig. 2A, compared with control group, reactive oxygen species (ROS) production was greatly increased in the SEV group. However, LiCl treatment reduced the level of ROS induced by

SEV. Moreover, LiCl treatment markedly increased the SOD1 and CAT levels in the hippocampus (Fig. 2B,C). These results demonstrate that LiCl suppresses oxidative stress induced by SEV in rat hippocampus.

LiCl reduces SEV-induced apoptosis in the hippocampus

Apoptosis plays a major part in the pathological mechanisms of SEV-induced memory impairment [27]. As shown in Fig. 3A, the results from the TUNEL assay demonstrated that SEV significantly increased the number of TUNEL⁺ cells/total number of cells ratio in rat hippocampus, whereas LiCl decreased the proportion in rat hippocampus.

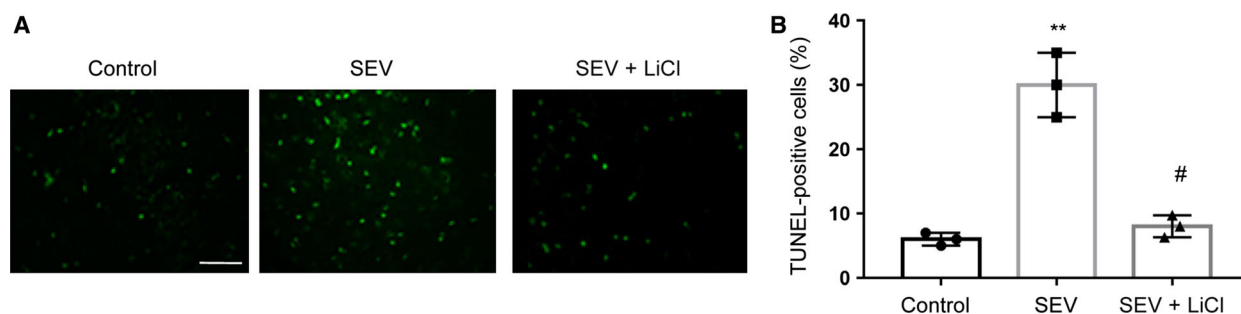


Fig. 3. LiCl reduces apoptosis induced by SEV in rat hippocampus. (A) In each group, photomicrographs presenting TUNEL-positive cells in rat hippocampus. (B) A chart for TUNEL-positive cells/total number of cells ratio in different groups, and five aspects were viewed in every rat. Scale bar: 40 μ m. Data are represented as mean \pm SEM; $n = 3$; one-way ANOVA, $F(2, 6) = 55.24$; ** $P < 0.01$ compared with control group; # $P < 0.05$ compared with SEV group.

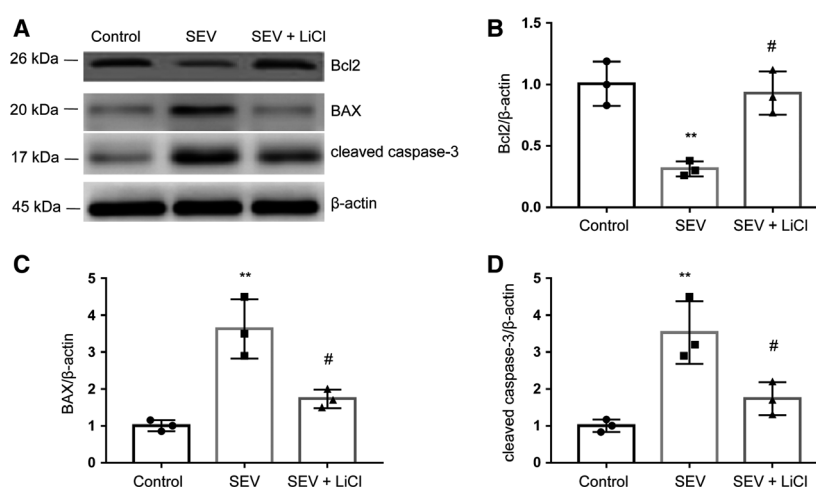


Fig. 4. LiCl decreases SEV-induced apoptosis-related proteins expression in rat hippocampus. (A–D) Immunoblots (A) and quantitative measurement of Bcl2 (B), BAX (C) and cleaved caspase-3 (D) in rat hippocampus. Data are demonstrated as mean \pm SEM; $n = 3$; one-way ANOVA, $F(2, 6) = 19.27$; ** $P < 0.01$ compared with control group; # $P < 0.05$ compared with SEV group.

