Journal of Inflammation Research Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

ZLN005 Reduces Neuroinflammation and Improves Mitochondrial Function in Mice with Perioperative Neurocognitive Disorders

Xiaofan Wu[1,2], Sheng Ding[1,2], Guizhi Wang[1,2], Wei Zhang[1], Keqiang He[1]

[1]Department of Anesthesiology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230001, People's Republic of China; [2]Department of Anesthesiology, Bengbu Medical College Graduate School, Bengbu, Anhui, 233000, People's Republic of China

Correspondence: Keqiang He; Wei Zhang, Department of Anesthesiology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230001, People's Republic of China, Email doctorhector@ustc.edu.cn; doctor_zw97079@163.com

Background: The decrease expression of PGC-1α contributes to perioperative neurocognitive disorders (PND). This study aimed to investigate the effects of the PGC-1α agonist ZLN005 in preventing PND and to explore the potential mechanism.

Methods: C57BL/6 mice were randomly divided into four groups: the control group (Group C), the surgery group (Group S), the surgery and ZLN005 (5 mg/(kg·d)) group (Group L), and the surgery and ZLN005 (7.5 mg/(kg·d)) group (Group H). Except for Group C, the other three groups received intraperitoneal injections of vehicle or ZLN005 once a day from 3 days before surgery to 3 days after surgery. The open field test, novel object recognition test and fear conditioning test were performed to measure anxiety behaviors, locomotor activity and memory. The levels of IL-6 and IL-1β were measured at 24 hours after surgery. ATP and ROS levels were measured at 3 days post-surgery. PGC-1α, NRF-1, Atp5d, Atp5k and Cox5a were measured at one day or three days post-surgery.

Results: ZLN005 treatment improved the cognitive function of mice in Group L and Group H compared with Group S. The expression of IL-6 and IL-1β in the hippocampus of the S group was increased after surgery, and ZLN005 reduced the expression of IL-6 and IL-1β in the hippocampus of mice one day after surgery. There were parallel decreases in the expression of PGC-1α/NRF-1 and mitochondrial function in the hippocampus of the Group S mice compared with the Group C mice. The expression of PGC-1α/NRF-1 and mitochondrial function were upregulated after ZLN005 treatment.

Conclusion: Neuroinflammation and mitochondrial damage are involved in the occurrence of PND. ZLN005 activates PGC-1α to increase the expression of mitochondrial proteins, improve mitochondrial function, and ultimately ameliorate the cognitive status of mice after surgery.

Keywords: ZLN005, perioperative neurocognitive disorders, neuroinflammation, PGC-1α, mitochondrial respiratory chain complex, respiratory function

Introduction

Perioperative neurocognitive disorder (PND) is defined as different types of cognitive impairment encountered during the perioperative period from immediately after surgery to 1 year.[1,2] Aging is an independent risk factor for PND in patients.[3] However, the aging population is accelerating. Patients with PND tend to have longer hospital stays, are more frequently discharged to professional nursing institutions, and face a higher risk of death, all of which increase the economic burden on both society and families.[4] In recent years, researchers have proposed several hypotheses to explain the primary mechanisms of PND, including neuroinflammation, oxidative stress, aberrant autophagy, synaptic damage, and insufficient nutrient supply to the nervous system.[5–9] Studies have shown that regional changes in brain function, particularly postoperative inflammation, are major contributors to memory impairment and behavioral disorders.[5] The central

Received: 8 August 2024
Accepted: 26 October 2024
Published: 4 November 2024

inflammatory reaction left by surgery and anesthesia may lead to the injury of neurons and the activation of central inflammatory cells. This process is related to mitochondrial dysfunction,[8] but the specific mechanism is still unknown.

Mitochondria are the center of energy metabolism, and peroxisome promoter activated receptor gamma coactivator 1α (PGC-1α) is related to mitochondrial biogenesis through nuclear respiratory factors, which act downstream. Some studies have proven that improving the expression of PGC-1α could reduce cognitive impairment.[10,11] Our previous study found that surgery causes PGC-1α decreased and mitochondrial oxidative respiratory chain damage, mainly manifested by down-regulated expression of mitochondrial subunit proteins (Atp5k, Atp5d, Cox5a). The damaged mitochondrial oxidative respiratory chain leads to mitochondrial dysfunction, including excessive accumulation of reactive oxygen species and impaired ATP regeneration.[5,12] This eventually causes the onset of PND. In our previous study, we used IL-1β to stimulate the HT22 cell line, simulating the inflammatory environment of neurons. This resulted in reduced PGC-1α levels and the downregulation of mitochondrial protein subunits such as Atp5k, Atp5d, and Cox5a. However, overexpression of PGC-1α reversed this downregulation, promoting the production of mitochondrial protein subunits, reducing reactive oxygen species, and enhancing ATP generation.

ZLN005 is a specific agonist of PGC-1α. It was first found to be beneficial to the treatment of type 2 diabetes by affecting energy metabolism.[13] Many studies have confirmed that ZLN005 can protect mitochondria from harmful factors and maintain mitochondrial homeostasis when applied to different in vitro and in vivo models.[14–17] At the same time, it was reported that ZLN005 can improve the neuronal injury induced by ischemia in vitro and in vivo.[18] However, whether ZLN005 can effectively act on the perioperative neurocognitive disorders model remains unknown.

In this study, we mainly explored whether ZLN005 could improve neuroinflammation and mitochondrial function in PND mice. Therefore, it may become a promising drug for preventing PND.

Materials and Methods

Animals

Male C57BL/6 wild-type mice aged 7–8 months were provided by Vital River Laboratory Animal Technology (Beijing, China). All animals were housed in a standard environment with free access to water and food. All procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences. All experimental protocols were approved by the Animal Studies Committee at The First Affiliated Hospital of the University of Science and Technology, Hefei, China. Before any operation or intervention, wild-type mice were randomly divided into four groups: the control group, the surgery and injection of vehicle group (Group S), the surgery and intraperitoneal injection of ZLN005 [Med Chem Express, USA, 5 mg/(kg·d)] group (Group L), and the surgery and intraperitoneal injection of ZLN005 [7.5 mg/(kg·d)] group (Group H). Except for the control group that received anesthesia-matched sham operation, the other three groups of mice underwent surgery and injection. The injection lasted from three days before the surgery to three days after the surgery, once per day. On the day of surgery, the injection was administered 30 minutes before surgery. Before injection, ZLN005 was dissolved in dimethyl sulfoxide (DMSO) as a stock solution, which was diluted to a solution of 5% DMSO, 40% PEG-300, 5% Tween-80 and 50% saline.

Establishment of a PND Animal Model

For the surgical procedure, C57BL/6 mice were anesthetized with 2.1% isoflurane (RWD Life Science) in a commercially available rodent inhalation anesthesia device (MIDTRX VIP2000, Midmark, Dayton, OH, United States). Tramadol (30 mg/kg) was administered subcutaneously to mice immediately for analgesia after anesthesia induction and before surgery.[19,20] Aseptic open tibial shaft fractures were performed in mice, and the fractures were treated with intramedullary nails. Control mice received the same dose of anesthesia and analgesia but did not undergo surgery. The body temperature was kept at 37°C±0.5°C using a warm pad and a temperature control lamp. The whole process from anesthesia induction to the end of the operation lasted 15±2 minutes.

