Short report

Prophylactic lithium alleviates postoperative cognition impairment by phosphorylating hippocampal glycogen synthase kinase-3β (Ser9) in aged rats

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ARTICLE INFO

Article history:
Received 5 June 2011
Received in revised form 24 August 2011
Accepted 6 September 2011
Available online 16 September 2011

Second Editor: Christian Humpel

Keywords:
Postoperative cognitive dysfunction
Lithium chloride
Spatial memory
Elderly patient
Phosphatidylinositol 3-kinase
Glycogen synthase kinase-3β
Interleukin-1β

ABSTRACT

Postoperative cognition impairment is a perishing complication in elderly patients undergone surgeries. Lithium is widely used in psychiatric patients for its role in neuronal protection, whereas whether or not it could attenuate surgery-associated postoperative cognition dysfunction used prophylactically is not well defined. After approval by the Institutional Animal Care and Use Committee, 48 male Sprague–Dawley rats aged 18 months old were randomly divided into three groups with 16 each: i, no surgeries and drugs were given; ii, surgical procedures were performed only without drug delivery; iii, prophylactic 2 mM/kg lithium chloride was given intraperitoneally once a day for seven days before surgeries. The change in spatial memory was assessed with Morris Water Maze (MWM), and the activation of PI3K/AKT/mTOR pathway was detected, and the levels of hippocampal glycogen synthase kinase-3β (p-GSK-3β) phosphorylation at serine 9 and interleukin-1β (IL-1β) were measured. The MWM detection showed that both swimming latency and distance were considerably prolonged by the surgeries, but these changes could be markedly shortened by prophylactic lithium administration. Meanwhile, the changes in the hippocampal PI3K cascades and p-GSK-3β and IL-1β expression displayed corresponding changes that were parallel to the alterations of spatial memory, and inhibition of PI3K and GSK-3β suggested upstream PI3K activation leads to downstream change in p-GSK-3β and IL-1β. These results indicate, at least in part, that prophylactic lithium can alleviate surgery-associated impairment of the spatial memory in aged rats which is strongly associated with the reduced levels of hippocampal p-GSK-3β and IL-1β resulted from the activation of PI3K/AKT/mTOR pathway.

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1. Introduction

Following surgery and anesthesia, up to over 60% older patients suffer from postoperative cognitive dysfunction (POCD) presenting with an impairment of recent memory, concentration, language comprehension or social integration (Coburn et al., 2010; Ramaiah and Lam, 2009), which is strongly associated with increased mortality (Steinmetz et al., 2010). POCD could present for weeks or months after surgery, and the precise mechanisms underlying this pathological condition are not well defined. It is hitherto known that cytokines from neuroinflammatory response are involved in such cognitive dysfunction (Thomson and Sutherland, 2005; Wilson et al., 2002). Previous animal studies showed that the peripheral innate immune system is involved in this process by inducing release of peripheral and central inflammatory cytokines, which then influences cognition via diverse mechanisms including the penetration of peripheral cytokines through the blood–brain barrier directly via active transport mechanisms or indirectly via vagal nerve stimulation (Coburn et al., 2010; Ramaiah and Lam, 2009; Rosczyk et al., 2008), and such surgical trauma induced neuroinflammation would be exacerbated in aged rodents (Cao et al., 2010). Besides, tissue trauma from surgical procedures, anesthesia, and stress responses all can induce release of a body of proinflammatory factors within the hippocampus which interfere with cognitive function as evidenced by abnormal memory and learning in the intact organism and inability to develop long-term potentiation in hippocampal slice preparation (Kurosawa and Kato, 2008; Rosczyk et al., 2008). Glycogen synthase kinase-3 (GSK-3), a constitutively active kinase, regulates a body of signaling molecules involved in inflammatory responses (Klamer et al., 2010). Inhibition of GSK-3β via phosphorylation reduced the expression of inflammatory cytokines induced by

Abbreviations: ANOVA, analysis of variance; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSK, glycogen synthase kinase; IL-1β, interleukin-1β; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; POCD, postoperative cognitive dysfunction.

