

α -lipoic acid attenuates spatial learning and memory impairment induced by hepatectomy

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Received December 06, 2017; Accepted November 19, 2018

DOI: 10.3892/etm.2019.7202

Abstract. The aim of the present study was to compare the effects of α -lipoic acid (ALA) on postoperative cognitive dysfunction (POCD) between wild type (WT) and leptin receptor-deficient (db/db) mice and to elucidate the underlying mechanism of treatment with ALA. The present study compared the effects of ALA on spatial learning and memory of WT and db/db mice using a Morris water maze following hepatectomy. The expression levels of proteins, including cyclin-dependent kinase 5 (Cdk5), tau, phosphorylated tau and amyloid β (A β) were measured in the hippocampus. Surgery impaired postoperative cognitive function in both WT and db/db mice. Furthermore, the expression levels of Cdk5 and A β , and the phosphorylation of tau in the hippocampus increased after the surgery in both WT and db/db mice. The ultrastructure of hippocampal neurons and synapses was analyzed by transmission electron microscopy and the results revealed that surgery damaged the structure of neurons and synapses in both WT and db/db mice. Treatment with ALA protected the postoperative cognitive function and the structure of hippocampal neurons and synapses, and prevented the increase in protein expression levels of Cdk5 and A β , and the phosphorylation of tau in the hippocampus of WT but not db/db mice. The results of the present study suggest that ALA may be used for the treatment of POCD. The molecular mechanisms underlying the activity of ALA require further investigation.

Introduction

Postoperative cognitive dysfunction (POCD) is a common postsurgical complication of the central nervous system. POCD occurs at an early stage of postoperative care and increases

the rates of perioperative mortality (1). The pathogenesis and prevention of POCD have been extensively studied. The mechanism underlying the behavioral alterations following surgery in animal models may be associated with the dysfunction of neurons and synapses (2-6). It has been hypothesized that the production and aggregation of amyloid β (A β), and the abnormal hyperphosphorylation and aggregation of tau protein may be involved in the pathogenesis of POCD (7). A β may alter the mitochondrial morphology and affect the steady state of calcium in the neurons (8). The hyperphosphorylation and aggregation of tau protein serves an important role in neurodegeneration (9).

The neuroprotective effect of α -lipoic acid (ALA) has been previously studied (10) and it was demonstrated that ALA promotes the secretion of leptin from adipocytes (11). A number of studies reported that leptin induces a protective effect on the cognitive function (12-15). Leptin regulates glucose and fat metabolism, and the leptin receptor activates brain-derived neurotrophic factor to regulate the synaptic plasticity and promote neural differentiation (12,13). Furthermore, leptin reduces the hyperphosphorylation of tau protein (14-16).

There are no effective treatment methods for patients with POCD and the underlying mechanism of this conditions remains to be elucidated. To the best of the authors' knowledge, the current study is the first to investigate the effect of ALA on POCD and the underlying mechanism of action. The present study aimed to investigate the effect of ALA on POCD induced by hepatectomy in wild type (WT) and leptin receptor-deficient (db/db) mice. Protein expression levels of Cdk5, phosphorylated tau (p-tau) and A β were determined by western blotting, and the ultrastructure of hippocampal neurons and synapses was analyzed by transmission electron microscopy.

Materials and methods

Experimental animals. A total of 60 WT C57BL/6 mice and 60 db/db C57BL/6 mice (all male; weight, 20-25 g; age, 14 weeks) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were housed with a 12 h light/dark cycle at a temperature of 24 \pm 1°C with access to food and water *ad libitum*. ALA (60 mg/kg; Wyeth Pharmaceutical Co., Ltd., Suzhou, China) or

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Key words: α -lipoic acid, postoperative spatial learning and memory impairment, cyclin-dependent kinase 5, tau, amyloid β , leptin

1% dimethyl sulfoxide in corn oil (vehicle) was administrated orally daily. At the end of the experiment, the animals were euthanized by intraperitoneal administration of a lethal dose of sodium pentobarbital (150 mg/kg). The present study was approved by the Institution Animal Care and Use Committee of Nanjing First Hospital (Nanjing, China).