Open Field Test (OFT)

We used an open field to measure the anxiety behaviors and general motor activities of the mice. Mice completed the open field task at 6 days post-surgery. Mice were gently placed in the center of the open field (45cm×45cm×40cm) and allowed to move freely for 5 minutes. The exploratory behaviors were recorded using a video tracking system (Smart 3.0 software, Panlab, Spain). The total distance traveled and the time spent in the center of the chamber were recorded. The arena was wiped with 75% ethanol after each test to avoid olfactory cues.

Novel Object Recognition Test (NORT)

The NORT measures the recognition memory and exploration propensity of rodents by their preference to explore novel rather than familiar objects.[21] On Day 6 post-surgery, mice were allowed to explore an empty test chamber (45cm×45cm×40cm; RWD Life Science) as mentioned in the OFT. At 7 days post-surgery, two identical objects (A + A) were placed in the opposite corner of the arena, and the mice were allowed to explore freely for 10 minutes. After a 4-hour retention interval, the animals were taken back to the arena, in which case object A remained the same and the other object was novel (B). If the nose of the mouse points to an object within 2 mm, the interaction is recognized. The detection time of each object was recorded. Data were analyzed using Smart 3.0 software (Panlab, Spain). The cognitive index was defined as the ratio of the time spent exploring the novel object to the total time spent exploring both objects.

Fear Conditioning Test (FCT)

As mentioned earlier, The FCT was conducted using a conditioning chamber (soft maze information technology). Briefly, the mice were placed in the chamber to acclimate for 100 s, and then conditional (20 seconds, 75–80 dB, 5 kHz tone conditional stimulation) and unconditional (2 seconds, 0.75 mA foot shock overlapped with conditional stimulation) stimuli were applied four times. The interval between each time is 100 s. The context testing was conducted 24 hours after the training sessions, and allowed mice to stay in the conditioning chamber without any stimulation for 5 minutes. The freezing behavior and freezing time during each test were recorded and analyzed.[12]

Western Blotting Analysis

Hippocampal tissues were harvested on Day 3 after surgery. Briefly, we measured the protein concentrations in the hippocampus with a BCA protein assay kit (Thermo Scientific, Cat #: P0010S). Then, SDS–PAGE was used to separate the proteins, which were subsequently transferred to nitrocellulose membranes (Poll, Cat# 66485). The membranes were incubated with 5% nonfat milk with 0.1% Tween-20 in TBS at room temperature for 1 h, followed by incubation with primary antibodies at 4°C overnight. The following primary antibodies were used: polyclonal rabbit anti-IL-1β (1:1,000, Proteintech, Cat#:16806-1-AP), monoclonal mouse anti-PGC-1α (1:5,000, Proteintech, Cat#:66369-1-Ig), polyclonal rabbit anti-NRF-1 (1:1,000, Proteintech, Cat#:12482-1-AP), polyclonal rabbit anti-Cox5a (1:500, Proteintech), polyclonal rabbit anti-Atp5d (1:1000, Proteintech, Cat#:14893-1-AP), polyclonal rabbit anti-Atp5k (1:500, Proteintech, Cat#:16483-1-AP), and monoclonal mouse β-actin antibody (1:5,000, Affinity, Cat#:AF7018). The immunoreactive bands were visualized by enhanced chemiluminescence (Thermo Scientific) and detected by a Chemiscope (CLiNX). Immunoreactive bands were quantified using ImageJ software (NIH).

Immunofluorescence Staining (IF)

Mice were perfused with PBS and PFA three days post-surgery, and brain tissue was collected. The brain tissue samples were dehydrated with 20% sucrose and 30% sucrose and then embedded in the optimal cutting temperature compound (Sakura). The tissues were permeabilized with PBS containing 0.2% Triton X-100 (PBST), followed by blocking with 2% BSA in PBST for 30 min. After blocking, brain tissues were incubated with primary antibodies overnight at 4°C, followed by incubation with an Alexa-conjugated secondary antibody (Invitrogen). Fluorescence was observed using a fluorescence microscope, and the results were analyzed using Image-Pro Plus software. The following antibodies were used for immunofluorescence staining: monoclonal mouse anti-PGC-1α (1:200, Proteintech, Cat#: 66369-1-Ig) and monoclonal rabbit anti-NRF-1 (1:200, Abcam, Cat#: ab175932).

RNA Isolation, Reverse Transcription, and Quantitative Polymerase Chain Reaction (qPCR)

Hippocampal tissues were harvested at Day 1 after surgery. Total RNA was extracted from hippocampal tissue using TRIzol (Invitrogen) and subjected to DNase I digestion to remove genomic DNA. Reverse transcription was carried out using Hiscript II Reverse Transcriptase (Vazyme) according to the manufacturer's instructions. Quantitative PCR was performed with AceQ qPCR SYBR Green Master Mix (Vazyme) on a Light Cycler 96 (Roche) instrument according to standard procedures. The real-time value for each sample was averaged and compared using the CT method, where the amount of target RNA ($2^{-\Delta\Delta CT}$) was normalized to an endogenous reference (ΔCT) and related to the amount of target gene in hippocampal tissues, which was set as the calibrator at 1.0. ANOVA and Student's t tests were applied for statistical analysis. The qPCR detection primers are listed in Supplementary Table 1.

Determination of Adenosine Triphosphate (ATP) Levels

ATP content levels were determined in hippocampal tissues using an ATP content assay kit (Beyotime, Cat#: S0026) according to the manufacturer's instructions.

Determination of Reactive Oxygen Species (ROS)

Hippocampal tissue reactive oxygen species (ROS) levels were measured using a commercially available kit (GENMED, Cat#: GMS10016.4) according to the manufacturer's instructions. Briefly, three days post-surgery, newly harvested hippocampal tissue was homogenized. A working solution containing DCFHDA (100 μL) was added to each sample in a 96-well plate. Fluorescence was measured using a SpectraMax i3x microplate reader (Molecular Devices) at an excitation/emission wavelength of 490/520 nm.

Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of IL-6 (R&D Systems, Cat#: EMC004) and IL-1β (NeoBioscience, Cat#: EMC001b) were measured in hippocampal tissues by an enzyme-linked immunosorbent assay (ELISA)-based approach according to the protocols. As previously described, one day post-surgery, mice were perfused with phosphate buffered saline (PBS), and hippocampal tissue was harvested.[22] Hippocampal proteins were extracted with RIPA buffer (Beyotime, Cat#: P0013B) containing 1% PMSF (Beyotime, Cat#: ST506) and 1% protease inhibitor cocktail (MCE, Cat#: C0001). The protein concentration was determined with a BCA protein assay kit (Thermo Scientific, Cat #: P0010S) and corrected with a standard curve.

Interventions

The experimental protocol is depicted in Figure 1.

Statistical Analysis

All of the results are presented as the mean ± standard error of the mean values. All quantified data represent an average of at least triplicate samples. Statistical significance was determined by one-way analysis of variance (ANOVA) in GraphPad Prism 9.0. $P < 0.05$ was considered significant (indicated by an asterisk in the figures).