LiCl decreases SEV-induced apoptosis-related protein expression in rat hippocampus

We measured the protein expressions of Bax, Bcl-2 and cleaved caspase-3, which are widely considered as apoptosis indicators in cells. The results demonstrated that SEV could remarkably increase the level of Bax and cleaved caspase-3, and reduced the level of Bcl-2 (Fig. 3B). LiCl greatly increased the expression of Bcl-2, reduced the expression level of Bax and cleaved caspase-3 induced by SEV (Fig. 4A–D).

LiCl inhibits the GSK-3 β / β -catenin signal channel activation in the hippocampus

To assess the effect of LiCl on GSK-3 β / β -catenin signal channel, which is involved in cognitive impairments, we examined the protein levels of β -catenin, JNK and GSK-3 β . Our results show that SEV could greatly increase the phosphorylated level of GSK-3 β (Ser9) and JNK (Thr183/Tyr185) (Fig. 5A,B). LiCl

significantly ameliorated the increased expression of JNK, GSK-3 β and β -catenin, which were induced by SEV (Fig. 5C,D). These results show that LiCl can inhibit the activation process of the GSK-3 β / β -catenin signal pathway in rat hippocampus.

Discussion

Cognitive impairment induced by anesthesia might be the most common type in postoperative cognitive disorder, which was found to influence signal pathways dysfunction, such as activation of GSK-3 β [28–33]. This research was aimed to reveal the role of GSK-3 β inhibition on cognitive impairment induced by SEV anesthesia. We identified that GSK-3 β inhibition had a neuroprotective effect from SEV anesthesia. LiCl reduced the neuroapoptosis induced by SEV, oxidative stress and the motor dysfunction in rats via inhibiting the GSK-3 β / β -catenin signal pathway.

GSK3 β is a kinase that, on activation, leads to the Tau phosphorylation process. The SEV induces GSK3 β

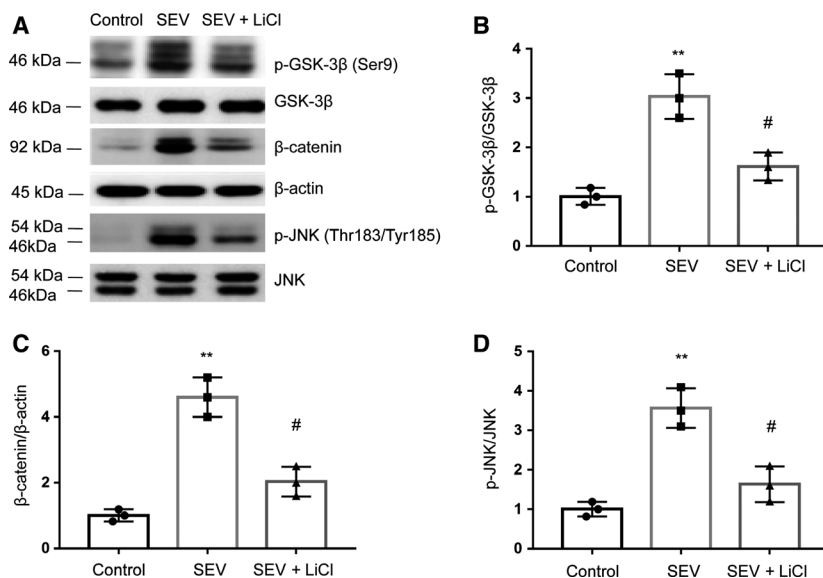


Fig. 5. LiCl inhibits the activation of GSK-3 β / β -catenin signal pathway in rat hippocampus. (A–D) Western blots results (A) and quantitative measurement of GSK-3 β (B), β -catenin (C) and JNK (D) in rat hippocampus. Data are represented as mean \pm SEM; $n = 3$; one-way ANOVA, $F(2, 6) = 31.33$; ** $P < 0.01$ compared with control group; # $P < 0.05$ compared with SEV group.

to activate Tau phosphorylation in rat hippocampus [30]. Strong evidence has demonstrated that GSK-3 β activation is involved in the excitotoxicity process of memory dysfunction in neurodegenerative diseases [34,35]. The GSK-3 β / β -catenin signal pathway is crucial for neuronal apoptosis, and its activation accelerates hippocampal neuroapoptosis by targeting proteins related to apoptosis or interacting with other signal pathways [36,37]. In this study, significantly activated GSK-3 β was identified in the hippocampus of rats given SEV, which was consistent with other studies. Lithium could inhibit GSK3 β . We found that lithium could reduce GSK3 β activation, induced by SEV anesthesia, neural apoptosis and cognition impairment in rats.