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lipopolysaccharide (Yuskaitis and Jope, 2009). Lithium chloride, a drug used in psychiatric patients for stabilizing mood, can phosphorylate the GSK-3β enzyme inside the brain to avoid disrupting routine schedules of many functions such as metabolism, sleep and body temperature (Pasquali et al., 2010; Young, 2009). Besides, lithium could improve spatial memory ability in gerbils after the cerebral ischemia–reperfusion injury or stress by suppressing GSK-3β activity (Bian et al., 2007; Silva et al., 2008), and lithium reduced production of major proinflammatory cytokines which in turn provides protection from apoptotic insults in the central nervous system (Beurel and Jope, 2006; Martin et al., 2005). Patrizia and colleagues reported that lithium attenuates the clinical symptoms of experimental autoimmune encephalomyelitis through reducing leukocyte infiltration, demyelination, and microglial activation (Patrizia et al., 2008). Given the involvement of phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling cascades in the regulation of glycoegen synthesis through phosphorylation and inactivation of GSK-3β (Camins et al., 2009), we hereby hypothesized that prophyllactic lithium treatment may attenuate POCD detected as the changes in spatial memory in aged rodents undergoing minor surgical procedures by suppressing GSK-3β activity and corresponding reduction in the expression of proinflammatory cytokines such as IL-1β in the hippocampus through inhibiting PI3K/AKT/mTOR signaling pathway.

2. Methods

2.1. Chemicals

To test whether or not the PI3K pathway is involved in lithium’s role in improving POCD, we used 30 nM PI-103 as a potent, cell-permeable and ATP-competitive PI3K inhibitor (Selleck Chem., Houston, TX, USA). Besides, 1 μM GSK-3β inhibitor VII (EMD Chemicals Inc., Gibbstown, NJ, USA), a phenyl α-bromomethyl ketone compound that acts as a cell-permeable and non-ATP competitive inhibitor of GSK-3β, was used to clarify the interrelationship between PI3K and GSK-3β.

2.2. Animals and study protocol

After approval by the Institutional Animal Care and Use Committee at Nanjing Medical University (Nanjing, China), forty eight 18-month-old male Sprague–Dawley rats (purchased from Jinling Hospital, Nanjing, China) were randomized into three groups with 16 each: the sham operated animals did not receive any surgical procedures and drugs except for the skin incision; the animals in the operation group merely underwent surgical intervention without drug delivery; and the rats in the prophylactic group received 2 mM/kg lithium chloride (Amresco, OH, USA) once a day for seven days (Rosczyk et al., 2008), and then the surgical procedures were performed. The rats in the groups sham and operation were injected with same volume of physiological saline without lithium treatment. The dose of lithium chloride used in this study is based on our previous study in which we found that lithium chloride 2 mM/kg is the optimal one in producing neuroprotective effect (Bian et al., 2007). The rats with dyskinesia were excluded from the study.

2.3. Surgical procedures

In the present study, the minor surgery animal model was established according to previous report, in which the degree of postoperative inflammation was declined to a lower point with considerable cognitive recovery (Rosczyk et al., 2008). In this model, minor surgical procedures do not cause profound learning and memory deficits in a reversal learning version of the Morris Water Maze (MWM), but in aged surgical animals it appears to persevere displaying spending less time swimming in a new target location. In brief, all animals were anesthetized with 2% pentobarbital sodium (0.25 ml/100 g, intraperitoneally). A 3-cm length incision was made in the upper left quadrant through the skin and muscle, and then a swab was inserted into the body cavity to gently manipulate the internal organs for 3 min. The dissolvable or silk sutures were used to close the muscle and skin respectively, and then were injected with buprenorphine 1.0 mg/10 g subcutaneously for postoperative analgesia.

2.4. Spatial memory detection

Morris Water Maze (Shanghai Jiliang Software Technology Co., Ltd., China) was used to detect animals’ spatial memory to display the changes in postoperative cognitive function after different interventions during the postoperative five days. A circular tank 100 cm in diameter and 30-cm deep was filled with water (24–26°C) to a depth of 25 cm. A transparent round platform 10 cm in diameter was placed at 0.5 cm below the surface of the water. During the test of spatial memory, the animals must learn to use distinctive distal visual cues surrounding the pool to navigate a direct path to the hidden platform. The platform remained in a constant location during the acquisition phase. Animals were placed on the platform for 30 s preceding the start of each training session. Animal training took place during a 3-day acquisition phase with three massed trials administered each day. Rats were allowed to swim freely for 90 s or until the platform was reached. If the platform was not located within the 90 s, the rats were guided to the platform and allowed to remain for 30 s. After completion of three consecutive trials, the rats were placed in their home cages under a heat lamp for 10 min. A video camera mounted to the ceiling directly above the center of the maze was used in conjunction with a computerized animal tracking system to monitor latency to the platform and distance swim.