Animal grouping. C57BL/6 WT and db/db mice were divided randomly into three groups each, including the control, surgery and ALA + surgery groups (20 mice/group). Control group mice received anesthesia only, the surgery group received 70% hepatectomy surgery, and the ALA + surgery group was intragastrically administrated 100 mg/kg ALA once daily for 12 weeks (17), and subsequently received 70% hepatectomy surgery. The control and surgery groups received the same volume of vehicle intragastrically (WT, 20 μ l/mice; db/db, 30 μ l/mice).

Hepatectomy surgery. Mice were anesthetized by an intraperitoneal injection of 50 mg/kg sodium pentobarbital (1% in saline). Mice were subsequently placed on a warming blanket and rectal temperatures were monitored. A roll of gauze was placed under the right scapula to provide adequate exposure of the liver. The abdomen of the mice was shaved, sterilized and draped. A 1-1.5-cm midline incision was made from the xiphoid inferiorly with micro dissecting scissors. The upper abdomen was opened and the three anterior lobes of the liver were isolated (~68% of the total liver weight), including the right upper lobe, left upper lobe and left lower lobe. Three knots were tied with moistened silk suture at the base of the lobes near the inferior vena cava. The tied lobes were subsequently cut immediately distal to the suture. The abdomen was irrigated with 2 ml of sterile saline (37°C) to ensure the removal of blood and to decrease contamination. Gentle pressure was applied on the abdomen with sterile gauze to remove residual irrigation. The wound was infiltrated with 0.25% bupivacaine to relieve pain. The peritoneum and the skin were subsequently closed separately with silk suture. The mice were placed under warming lights while waking up from anesthesia, and subsequently housed individually (18).

Morris water maze (MWM). Cognitive function was assessed using a 5-day MWM test as previously described (19). A round tub was used for the MWM task and filled with water mixed with nontoxic black paint to submerge a platform 1 cm below the water surface (water temperature, 19-24°C). Visual distal cues were located on the walls. During the first four days, mice were given four trials/day using a random starting location. If mice did not reach the platform within 60 sec, they were gently guided to the hidden platform. The latency to reach the platform was recorded from all sessions, and averaged to calculate the escape latency for each day. On day 5, the platform was removed for probe testing. Mice were allowed to swim freely for 60 sec. The number of crossings over the platform that had served as the target on days 1-4 was recorded. The time spent in the quadrant where the target platform was located was recorded. Data were collected and analyzed using motion detection software (DigBehv-MM; Shanghai Jiliang Software Technology Co., Ltd., Shanghai, China).

Transmission electron microscopy. Mice were intraperitoneally injected with 1% pentobarbital sodium (35 mg/kg) and then fixed on a foam plate. A large U-shaped incision was cut into the chest to expose the heart. The perfused needle was inserted into the apical part with 5 mm depth and fixed by hemostatic clamp. Heparin saline was infused until liver became pale, followed with 3% glutaraldehyde perfusion. The brain was removed, and the hippocampus was isolated and post-fixed in 2.5% glutaraldehyde solution for 2 h in 4°C, followed by embedding in the epoxy resin for 12 h at 45°C. The embedded tissues were cut into 50-70-nm-thick sections and mounted on 150 mesh copper grids. Following staining with uranyl acetate and lead citrate for 12 h in 4°C, the specimens were observed under a transmission electron microscope (HITACHI-7650; Hitachi, Ltd., Tokyo, Japan).

Western blotting. The hippocampal tissue was homogenized with Tris-HCl (pH 7.4; 50 mM), 1% Triton X-100, 0.2% sodium deoxycholate, 0.2% SDS and 1 mM EDTA (Beyotime Institute of Biotechnology, Shanghai, China), and centrifuged at 8,000 x g for 20 min at 4°C. The protein concentration from each mouse was tested using the bicinchoninic acid method. Protein (40 μ g/lane) was separated by SDS-PAGE (12% gel) and transferred to a nitrocellulose membrane (Hybond[®] ECL[™]; GE Healthcare, Chicago, IL, USA). Membranes were blocked with 5% milk and 0.1% Tween-20 in PBS for 1 h at room temperature. Membranes were then incubated with primary antibodies against Cdk5 (cat. no. ab21249), tau (cat. no. ab80579), β -actin (cat. no. ab8227; all 1:1,000), A β (cat. no. ab10148; 1:7,000; all Abcam, Cambridge, MA, USA) and p-tau (cat. no. 44-750G; 1:1,000; Thermo Fisher Scientific, Inc., Waltham, MA, USA) overnight at 4°C. After washing with tris buffered saline with Tween-20 (TBST) three times, membranes were incubated with anti-rabbit secondary antibodies (cat. no. ab6721; 1:2,000; Abcam) for 2 h at room temperature. Between steps, blots were washed with TBST. Immunodetection was performed using a LumiGLO chemiluminescence kit (Amersham; GE Healthcare). Bands were analyzed by Quantity One software (version 4.6.2; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. All data are presented as the mean \pm standard deviation. Data were analyzed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA) or GraphPad Prism (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). Two-way analysis of variance (ANOVA) was used to analyze the spatial memory data obtained from the MWM test. The levels of protein and gene expression were analyzed using one-way ANOVA. ANOVA analyses were followed by the Bonferroni post-hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