Results

ZLN005 Treatment Reversed the Cognitive Impairment in PND Mice

In the open field test, the total distance of movement and the time spent in the center were recorded for each mouse. We found that there was no significant difference in locomotor activities and anxiety behavior among the four groups (Supplementary Figure 1). Cognitive function was evaluated by a new object recognition test and fear conditioning test. At 7 days post-surgery, the mouse was subjected to the NORT, and the context test was performed 4 hours after the training session. In the training session, there was no significant difference between the four groups. However, 4 hours later, in the context phase, the S group mice spent significantly less time exploring the new object than the control (C)

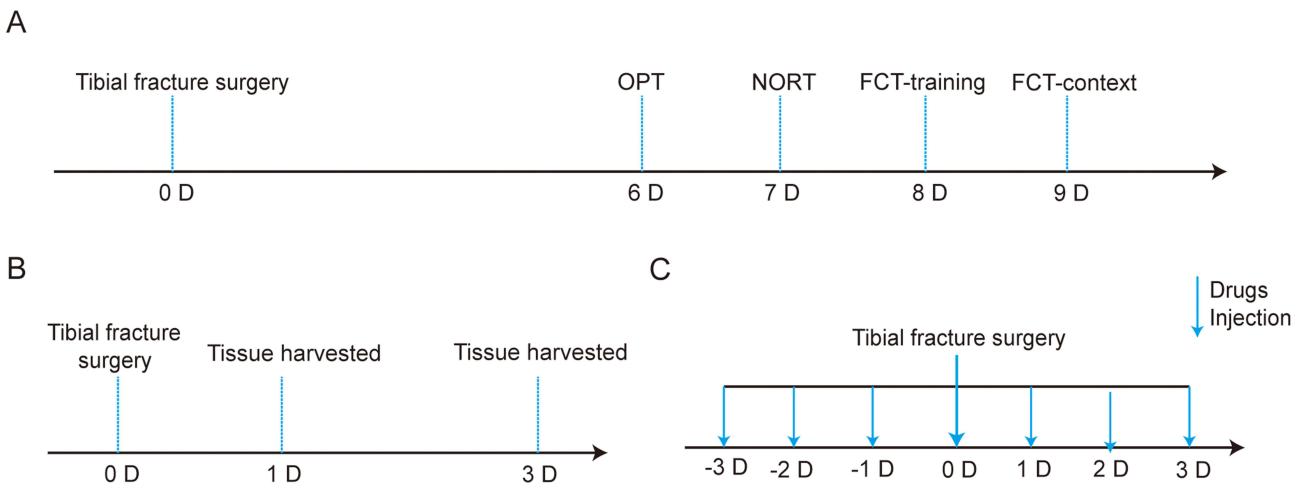

Figure 1 Experimental diagrams. (**A**) The anxiety level and locomotor activities of the mice were measured in the open field test 6 days post-surgery. The old/new object recognition test was performed 7 days post-surgery, and the training and context tests of the fear conditioning test were performed 8 days and 9 days post-surgery, respectively. (**B**) On day one- and three-days post-surgery, hippocampal tissues were harvested to detect inflammatory factors, target RNA, and target proteins and to evaluate mitochondrial function in each group. (**C**) In the groups requiring drug administration, intraperitoneal injection was started from three days before the operation to 3 days after the operation, once a day. Among them, the control group (sham), the S group (surgery and DMSO), the L group (surgery and ZLN005 5 mg/(kg·d)) and the H group (surgery and ZLN005 7.5 mg/(kg·d)) were treated differently, as shown in the Methods section.

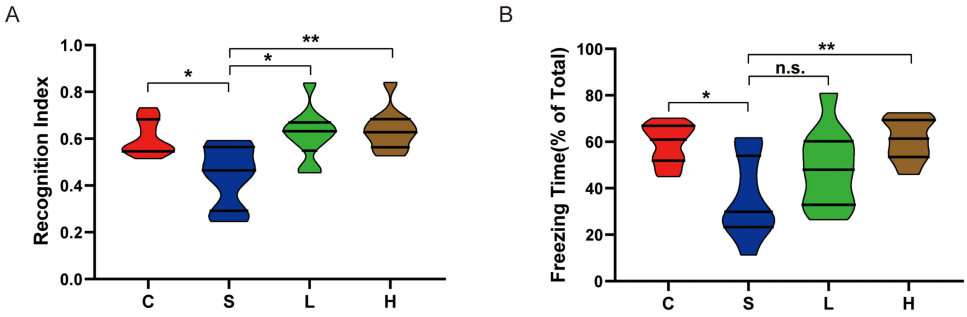

Figure 2 Recognition and memory in mice were evaluated after surgery and ZLN005 treatment. Behaviour tests were performed on postoperative Days 6–9. (**A**) On Day 7 post-surgery, long-term memory was evaluated by the novel object recognition test (NORT). The recognition index was calculated for each mouse in the four groups at 4 hours after the training. (**B**) Long-term memory was evaluated by the fear conditioning task. Freezing time was recorded, and the ratio of freezing time to the total testing time was calculated on Day 9 post-surgery. *P < 0.05; **P < 0.01 by one-way analysis of variance (ANOVA); n. s. not significant; error bars denote the standard error of mean (SEM).

mice, which did not undergo surgery. Surgery and anesthesia can result in memory destruction and cognitive dysfunction. The above results show that the construction of the PND model is effective, which is consistent with previous research. The mice treated with ZLN005 (L/H groups) spent significantly more time exploring the new object than those in the S groups. ZLN0005 reversed the hippocampus-dependent cognitive dysfunction caused by surgery (Figure 2A). Consistently, the FCT task also showed that the freezing time was significantly shorter in the surgery (S) group than in the control (C) group, and the ZLN005 treatment group (H groups) had significantly longer freezing times than the surgery group (Figure 2B). As a result, ZLN005 treatment improved the cognitive impairment of PND mice.

ZLN005 Treatment Ameliorated Neuroinflammation in PND Mice

IL-6 and IL-1β are well-known inflammatory factors that indicate mouse inflammation status. In this study, we collected mouse brain tissue, including the cortex and hippocampus, 24 h after surgery. In the hippocampus and cortex, IL-1β and IL-6 levels were significantly increased in the S group and obviously decreased in the H group treated with ZLN005. In the hippocampus, IL-1β levels were significantly reduced in L group and IL-6 levels showed a decreasing trend. In the cortex, IL-6 levels were significantly reduced in L group and IL-1β levels showed a decreasing trend. (Figure 3A–D). In