2.5. Immunoblotting analysis

Hippocampus samples were placed in sample buffer (0.5 M Tris–HCl pH = 6.8, 10% glycerol, 2% (w/v) SDS, 5% (v/v) 2-β-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95–100 °C for 2 min. Samples were separated by electrophoresis on 15% acrylamide and were transferred to polyvinylidene fluoride sheets using a transblot apparatus. Membranes were blocked for 1 h at room temperature with 5% non-fat milk dissolved in TBS-T buffer (Tris 50 mM, NaCl 1.5%, Tween 20, 0.05%, pH = 7.5) and then incubated with following primary antibodies: p-PI3K p85α, rabbit-anti-rat polyclonal antibody, 1:1 000 (Bio-world Tech., Louis Park, MN, USA); p-AKT (Ser473), rabbit-anti-rat polyclonal antibody, 1:1000 (Cell Signaling, Boston, USA); mTOR, rabbit-anti-rat polyclonal antibody, 1:2000 (Bethyl Laboratories Inc., Montgomery, TX, USA); Rictor, rabbit-anti-rat polyclonal antibody, 1:5000 (Bethyl Laboratories Inc.); Raptor, rabbit-anti-rat polyclonal antibody, 1:3000 (Bethyl Laboratories Inc.); Rictor, rabbit-anti-rat polyclonal antibody, 1:5000 (Bethyl Laboratories Inc.); p-GSK-3β (Ser9), rabbit-anti-rat monoclonal antibody, 1:500 (Cell Signaling). After 4-h incubation, blots were washed thoroughly with TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG antibody. Immunoreactive protein was visualized using a chemiluminescence-based detection kit following the manufacturer’s protocol, and followed with film exposure and relative density analysis with the Typhoon Imaging System (GE Healthcare, Piscataway, NJ, USA). The immunoblots were washed briefly and then incubated with a monoclonal rabbit anti-rat GAPDH (glyceraldehyde 3-phosphate dehydrogenase) antibody at 1:5000 for 40 min at room temperature followed by a horseradish peroxidase-conjugated rabbit anti-mouse antibody. GAPDH protein was then visualized and detected as above as the loading biomarker.

2.6. Enzyme-linked immunosorbent assay (ELISA) for IL-1β measurement

Hippocampus was collected 24 h later after the surgery for ELISA detection of IL-1β. Added lysis solution with 1:150 in hippocampus tissue fragments, then grinded for 5 min in ice, and spin at 12,000 rpm/10 min at 4 °C 10 min after grinding. The supernatant was
reserved for IL-1β measurement. All reagents and samples were prepared as the protocol described in the IL-1β ELISA kit (USCN, Wuhan, China). Appropriate concentrations were determined according to the standard curve. After 100 μl of Standard, Blank, or Sample solutions was added into each well, and the plates were covered with sealer and incubated for 2 h at 37 °C. After removing the liquid in each well and adding detection reagent A 100 μl to the well, then the plate was sealed and incubated for 1 h at 37 °C. After three times of aspirating and washing with wash buffer (approximately 400 μl), detection reagent B working solution 100 μl was added to each well, incubated for another 1 h at 37 °C. Substrate solution 90 μl was then added to each well and incubated for 30 min at 37 °C after repeated aspiration and washing, followed by adding stop solution 50 μl to each well. We determined the optical density of each well at the wave length of 450 nm.

2.7 Statistics

Data are presented as the means ± SDs and analyzed using GraphPad Prism v5.0 (GraphPad Software Inc., San Diego, CA, USA) or PASW Statistics v18.0 (IBM Co., Armonk, NY, USA). The levels of different protein expression were analyzed using one-way analysis of variance (ANOVA). For spatial working memory, the data were compared with two-way ANOVA. The ANOVA tests were always followed by the Bonferroni post hoc tests. All reported P values are two-sided and a P value of less than 0.05 is accepted for statistical significance.