ALA rescues the impaired cognitive function in WT but not db/db mice. To study cognitive function, MWM was used to examine spatial learning and memory in both WT and db/db mice with or without treatment with ALA. Among the WT mice, the surgery group (day 2, 53.0 \pm 5.3; day 3, 40.1 \pm 6.7; day 4, 33.8 \pm 4.1) exhibited significantly longer latency to find the platform compared with the control group (day 2, 27.7 \pm 3.7;

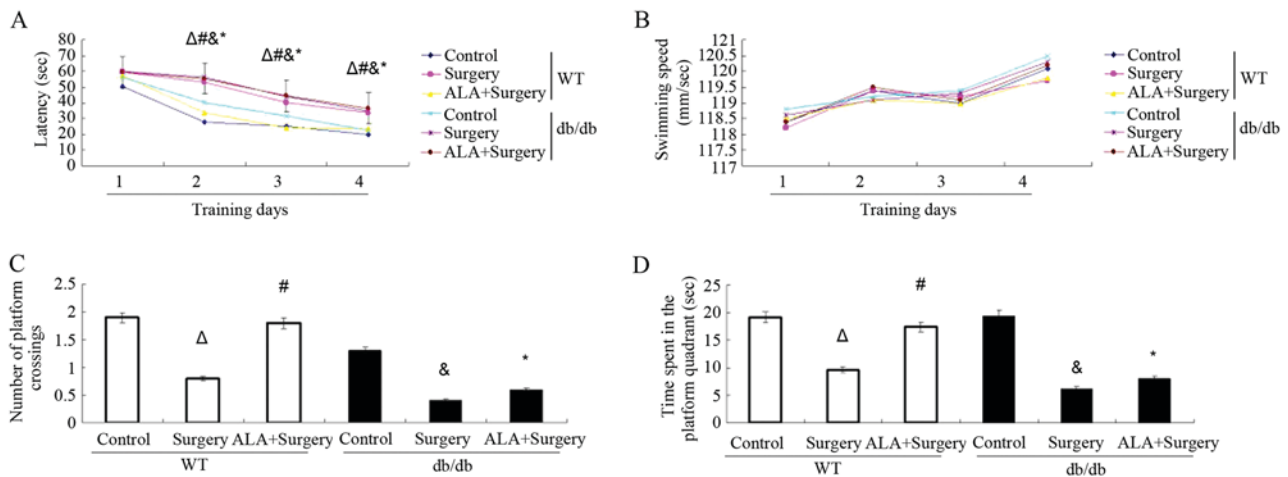


Figure 1. Effects of pretreatment with ALA on surgery-induced behavioral deficits measured using the Morris water maze. (A) The latency to reach the target platform during training days 1-4. (B) The swimming speed during training days 1-4. (C) The time spent in the target quadrant on the testing day (day 5). (D) The number of crossings over the original target platform on the testing day (day 5). Data are represented as the mean \pm standard deviation (n=20/group). ^ΔP<0.01 compared with the WT control group; ^{*}P<0.05 compared with the WT surgery group; [&]P<0.01 compared with the db/db control group; [#]P<0.01 compared with the WT ALA + surgery group. ALA, α -lipoic acid; WT, wild type; db/db, leptin receptor-deficient mice.