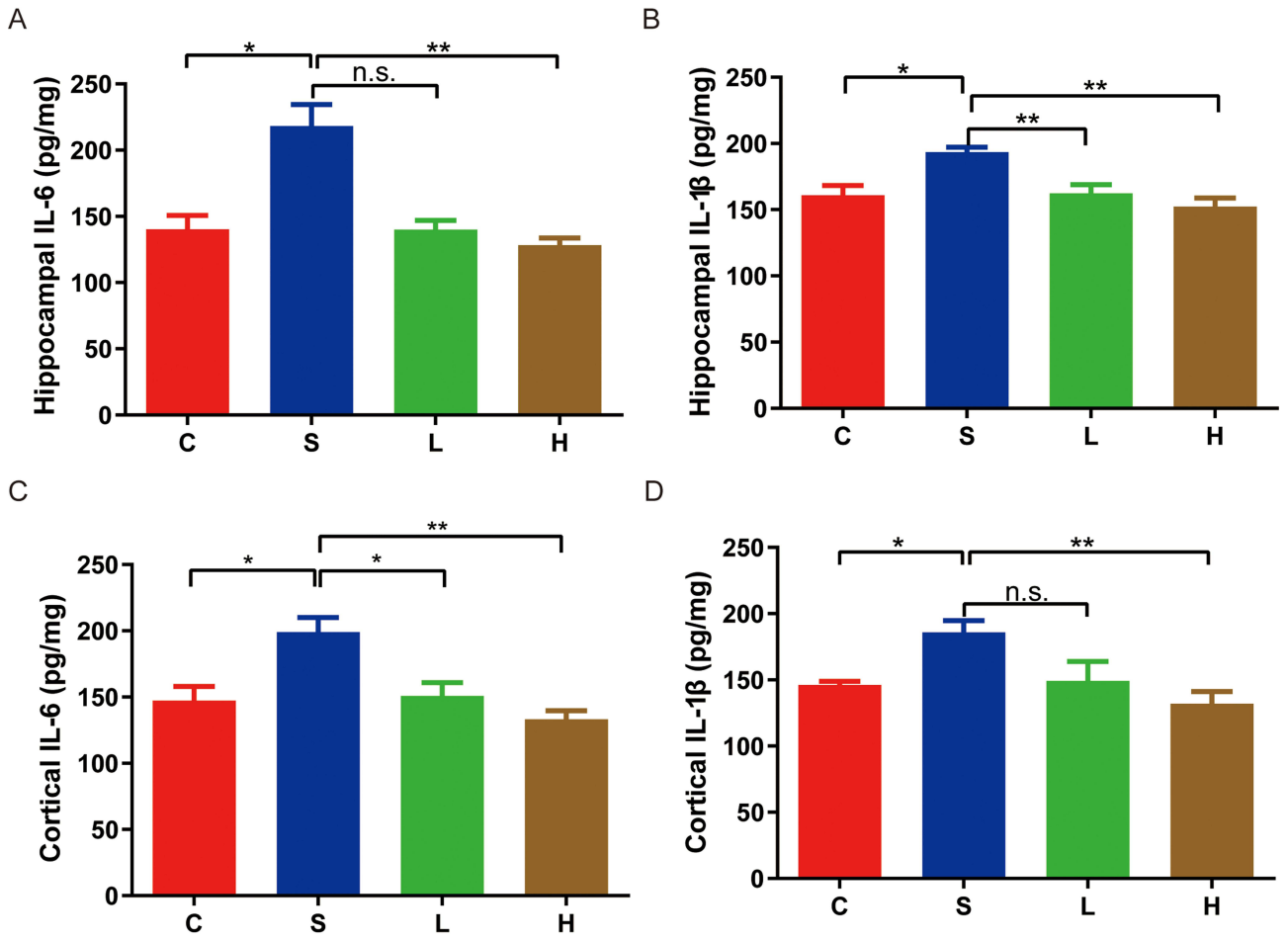

Figure 3 Evaluation of neuroinflammation in mice after tibial fracture surgery and drug treatment. Wild-type mice (7–8 months old) were divided into four groups as mentioned before (n=6-8). For the S group, L group and H group, intraperitoneal injections were all performed from 3 days preoperatively to 1 day postoperatively once a day. Hippocampal and cortical tissues were collected 24 hours post-surgery and 30 minutes after drug injection. (**A** and **B**) Levels of IL-6 and IL-1β in the hippocampus were determined by ELISA. (**C** and **D**) Levels of IL-6 and IL-1β in the cortex were determined by ELISA. *P < 0.05; **P < 0.01 by one-way analysis of variance (ANOVA); n. s. not significant; error bars denote the standard error of mean (SEM).

short, surgery and anesthesia cause neuroinflammation, while ZLN005 treatment ameliorates neuroinflammation in PND mice.

ZLN005 Treatment Increased the Expression of PGC-1α, NRF-1 and Mitochondrial-Related Genes in PND Mice

PGC-1α plays an important role in mitochondrial biogenesis,[23] and nuclear respiratory factor 1 (NRF-1) is an important regulator of mitochondrial DNA transcription and replication.[24] Atp5k, Atp5d, and Cox5a are located in the oxidative respiratory chain and are closely related to ATP synthesis and mitochondrial oxidative respiratory function. Our previous study showed that PGC-1α and NRF-1 expression were decreased and mitochondrial function was damaged in PND mice.[12] In this study, we extracted RNA from hippocampal tissue 24 hours post-surgery. The RNA levels of PGC-1α and NRF-1 in the S group were significantly decreased. The use of ZLN005 improved the transcription of PGC-1α and NRF-1 (Figure 4A). At 3 days post-surgery, hippocampal tissues were taken to detect PGC-1α and NRF-1 expression in each group by Western blotting or immunofluorescence. The results indicated that compared with that in the S group, the expression of PGC-1α and NRF-1 was significantly increased in the C group and the H group. The expression of PGC-1α in the L group exhibited an increasing trend but was not significantly different from that in the S group. However, NRF-1 was obviously increased in the L group compared with the S group