The process of learning and memory appears to involve complex mechanisms that are affected by many aspects. Neural death through apoptosis can result in learning dysfunction and deficits in memory consolidation [38,39]. Cognitive impairment induced by anesthesia has been related with neuronal apoptosis caused by activated GSK-3 β in some parts of the central nervous system [40]. Here, we proved that LiCl could inhibit the GSK-3 β activation in rat hippocampus and decrease neuron apoptosis and ROS production. Thus, we speculated that LiCl suppressed the GSK-3 β activation, which resulted in reduced neuron apoptosis and oxidative stress, ultimately contributing to improvement of cognitive deficits.

Conclusions

LiCl improved the SEV-induced cognitive impairment by inhibiting apoptosis and oxidative stress through suppression of the GSK-3 β / β -catenin signaling pathway,

and LiCl could become a therapeutic agent in treating SEV anesthesia-induced neurodegeneration.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

YW and ZY designed experiments. XA and XZ carried out experiments. JL and JW analyzed experimental results. YW wrote the manuscript. ZY revised the manuscript. All authors approved the final manuscript.

References

- 1 Czyz-Szypenbejl K, Medrzycka-Dabrowska W, Kwicien-Jagus K and Lewandowska K (2019) The occurrence of postoperative cognitive dysfunction (POCD) - systematic review. *Psychiatr Pol* **53**, 145–160.
- 2 Shao LJ, Xue FS, Guo RJ and Zheng L (2019) Assessing effect of anaesthetic methods on postoperative cognitive dysfunction. *Anaesth Intensive Care* **47**, 202–203.
- 3 Shao LJ, Xue FS, Guo RJ and Zheng L (2019) Comparing the effects of different drugs on postoperative cognitive dysfunction in elderly patients. *Chin Med J* **132**, 1007–1008.
- 4 Meng F, Li N, Li D, Song B and Li L (2019) The presence of elevated circulating trimethylamine N-oxide exaggerates postoperative cognitive dysfunction in aged rats. *Behav Brain Res* **368**, 111902.
- 5 Chen C, Gao R, Li M, Wang Q, Chen H, Zhang S, Mao X, Behensky A, Zhang Z, Gan L *et al.* (2019)