day 3, 25.0 ± 5.9 ; day 4, 19.9 ± 3.9 ; all $P < 0.01$). Among the db/db mice, the surgery group (day 2, 56.3 ± 2.1 ; day 3, 43.8 ± 3.0 ; day 4, 35.0 ± 1.5) exhibited significantly longer latency to find the platform compared with the control group (day 2, 40.3 ± 6.9 ; day 3, 31.8 ± 6.3 ; day 4, 22.3 ± 3.9 ; $P < 0.01$). The db/db ALA + surgery group (day 2, 55.4 ± 2.2 ; day 3, 44.5 ± 4.8 ; day 4, 36.7 ± 4.3) did not exhibit altered latency compared with the surgery only group ($P > 0.05$; Fig. 1A). However, the db/db ALA + surgery group exhibited significantly longer latency compared with the WT ALA + surgery group ($P < 0.01$; Fig. 1A). These results suggest that hepatectomy surgery prolonged the escape latency during the MWM test in both WT and db/db mice. Treatment with ALA alleviated the effect induced by surgery in WT mice but not in db/db mice. The swimming speed was recorded during the learning sessions in the MWM test. The results revealed that there were no differences in the swimming speed among the control, surgery and ALA + surgery groups in WT and db/db mice (Fig. 1B).

On probe testing day, WT mice in the surgery group crossed the target quadrant fewer times (0.8 ± 0.5 vs. 1.9 ± 0.8 ; $P < 0.01$) and spent less time in the platform quadrant (9.6 ± 7.7 vs. 19.2 ± 6.3 ; $P < 0.01$) compared with the control group. Treatment with ALA increased the time spent in the platform quadrant (17.4 ± 8.0 vs. 9.6 ± 7.7 ; $P < 0.05$) and crossing number (1.8 ± 0.6 vs. 0.8 ± 0.5 ; $P < 0.05$) in the target quadrant compared with the surgery group. Among the db/db mice, the surgery group crossed the target quadrant fewer times (0.4 ± 0.3 vs. 1.3 ± 0.9 ; $P < 0.01$) and spent less time in the platform quadrant (6.2 ± 3.0 vs. 19.5 ± 6.9 ; $P < 0.01$) compared with the control group. However, the crossing number (0.6 ± 0.4 vs. 0.4 ± 0.3 ; $P > 0.05$) and time spent in the platform quadrant (8.0 ± 5.4 vs. 6.2 ± 3.0 ; $P > 0.05$) remained unaltered following administration of ALA compared with the surgery group among the db/db mice (Fig. 1C and D). The ALA + surgery group db/db mice spent less time in the platform quadrant (8.0 ± 3.1 vs. 17.4 ± 8.0 ; $P < 0.01$) and crossed the platform fewer times (0.6 ± 0.3 vs. 1.8 ± 0.6 ; $P < 0.05$) compared to ALA + surgery group WT mice ($P < 0.01$; Fig. 1C and D).

Hepatectomy increases Cdk5, p-tau and A β protein levels in both WT and db/db mice, and this effect is reversed following treatment with ALA among WT mice but not db/db mice. Western blotting was used to assess the expression levels of cognitive function-associated proteins Cdk5 and A β and the phosphorylation levels of tau. Among the WT mice, surgery significantly increased the protein expression of Cdk5 and A β and the p/total (t) tau ratio in the hippocampus compared with the control group ($P < 0.01$). Administration of ALA following surgery significantly reduced the protein expression of Cdk5 and A β , and the p/t tau ratio in the hippocampus compared with the surgery group ($P < 0.05$; Fig. 2).

Among the db/db mice, surgery significantly increased the protein expression of Cdk5 and A β and the p/total (t) tau ratio in the hippocampus compared with the control group ($P < 0.01$). However, treatment with ALA did not alter the protein expression of Cdk5 and A β or the p/t tau ratio compared with the surgery group. The db/db ALA + surgery group mice exhibited significantly increased expression of Cdk5 and A β , and p/t tau ratio in the hippocampus compared with the WT ALA + surgery group in mice ($P < 0.01$; Fig. 2).

Hepatectomy impairs cellular structure of the hippocampus of WT and db/db mice, and this effect is reversed following treatment with ALA among WT mice but not db/db mice. The ultrastructure of the CA3 hippocampal region was analyzed using transmission electron microscopy (Fig. 3). The images revealed that among the WT mice, surgery (Fig. 3B) impaired the ultrastructure of hippocampal neurons, which exhibited pyknosis, shrinkage of nuclear membrane, broadened perinuclear space and reduced synaptic density. Administration of ALA after surgery (Fig. 3C) improved the neuronal structure in the hippocampus, which appeared similar to the control group (Fig. 3A), exhibiting a smooth nuclear membrane and normal synaptic density and structure.