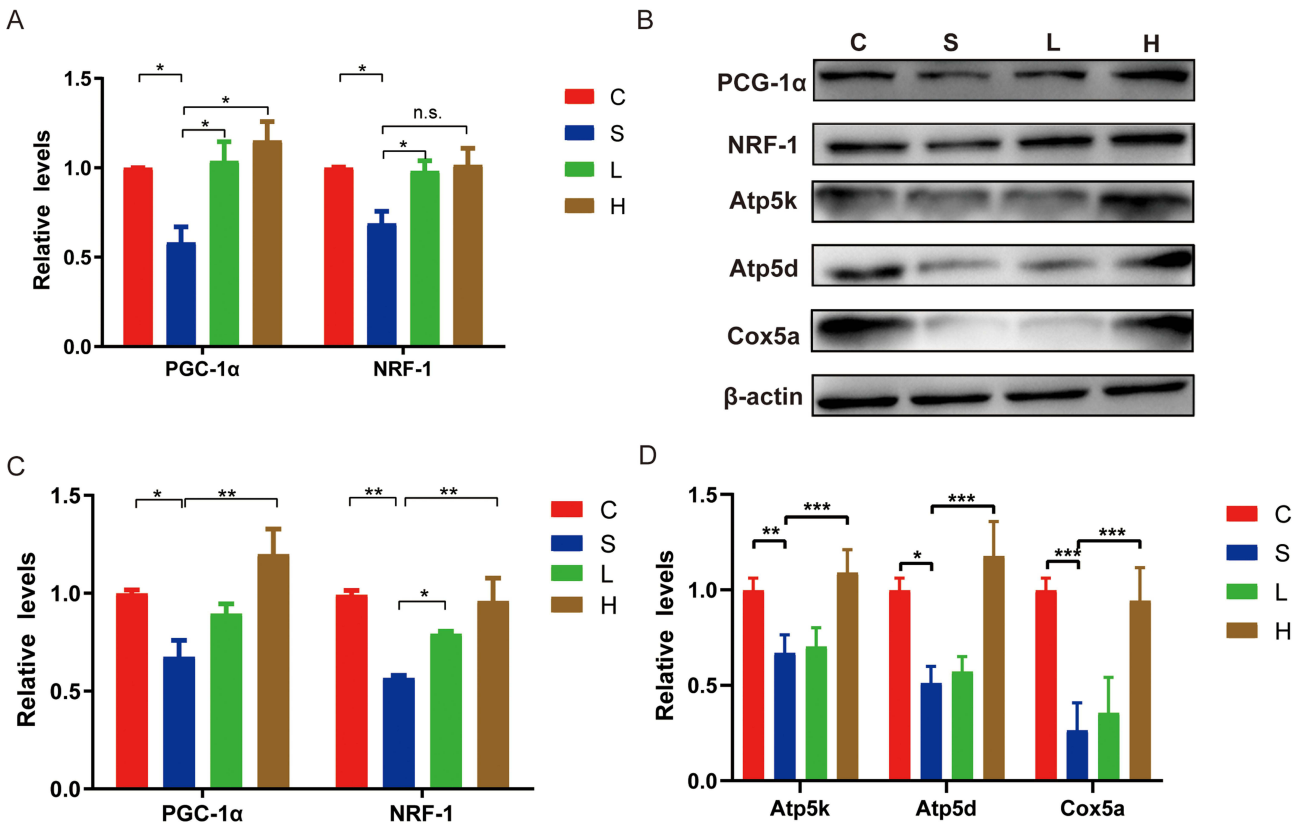

Figure 4 The expression levels of PGC-1α/NRF-1 and oxidative respiratory chain proteins were measured by Western blotting and qPCR. (**A**) One day after surgery, the hippocampus was harvested to detect the transcription of PGC-1α and NRF-1 (n=6-7). The grouping is shown before. (**B–D**) The expression levels of PGC-1α, NRF-1, Atp5k, Atp5d, and Cox5a were measured three days post-surgery in the hippocampus in the four groups (n=4-5). The expression of PGC-1α, NRF-1, Atp5k, Atp5d, and Cox5a was normalized to that of the β-actin internal control. *P < 0.05; **P < 0.01; ***P < 0.001. Mean ± standard error of the mean values is presented for each group.

(Figure 4B and C). The expression of Atp5k, Atp5d, and Cox5a in the S group and L group was obviously reduced compared with that in the C group and H group. This result shows that surgery causes a decline in the expression of oxidative respiratory chain protein and that a slightly higher dose of ZLN005 treatment can reverse this impairment (Figure 4B and D). The expression of PGC-1α and NRF-1 showed similar results as Figure 4 by immunofluorescence (Figure 5A–C).

ZLN005 Treatment Reversed Mitochondrial Function Damage in PND Mice

Mitochondria are the center of energy metabolism, and the normal operation of their functions is the basis for ensuring the health of the body. We collected fresh hippocampal tissue three days after surgery to detect the levels of adenosine triphosphate (ATP) and reactive oxygen species (ROS) in each group. We found that the operation combined with anesthesia can significantly reduce the synthesis of mitochondrial ATP and lead to significant accumulation of ROS. The use of ZLN005 can reverse this process. The synthesis of ATP in the L and H groups was significantly higher than that in the S group, and the accumulation of ROS was significantly lower than that in the S group (Figure 6A and B). These findings suggest that PGC-1α activator (ZLN005) treatment reverses the damage to mitochondrial function in mice with perioperative cognitive dysfunction.

We would like to summarize the results mentioned above. Neuroinflammation caused by surgery /anesthesia can lead to a reduction in PGC-1α level, which in turn impairs mitochondrial function and results in cognitive decline. ZLN005 has been shown to reduce neuroinflammation, promote PGC-1α production, improve mitochondrial function, and ultimately decrease the risk of cognitive impairment (Figure 7).