- Extracellular RNAs-TLR3 signaling contributes to cognitive decline in a mouse model of postoperative cognitive dysfunction. *Brain Behav Immun* **80**, 439–451.
- 6 Lu Y, Xu X, Dong R, Sun L, Chen L, Zhang Z and Peng M (2019) MicroRNA-181b-5p attenuates early postoperative cognitive dysfunction by suppressing hippocampal neuroinflammation in mice. *Cytokine* **120**, 41–53.
 - 7 Schenning KJ, Murchison CF, Mattek NC, Kaye JA and Quinn JF (2019) Sex and genetic differences in postoperative cognitive dysfunction: a longitudinal cohort analysis. *Biol Sex Differ* **10**, 14.
 - 8 Fang F, Xue Z and Cang J (2012) Sevoflurane exposure in 7-day-old rats affects neurogenesis, neurodegeneration and neurocognitive function. *Neurosci Bull* **28**, 499–508.
 - 9 Zhang Y, Zhen Y, Dong Y, Xu Z, Yue Y, Golde TE, Tanzi RE, Moir RD and Xie Z (2011) Anesthetic propofol attenuates the isoflurane-induced caspase-3 activation and A β oligomerization. *PLoS ONE* **6**, e27019.
 - 10 Kristek G, Rados I, Kristek D, Kapural L, Neskovic N, Skiljic S, Horvat V, Mandic S and Harsanji-Drenjancevic I (2019) Influence of postoperative analgesia on systemic inflammatory response and postoperative cognitive dysfunction after femoral fractures surgery: a randomized controlled trial. *Reg Anesth Pain Med* **44**, 59–68.
 - 11 Zhang Y, Bao HG, Lv YL, Si YN, Han L, Wang HY, Gao YJ, Jiang WQ and Zhang C (2019) Risk factors for early postoperative cognitive dysfunction after colorectal surgery. *BMC Anesthesiol* **19**, 6.
 - 12 Cottrell JE and Hartung J (2012) Developmental disability in the young and postoperative cognitive dysfunction in the elderly after anesthesia and surgery: do data justify changing clinical practice? *Mt Sinai J Med* **79**, 75–94.
 - 13 Konishi Y, Evered LA, Scott DA and Silbert BS (2018) Postoperative cognitive dysfunction after sevoflurane or propofol general anaesthesia in combination with spinal anaesthesia for hip arthroplasty. *Anaesth Intensive Care* **46**, 596–600.
 - 14 Huang S, Hu H, Cai YH and Hua F (2019) Effect of parecoxib in the treatment of postoperative cognitive dysfunction: a systematic review and meta-analysis. *Medicine* **98**, e13812.
 - 15 Zhang H, Wu Z, Zhao X and Qiao Y (2018) Role of dexmedetomidine in reducing the incidence of postoperative cognitive dysfunction caused by sevoflurane inhalation anesthesia in elderly patients with esophageal carcinoma. *J Cancer Res Ther* **14**, 1497–1502.
 - 16 Zhang BJ and Yuan CX (2019) Effects of ADAM2 silencing on isoflurane-induced cognitive dysfunction via the P13K/Akt signaling pathway in immature rats. *Biomed Pharmacother* **109**, 217–225.
 - 17 Tu Y, Liang Y, Xiao Y, Lv J, Guan R, Xiao F, Xie Y and Xiao Q (2019) Dexmedetomidine attenuates the neurotoxicity of propofol toward primary hippocampal neurons *in vitro* via Erk1/2/CREB/BDNF signaling pathways. *Drug Des Devel Ther* **13**, 695–706.
 - 18 Tao G, Zhang J, Zhang L, Dong Y, Yu B, Crosby G, Culley DJ, Zhang Y and Xie Z (2014) Sevoflurane induces tau phosphorylation and glycogen synthase kinase 3 β activation in young mice. *Anesthesiology* **121**, 510–527.
 - 19 Liu XS, Xue QS, Zeng QW, Li Q, Liu J, Feng XM and Yu BW (2010) Sevoflurane impairs memory consolidation in rats, possibly through inhibiting phosphorylation of glycogen synthase kinase-3 β in the hippocampus. *Neurobiol Learn Mem* **94**, 461–467.
 - 20 Liu J, Yang J, Xu Y, Guo G, Cai L, Wu H, Zhao Y and Zhang X (2017) Roscovitine, a CDK5 inhibitor, alleviates sevoflurane-induced cognitive dysfunction via regulation Tau/GSK3 β and ERK/PPAR γ /CREB signaling. *Cell Physiol Biochem* **44**, 423–435.
 - 21 Zhou X, da Li W, Yuan BL, Niu LJ, Yang XY, Zhou ZB, Chen XH and Feng X (2016) Lithium treatment prevents apoptosis in neonatal rat hippocampus resulting from sevoflurane exposure. *Neurochem Res* **41**, 1993–2005.
 - 22 Kim Y, Kim J, Ahn M and Shin T (2017) Lithium ameliorates rat spinal cord injury by suppressing glycogen synthase kinase-3 β and activating heme oxygenase-1. *Anat Cell Biol* **50**, 207–213.
 - 23 Liu Z, Li R, Jiang C, Zhao S, Li W and Tang X (2018) The neuroprotective effect of lithium chloride on cognitive impairment through glycogen synthase kinase-3 β inhibition in intracerebral hemorrhage rats. *Eur J Pharmacol* **840**, 50–59.
 - 24 Dahari DE, Salleh RM, Mahmud F, Chin LP, Embi N and Sidek HM (2016) Anti-malarial activities of two soil actinomycete isolates from Sabah via inhibition of glycogen synthase kinase 3 β . *Trop Life Sci Res* **27**, 53–71.
 - 25 Ma Z, Zhong Z, Zheng Z, Shi XM and Zhang W (2014) Inhibition of glycogen synthase kinase-3 β attenuates glucocorticoid-induced suppression of myogenic differentiation *in vitro*. *PLoS ONE* **9**, e105528.
 - 26 Qin Y, Ni J, Kang L, Zhong Z, Wang L and Yin S (2019) Sevoflurane effect on cognitive function and the expression of oxidative stress response proteins in elderly patients undergoing radical surgery for lung cancer. *J Coll Physicians Surg Pak* **29**, 12–15.
 - 27 Zhou X, Lu D, Li WD, Chen XH, Yang XY, Chen X, Zhou ZB, Ye JH and Feng X (2018) Sevoflurane affects oxidative stress and alters apoptosis status in children