Among the db/db mice, surgery (Fig. 3E) impaired the ultrastructure of hippocampal neurons and synapses compared with the control group. However, treatment with ALA + surgery

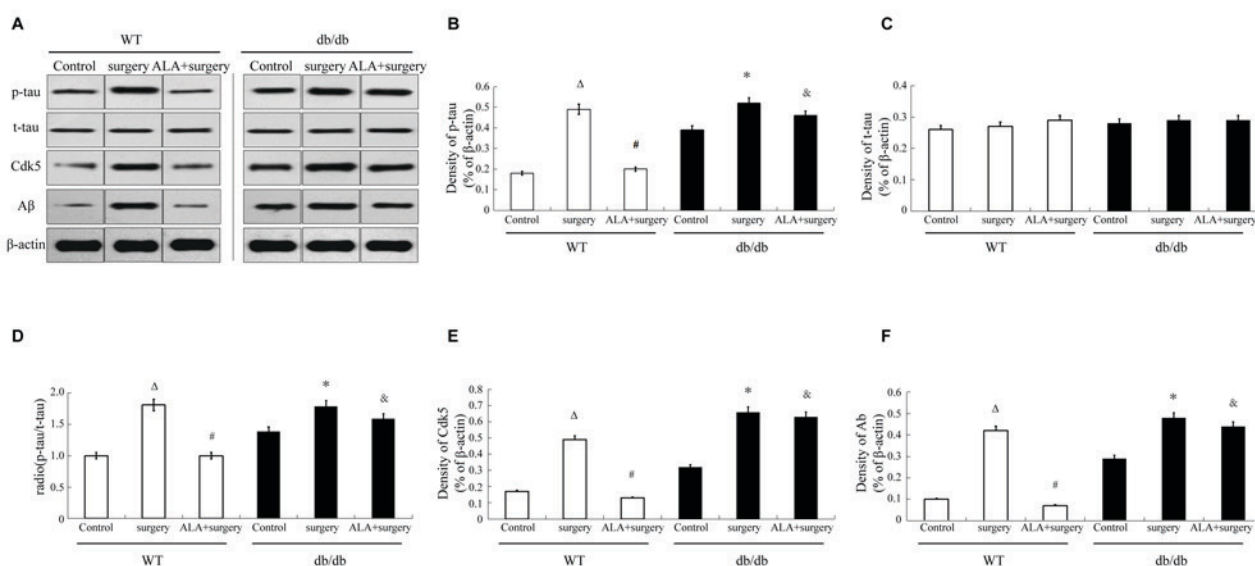


Figure 2. Effects of ALA on surgery-induced alterations in Cdk5 and A β protein expression levels and p/t tau ratio in the hippocampus. (A) Representative images of western blotting for the analysis of p-tau, t-tau, Cdk5, A β and β -actin protein expression. (B) Quantitative analysis of p-tau protein expression relative to β -actin. (C) Quantitative analysis of t-tau protein expression relative to β -actin. (D) Quantitative analysis of p-tau to t-tau protein expression ratio. (E) Quantitative analysis of Cdk5 protein expression relative to β -actin. (F) Quantitative analysis of A β protein expression relative to β -actin. Data are presented as the mean \pm standard deviation (n=5/group). $^{\Delta}$ P<0.01 compared with the WT control group; *P<0.05 compared with the surgery group; $^{\#}$ P<0.01 compared with the db/db control group; $^{\&}$ P<0.01 compared with the WT ALA + surgery group. ALA, α -lipoic acid; WT, wild type; db/db, leptin receptor-deficient mice; p, phosphorylated; t, total; Cdk5, cyclin-dependent kinase 5; A β , amyloid β .