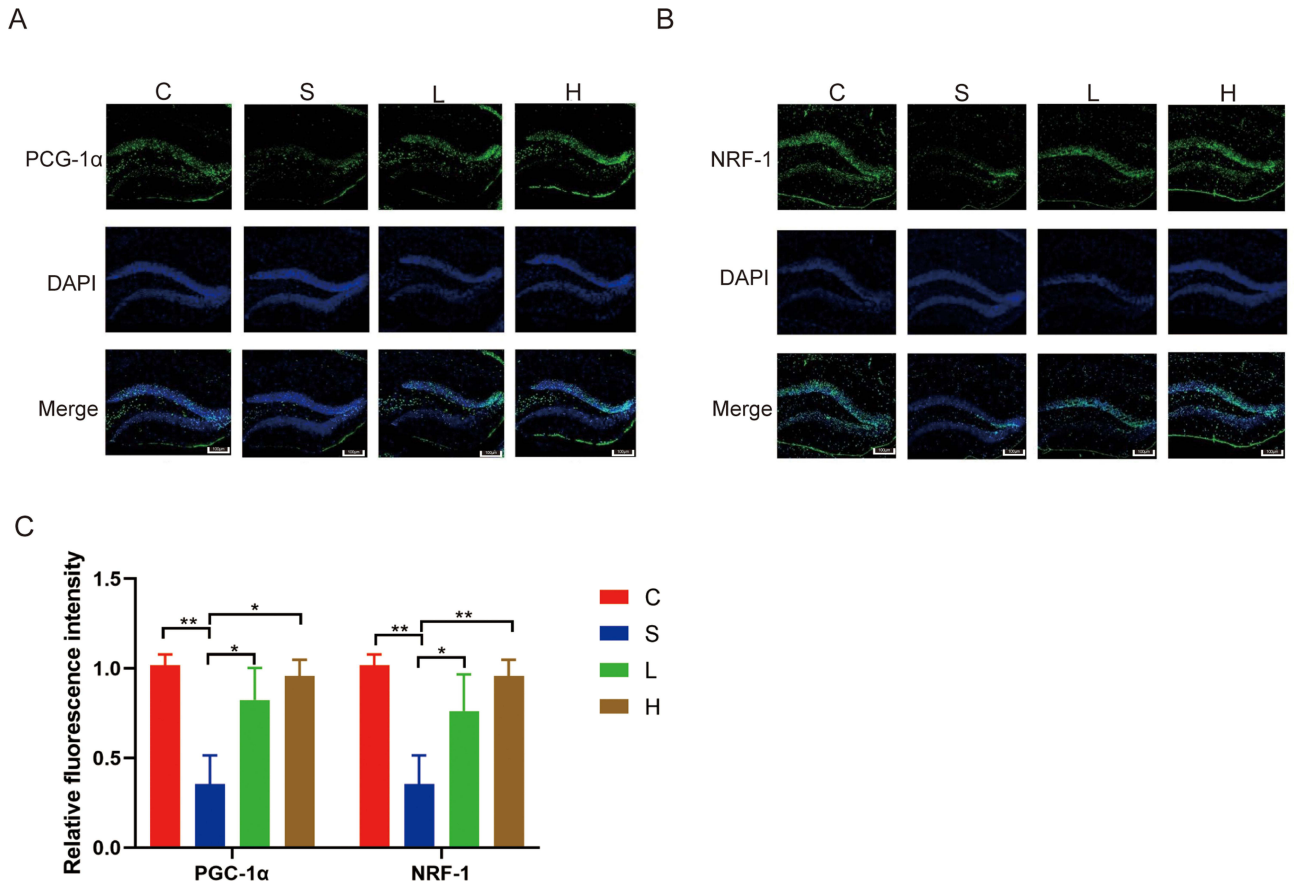

Figure 5 At 3 days post-surgery, the brain was harvested to detect the expression of PGC-1α and NRF-1 in different groups of mice by immunofluorescence (n=4). The grouping is as shown before. (**A** and **B**) Representative images for PGC-1α and NRF-1 in the hippocampus. (**C**) Quantification of PGC-1α and NRF-1 fluorescence in the hippocampus. *P < 0.05; **P < 0.01. Mean ± standard error of the mean values is presented for each group. Scale bar, 100 μm.

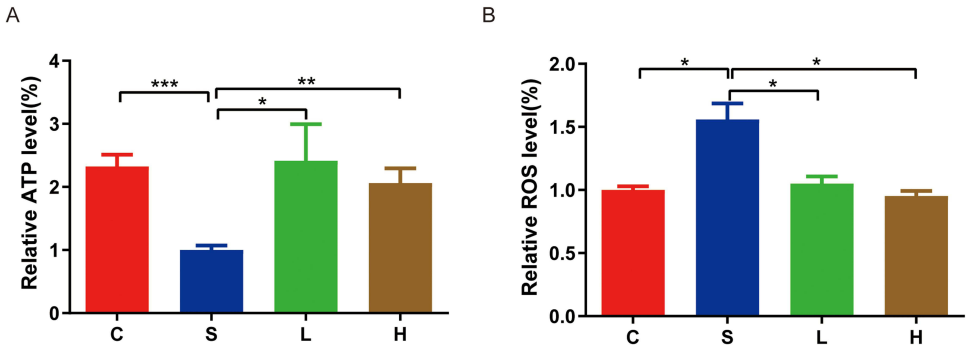

Figure 6 Mitochondrial function was evaluated three days post-surgery in each group. (**A** and **B**) On the third day, the fresh hippocampus was harvested to measure ATP (n=9) and ROS (n=6) in the sham (C group), the surgery and DMSO (S group), the surgery and ZLN005 (5 mg/(kg·d)) (L group), and the surgery and ZLN005 (7.5 mg/(kg·d)) groups (H group). *P < 0.05; **P < 0.01; ***P < 0.001 by one-way analysis of variance (ANOVA); error bars denote the SEM.

Discussion

PND is a serious perioperative complication that mainly manifests as persistent cognitive and memory impairment.[2] In this study, we chose the cognitive dysfunction model of tibial fracture surgery, which is widely used in the study of PND.[25,26]

Neuroinflammation is involved in the pathological processes of various diseases, and it is crucial to successfully treat individuals based on the pathological mechanisms of neuroinflammation.[27,28] It is currently believed that

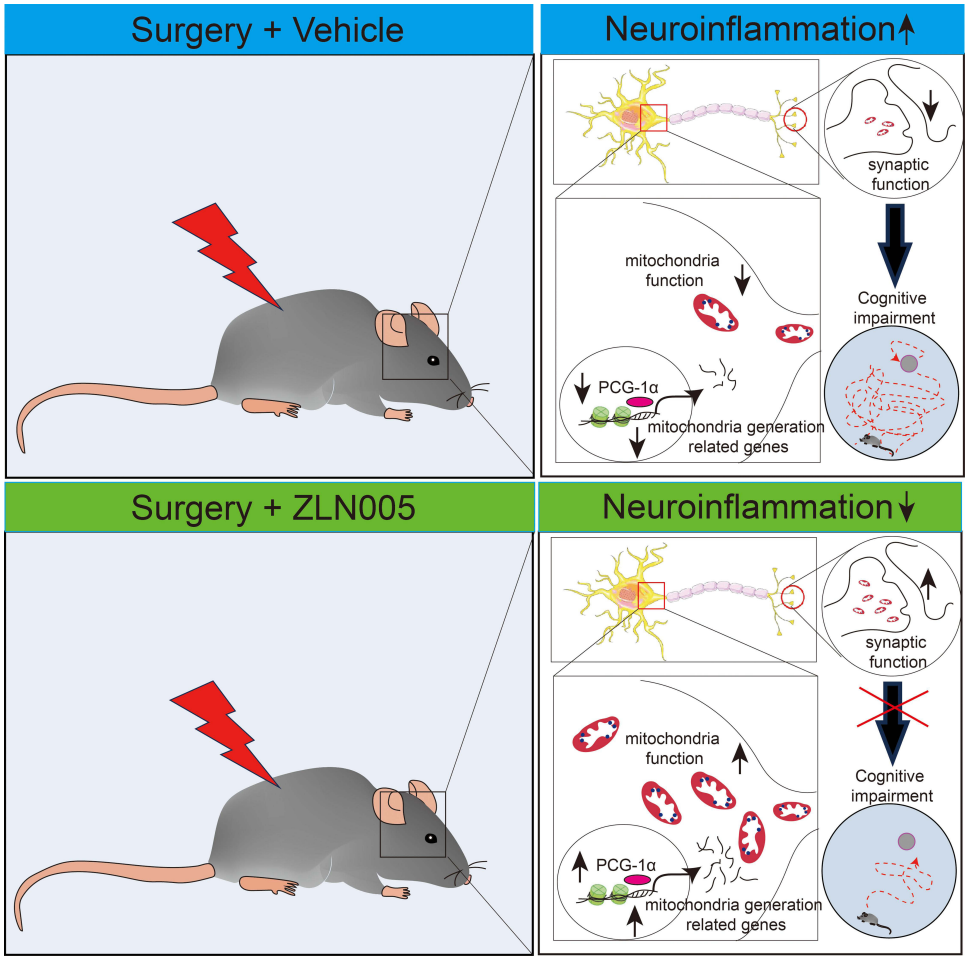

Figure 7 The role of ZLN005 in perioperative neurocognitive disorders.

neuroinflammation is the key pathogenesis of neurodegenerative disorders. Some drugs with anti-inflammatory effects can alleviate neuroinflammation and have potential therapeutic effects on neurodegenerative diseases, such as Atsttrin.[29] The levels of IL-6 and IL-1β in cerebrospinal fluid were related to a decrease in postoperative cognitive function in both cardiac surgery and noncardiac surgery.[30,31] Consistently, in our previous study, we observed that IL-6 and IL-1β expression increased in the brain at 1 d post-surgery, and later mitochondrial dysfunction occurs in PND mice.[12] Mitochondrial damage affects the production of ATP and the accumulation of peroxide, both of which are important for maintaining cell homeostasis and activity.[7]