- and cultured neural stem cells. *Neurotox Res* **33**, 790–800.
- 28 Chen Y, Dai CL, Wu Z, Iqbal K, Liu F, Zhang B and Gong CX (2017) Intranasal insulin prevents anesthesia-induced cognitive impairment and chronic neurobehavioral changes. *Front Aging Neurosci* **9**, 136.
- 29 Xiao H, Run X, Cao X, Su Y, Sun Z, Tian C, Sun S, Liang Z and Neurosciences C (2014) Temperature control can abolish anesthesia-induced tau hyperphosphorylation and partly reverse anesthesia-induced cognitive impairment in old mice. *Psychiatry Clin Neurosci* **67**, 493–500.
- 30 Boscolo A, Starr JA, Sanchez V, Lunardi N, DiGrucio MR, Ori C, Erisir A, Trimmer P, Bennett J and Jevtovic-Todorovic V (2012) The abolishment of anesthesia-induced cognitive impairment by timely protection of mitochondria in the developing rat brain: the importance of free oxygen radicals and mitochondrial integrity. *Neurobiol Dis* **45**, 1031–1041.
- 31 Wang S, Xue H, Xu Y, Niu J and Zhao P (2019) Sevoflurane postconditioning inhibits autophagy through activation of the extracellular signal-regulated kinase cascade, alleviating hypoxic-ischemic brain injury in neonatal rats. *Neurochem Res* **44**, 347–356.
- 32 Wang F, Wang J, An J, Yuan G, Hao X and Zhang Y (2018) Resveratrol ameliorates depressive disorder through the NETRIN1-mediated extracellular signal-regulated kinase/cAMP signal transduction pathway. *Mol Med Rep* **17**, 4611–4618.
- 33 Liu J, Yang J, Xu Y, Guo G, Cai L, Wu H, Zhao Y and Zhang X (2017) Roscovitine, a CDK5 inhibitor, alleviates sevoflurane-induced cognitive dysfunction via regulation Tau/GSK3beta and ERK/PPARgamma/CREB signaling. *Cell Physiol Biochem* **44**, 423–435.
- 34 Forlenza OV, De-Paula VJ and Diniz BS (2014) Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders. *ACS Chem Neurosci* **5**, 443–450.
- 35 Terstappen GC, Gaviraghi G and Caricasole A (2006) The Wnt signaling pathway as a target for the treatment of neurodegenerative disorders. *IDrugs* **9**, 35–38.
- 36 Pan C, Liu N, Zhang P, Wu Q, Deng H, Xu F, Lian L, Liang Q, Hu Y, Zhu S *et al.* (2018) EGb761 ameliorates neuronal apoptosis and promotes angiogenesis in experimental intracerebral hemorrhage via RSK1/GSK3beta pathway. *Mol Neurobiol* **55**, 1556–1567.
- 37 He H, Li X and He Y (2019) Hyperbaric oxygen therapy attenuates neuronal apoptosis induced by traumatic brain injury via Akt/GSK3beta/beta-catenin pathway. *Neuropsychiatr Dis Treat* **15**, 369–374.
- 38 Alhowail AH, Bloemer J, Pinky PD, Bhattacharya S, Majrashi M, Yongli Z, Bhattacharya D, Eggert M, Woodie L, Buabeid MA *et al.* (2019) Doxorubicin-induced neurotoxicity is associated with acute alterations in synaptic plasticity, apoptosis, and lipid peroxidation. *Toxicol Mech Methods* **29**, 457–466.
- 39 Fang H, Li HF, Yang M, Liao R, Wang RR, Wang QY, Zheng PC, Zhang FX and Zhang JP (2019) NF-kappaB signaling pathway inhibition suppresses hippocampal neuronal apoptosis and cognitive impairment via RCAN1 in neonatal rats with hypoxic-ischemic brain damage. *Cell Cycle* **18**, 1001–1018.
- 40 Morroni F, Sita G, Tarozzi A, Rimondini R and Hrelia P (2016) Early effects of Abeta1-42 oligomers injection in mice: involvement of PI3K/Akt/GSK3 and MAPK/ERK1/2 pathways. *Behav Brain Res* **314**, 106–115.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. LiCl improves SEV-induced memory impairment in rats. The rats were intraperitoneally injected with LiCl (30, 60, 100 mg·kg⁻¹) twice a day and then treated with SEV. After removing the platform on the sixth day, we counted the number of platform crossings within 90 s in MWM. Data are represented as mean ± SEM; *n* = 10; one-way ANOVA, **P* < 0.05 compared with control group; #*P* < 0.05 compared with the SEV group.