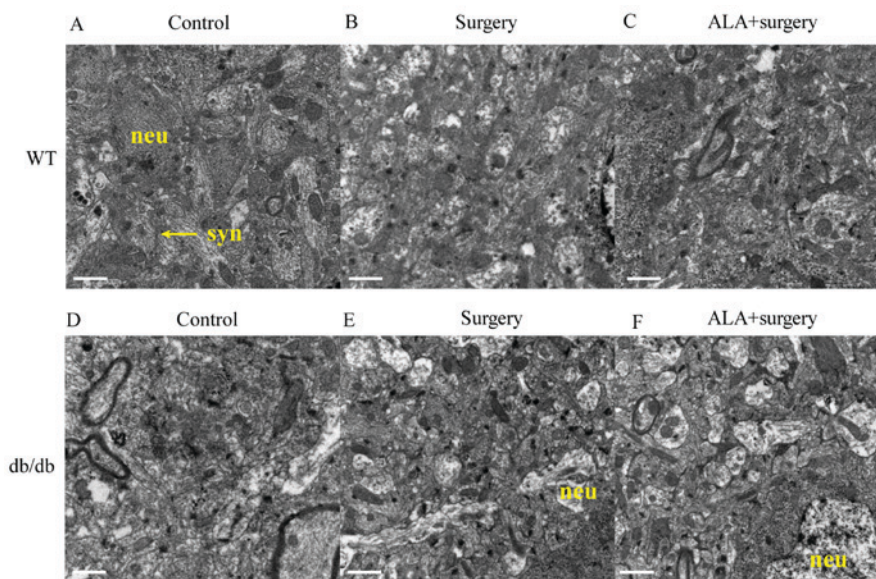


Figure 3. Ultrastructure of neurons and synapses in the hippocampus. The ultrastructure images from electron microscopy presenting the results for (A) WT control group, (B) WT surgery group, (C) WT ALA + surgery group, (D) db/db control group, (E) db/db surgery group, (F) db/db ALA+surgery group. Scale bar=1 μ m. Neu, neuron; syn, synapse; ALA, α -lipoic acid; WT, wild type; db/db, leptin receptor-deficient mice.

(Fig. 3F) did not repair the neuronal structure in the hippocampus, which was different compared with the normal structure of the control group (Fig. 3D).

Discussion

A previous study reported that ALA can increase the level of leptin in serum through the regulation of Cdk5. Cdk5 serves an important role in neuron differentiation and synaptic plasticity by phosphorylating cytoskeleton proteins and signaling pathway

molecules (16). Cdk5 is a serine/threonine protein kinase that forms complexes with p35 or p39 and is essential for neural development and function (20). Downregulation of CDK5 has been demonstrated to mitigate hippocampal degeneration and cognitive dysfunction (16).

The effects of ALA on the cognitive function following hepatectomy and the possible underlying mechanism were studied using db/db and WT mice. The results revealed that surgery impaired postoperative cognitive function, increased the hippocampal expression of Cdk5 and A β proteins and the

phosphorylation of tau, and damaged the structure of hippocampal neurons and synapses in both WT and db/db mice. ALA rescued the cognitive function of WT mice after surgery, as revealed by the results of the MWM learning and test.

In addition, analysis of protein expression in the hippocampus revealed that treatment with ALA decreased the elevated protein expression levels of Cdk5 and A β and the p/tau ratio in WT mice subjected to surgery. However, this effect was not observed among the db/db mice. The ultrastructure of neurons and synapses in the hippocampus was observed in mice following surgery and treatment with ALA. Hepatectomy markedly damaged the structure of neurons and synapses in both WT and db/db mice. However, treatment with ALA repaired the structure of neurons and synapses in WT mice, but not the db/db mice. These results suggested that the improvement in cognitive function following administration of ALA may be associated with reduced protein expression levels of Cdk5 and A β , and the phosphorylation level of tau. Furthermore, treatment with ALA enabled maintenance of the normal structure of hippocampal neurons and synapses.

Treatment with ALA did not improve the cognitive function of db/db mice following hepatectomy, which may suggest that the leptin signaling pathway may be a potential target of ALA. ALA may regulate the expression of leptin or regulate the binding to leptin receptor to alter the expression levels of Cdk5 and A β , and the phosphorylation level of tau to maintain the normal structure of hippocampal neurons and synapses. Future studies should investigate the effect of ALA on the leptin signaling pathway and cognitive function in WT mice with hepatectomy.

Acknowledgements

Not applicable.

Funding

The current study was supported by grants from the National Natural Science Foundation (grant no. 81873954) and the Medicine Development Project of Nanjing Scientific Committee (grant no. YKK15089).

Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Authors' contributions

YZ, HGB conceived the study design. YZ and YLL performed the experiments. YNS, JWZ and YNQ participated in the data analysis and interpretation.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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