PGC-1α plays an important role in mitochondrial oxidative metabolism and maintains the balance of energy.[32,33] NRF-1 is a nuclear gene required for mitochondrial DNA transcription and replication. PGC-1 α combined with nuclear respiratory factor (NRF1/NRF2) increases mitochondrial gene replication and transcription, promotes the expression of mitochondrial structural genes, and stabilizes mitochondrial function.[13,24] Similar to previous studies, we also observed decreased PGC-1α/NRF-1 expression and mitochondrial dysfunction in PND mice 1 or 3 days after surgery.[12]

In 2013, Zhang LN first proposed that the small molecule compound ZLN005 selectively elevated the expression of PGC-1α in myotubes and skeletal muscle through myocyte enhancer factor 2(MEF2) and exerted promising therapeutic effects for treating type 2 diabetes.[34] Pretreatment with ZLN005 in mouse spermatocyte-derived cells (GC-2 cells) effectively alleviated the mitochondrial dysfunction of GC-2 cells caused by benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE). This process is mediated by the SIRT1/TERT/PGC-1α pathway.[14] In this study, ZLN005 was applied to mice in the perioperative period to prevent PGC-1α from decreasing after surgery. Our results showed that ZLN005 increases the transcription and translation of PGC-1α/NRF-1 in the hippocampus in surgical mice 1 or 3 days after surgery. In

a rotenone-induced neurotoxicity experiment in PC12 cells, PGC-1α upregulation by ZLN005 maintained mitochondrial homeostasis by increasing the protein levels of multiple mtDNA repair proteins.[17] After treatment with ZLN005 in PND mice, we found that the expression of multiple mitochondrial respiratory complex proteins (Atp5d, Atp5k and Cox5a) increased and repaired the damage to mitochondrial function in the hippocampus 3 days after surgery. The recovery of mitochondrial function was manifested by increased production of ATP and decreased accumulation of ROS. ZLN005 could improve neuronal damage induced by ischemia in vitro and in vivo and reduce the neurotoxicity associated with Alzheimer's disease.[18,35] Our research indicates that perioperative ZLN005 treatment in surgery mice can reduce memory loss.

When PGC-1α was increased in Group L and Group H, we observed a decrease in IL-1β and IL-6 levels in the mouse brain. This effect may be related to the process by which ZLN005 crosses the blood–brain barrier and inhibits microglia to reduce the production of inflammatory factors.[36] Overall, our results show that ZLN005 inhibits inflammatory factor production, increases the level of PGC-1α/NRF-1, repairs mitochondrial function damage, and thus attenuates cognitive dysfunction in PND mice.

Regarding the potential mechanisms of ZLN005 in preventing PND, ZLN005 may mitigate neuroinflammation by inhibiting the activation of the NLRP3 inflammasome and reducing the production of inflammatory mediators such as IL-1β and IL-6. Furthermore, it enhances the expression of PGC-1α, which subsequently increases the production of mitochondrial respiratory chain protein subunits, thereby alleviating mitochondrial dysfunction. Through these dual mechanisms, ZLN005 may play a role in preventing the onset of PND. This is also supported by recent literature,[37] and our findings are consistent with this evidence.

In this experiment, we chose to inject a smaller dose of ZLN005 intraperitoneally into mice and did not choose the previously reported 15 mg/(kg·d) or an even higher dose, because we found deaths of mice after injections of 10 mg/(kg·d) or 15 mg/(kg·d). This result suggests that the range of safe and effective concentrations of ZLN005 is narrow, which has not been reported before. The difference in results could be due to differences in experimental animals. Wang used Sprague Dawley rats in the study,[14] while we used 8-month-old C57BL/6 mice. In addition to experimental animals, there are also differences in the use of medications. Due to the elimination of the first pass of oral drugs, the potential dosage required for intragastric administration to achieve the same blood drug concentration is greater.[13,18] Small doses of ZLN005 were used for 7 consecutive days to maintain a high level of PGC-1α to explore the effect of elevated PGC-1α on postoperative cognition.

Conclusion

Our results indicate that surgery leads to neuroinflammation and mitochondrial dysfunction. Furthermore, the application of ZLN005 to PND mice attenuates cognitive damage. This results from the fact that ZLN005 can not only inhibit the expression of brain inflammatory factors but can also activate the expression of PGC-1α/NRF-1 to improve mitochondrial function in mice with PND. ZLN005 can be used as a promising drug for preventing PND.

Acknowledgments

We thank Professor Qiang Liu from the University of Science and Technology of China for providing us with experimental resources.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the "Rui" Special Fund for Scientific Research from Hubei Chen Xiaoping Science and Technology Development Foundation (CXPJJH1200000-07-113); Health Research Project of Anhui Provincial Health Commission, AHWJ2023BAc20089; Anhui Province Health "Backbone Talents" Project; Shou Zheng Innovation - Bethune anesthesia depth monitoring research support project, 07.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Eckenhoff RG, Maze M, Xie Z, et al. Perioperative neurocognitive disorder: state of the preclinical science. *Anesthesiology*. 2020;132(1):55–68. doi:10.1097/ALN.0000000000002956
2. Kong H, Xu L-M, Wang D-X. Perioperative neurocognitive disorders: a narrative review focusing on diagnosis, prevention, and treatment. *CNS Neurosci Ther*. 2022;28(8):1147–1167. doi:10.1111/cns.13873
3. Lin X, Chen Y, Zhang P, Chen G, Yu X. The potential mechanism of postoperative cognitive dysfunction in older people. *Exp Gerontology*. 2019;130:110791. doi:10.1016/j.exger.2019.110791
4. Witlox J, Eurelings LSM, de Jonghe JFM, Kalisvaart KJ, Eikelenboom P, van Gool WA. Delirium in elderly patients and the risk of postdischarge mortality, institutionalization, and dementia: a meta-analysis. *JAMA*. 2010;304(4):443–451. doi:10.1001/jama.2010.1013
5. Subramaniyan S, Terrando N. Neuroinflammation and perioperative neurocognitive disorders. *Anesth Analg*. 2019;128(4):781–788. doi:10.1213/ANE.0000000000004053
6. Liu Q, Sun Y-M, Huang H, et al. Sirtuin 3 protects against anesthesia/surgery-induced cognitive decline in aged mice by suppressing hippocampal neuroinflammation. *J Neuroinflammation*. 2021;18(1):41. doi:10.1186/s12974-021-02089-z
7. Back NM, Neri D, Mariana G, et al. Oxidative stress and mitochondrial dysfunction contributes to postoperative cognitive dysfunction in elderly rats. *Brain Behav Immun*. 2018;73:S0889159118303593.
8. Yang Y, Liu Y, Zhu J, et al. Neuroinflammation-mediated mitochondrial dysregulation involved in postoperative cognitive dysfunction. *Free Radic Biol Med*. 2022;178:134–146. doi:10.1016/j.freeradbiomed.2021.12.004
9. Gonzales MM, Garbarino VR, Pollet E, et al. Biological aging processes underlying cognitive decline and neurodegenerative disease. *J Clin Invest*. 2022;132(10). doi:10.1172/JCI158453
10. Panes JD, Godoy PA, Silva-Grecchi T, et al. Changes in PGC-1α/SIRT1 signaling impact on mitochondrial homeostasis in amyloid-beta peptide toxicity model. *Front Pharmacol*. 2020;11:709. doi:10.3389/fphar.2020.00709
11. Han B, Jiang W, Liu H, et al. Upregulation of neuronal PGC-1α ameliorates cognitive impairment induced by chronic cerebral hypoperfusion. *Theranostics*. 2020;10(6):2832–2848. doi:10.7150/thno.37119
12. He K, Zhang J, Zhang W, et al. Hippocampus-based mitochondrial respiratory function decline is responsible for perioperative neurocognitive disorders. *Front Aging Neurosci*. 2022;14:772066. doi:10.3389/fnagi.2022.772066
13. Zhang L-N, Zhou H-Y, Fu -Y-Y, et al. Novel small-molecule PGC-1α transcriptional regulator with beneficial effects on diabetic db/db mice. *Diabetes*. 2013;62(4):1297–1307. doi:10.2337/db12-0703
14. Yang W, Zhang G, Jiang F, et al. BPDE and B[a]P induce mitochondrial compromise by ROS-mediated suppression of the SIRT1/TERT/PGC-1α pathway in spermatogenic cells both in vitro and in vivo. *Toxicol Appl Pharmacol*. 2019;376:17–37. doi:10.1016/j.taap.2019.05.004
15. Zhang T, Liu C-F, Zhang T-N, Wen R, Song W-L. Overexpression of peroxisome proliferator-activated receptor γ coactivator 1-α protects cardiomyocytes from lipopolysaccharide-induced mitochondrial damage and apoptosis. *Inflammation*. 2020;43(5):1806–1820. doi:10.1007/s10753-020-01255-4
16. Satish S, Philipose H, Rosales MAB, Saint-Geniez M. Pharmaceutical induction of PGC-1 promotes retinal pigment epithelial cell metabolism and protects against oxidative damage. *Oxid Med Cell Longev*. 2018;2018:9248640. doi:10.1155/2018/9248640
17. Xiao J, Dong X, Peng K, et al. PGC-1α mediated-EXOG, a specific repair enzyme for mitochondrial DNA, plays an essential role in the rotenone-induced neurotoxicity of PC12 cells. *J Mol Neurosci*. 2021;71(11):2336–2352. doi:10.1007/s12031-020-01775-6
18. Xu Y, Kabba JA, Ruan W, et al. The PGC-1α activator ZLN005 ameliorates ischemia-induced neuronal injury in vitro and in vivo. *Cell Mol Neurobiol*. 2018;38(4):929–939. doi:10.1007/s10571-017-0567-0
19. Vacas S, Degos V, Tracey KJ, Maze M. High-mobility group box 1 protein initiates postoperative cognitive decline by engaging bone marrow-derived macrophages. *Anesthesiology*. 2014;120(5):1160–1167. doi:10.1097/ALN.0000000000000045
20. Feng X, Valdearcos M, Uchida Y, Lutrin D, Maze M, Koliwad SK. Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. *JCI Insight*. 2017;2(7):e91229. doi:10.1172/jci.insight.91229
21. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process*. 2012;13(2):93–110. doi:10.1007/s10339-011-0430-z
22. Wu J, Cai Y, Wu X, Ying Y, Tai Y, He M. Transcardiac perfusion of the mouse for brain tissue dissection and fixation. *Biol Protoc*. 2021;11(5):e3988. doi:10.21769/BioProtoc.3988
23. Qian X, Li X, Shi Z, et al. KDM3A senses oxygen availability to regulate PGC-1α-mediated mitochondrial biogenesis. *Molecular Cell*. 2019;76(6):885–895.e7. doi:10.1016/j.molcel.2019.09.019
24. Scarpulla RC. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. *Physiol Rev*. 2008;88(2):611–638. doi:10.1152/physrev.00025.2007
25. Yang T, Velagapudi R, Terrando N. Neuroinflammation after surgery: from mechanisms to therapeutic targets. *Nat Immunol*. 2020;21(11):1319–1326. doi:10.1038/s41590-020-00812-1
26. Xiong C, Zhang Z, Baht GS, Terrando N. A mouse model of orthopedic surgery to study postoperative cognitive dysfunction and tissue regeneration. *J Vis Exp*. 2018;(132). doi:10.3791/56701
27. Jurcau A, Simion A. Neuroinflammation in cerebral ischemia and ischemia/reperfusion injuries: from pathophysiology to therapeutic strategies. *Int J Mol Sci*. 2021;23(1). doi:10.3390/ijms23010014
28. Poniatowski ŁA, Woźnica M, Wojdasiewicz P, et al. The role of progranulin (PGRN) in the pathogenesis of glioblastoma multiforme. *Cells*. 2024;13(2):124. doi:10.3390/cells13020124

29. Poniatowski ŁA, Joniec-Maciejak I, Wawer A, et al. Dose-ranging effects of the intracerebral administration of atsttrin in experimental model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. *Mol Neurobiol*. 2024;61:9432–9458. doi:10.1007/s12035-024-04161-0
30. Luo G, Wang X, Cui Y, Cao Y, Zhao Z, Zhang J. Metabolic reprogramming mediates hippocampal microglial M1 polarization in response to surgical trauma causing perioperative neurocognitive disorders. *J Neuroinflammation*. 2021;18(1):267. doi:10.1186/s12974-021-02318-5
31. Qiu -L-L, Pan W, Luo D, et al. Dysregulation of BDNF/TrkB signaling mediated by NMDAR/Ca2+/calpain might contribute to postoperative cognitive dysfunction in aging mice. *J Neuroinflammation*. 2020;17(1):23. doi:10.1186/s12974-019-1695-x
32. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochimica Et Biophysica Acta*. 2011;1813(7):1269–1278. doi:10.1016/j.bbamcr.2010.09.019
33. Abu Shelbayeh O, Arroum T, Morris S, Busch KB. PGC-1α is a master regulator of mitochondrial lifecycle and ROS stress response. *Antioxidants*. 2023;12(5):1075. doi:10.3390/antiox12051075
34. Li W, Li X, Wang B, et al. ZLN005 protects cardiomyocytes against high glucose-induced cytotoxicity by promoting SIRT1 expression and autophagy. *Exp Cell Res*. 2016;345(1):25–36. doi:10.1016/j.yexcr.2016.05.012
35. Dong YT, Cao K, Xiang J, Shan L, Guan ZZ. Silent mating–type information regulation 2 homolog 1 attenuates the neurotoxicity associated with Alzheimer disease via a mechanism which may involve regulation of peroxisome proliferator-activated receptor gamma coactivator 1-α. *Am J Pathol*. 2020;190(7):1545–1564. doi:10.1016/j.ajpath.2020.03.015
36. Sun W, Nguyen KD, Fitch WL, et al. In vitro and in vivo metabolite identification of a novel benzimidazole compound ZLN005 by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom*. 2018;32(6):480–488. doi:10.1002/rcm.8060
37. Zhang W, Wu -C-C, Ge -M-M, et al. The PGC-1α/ERRα/ULK1 pathway contributes to perioperative neurocognitive disorders by inducing mitochondrial dysfunction and activating NLRP3 inflammasome in aged mice. *Neuropharmacology*. 2024;260:110119. doi:10.1016/j.neuropharm.2024.110119