3. Results

3.1. Prophylactic lithium alleviated surgery-associated memory impairment

A change in memory is one of the major characteristics of POCD (Coburn et al., 2010; Ramaiah and Lam, 2009). Here we used MWM as the tool to reveal spatial memory deficit through examining the swimming latency and distance in aged rats after surgical procedures. Surgical interventions prolonged the swimming latency than that of the sham operated animals in a time dependent manner (Fig. 1A, P < 0.05), but such changes could be markedly alleviated by prophylactic administration of lithium (Fig. 1A, P < 0.05). Correspondingly, the swimming distance in the sham-operated animals was significantly shorter than that of the operative ones (Fig. 1B, P < 0.05), and the preoperative lithium attenuated such change considerably, even to the normal levels as the sham-treated animals shown (Fig. 1B, P = 0.05). These data indicate that prophylactic lithium is an effective means in alleviating surgery-associated impairment in memory in aged rats.

3.2. PI3K/AKT/mTORC2 activated by surgical procedures but inhibited by prophylactic lithium

In consideration of the involvement of PI3K/AKT/mTORC2 signaling pathway in the regulation of GSK-3β activity by lithium (Camins et al., 2009), we next tested the changes of this signaling cascade in the operation-treated hippocampus. Surgical procedures upregulated the expression of proteins of p-PI3K p85α, p-AKT (Ser374), mTOR and Rictor, but not the expression of Raptor (Fig. 2A and B, P < 0.05), and these changes could be significantly inhibited by the administration of lithium (Fig. 2A and B, P < 0.05). These results indicate that surgical intervention is an essential activator of PI3K/AKT/mTORC2 pathway, and this activation can be effectively inhibited by preoperative lithium.

3.3. Preoperative lithium increased the hippocampal GSK-3β phosphorylation

A body of literatures reported the role of phosphorylated GSK-3β (p-GSK-3β) in memory improvement (Forlenza et al., 2011; Li et al., in press; Liu et al., 2010). To explore whether or not the changes in the hippocampal GSK-3β phosphorylation were associated with surgical intervention induced cognition deficit, we detected the expression of hippocampal p-GSK-3β (Ser9), and found that the level of p-GSK-3β (Ser9) in the operative animals was significantly lower than that of the sham-treated animals (Fig. 2A and B, P < 0.05), and this change could be reversed by prophylactic administration of lithium, i.e. the expression p-GSK-3β (Ser9) was upregulated markedly (Fig. 2A and B, P < 0.0001). These data indicate surgical intervention induced downregulation of hippocampal p-GSK-3β (Ser9) is contribut to the impairment of POCD, but such change can be attenuated by preoperative administration of lithium.

3.4. Preoperative lithium downregulated hippocampal IL-1β expression induced by surgical procedures

Previous study showed that hippocampal expression of IL-1β is involved in the process of neuroinflammation-related impairment of memory induced by peripheral surgery (Cibelli et al., 2010). We measured the expression of IL-1β at 24 h after surgical intervention and the results were in accordant to previous reports that operative procedures considerably upregulated IL-1β expression compared with the sham animals (Fig. 2C, P = 0.045), but this increased level of IL-1β could be inhibited by prophylactic lithium administration (Fig. 2C, P = 0.023). These findings showed that preoperative lithium treatment can decrease hippocampal IL-1β which may be involved in the regulation of surgery-associated memory impairment.

3.5. Operation induced changes in p-GSK-3β and IL-1β is the effector of PI3K activation

To clarify the interrelationship between PI3K activation and changes in p-GSK-3β and IL-1β, we used competitive inhibitors of PI3K and
GSK-3β. As shown in Fig. 3, PI-103 significantly inhibited the expression of both p-Pi3K and p-GSK-3β (A and B, \( P<0.05 \)). However, GSK-3β inhibitor VII produced no role in inhibiting p-Pi3K, but only in the expression of p-GSK-3β (A and B, \( P>0.05 \)). Moreover, both inhibitors all could decrease the level of IL-1β as did of lithium (Fig. 3C, \( P<0.01 \)). These data give us the information that changes in GSK-3β (Ser9) phosphorylation and IL-1β expression are downstream effectors of the activation of PI3K signaling pathway.

4. Discussion

In this preliminary study, we demonstrated that surgical procedures used as exploratory laparotomy impaired the cognition function detected with spatial memory deficit in aged rats, but such change could be considerably alleviated by prophylactic administration of lithium. Meanwhile, the surgery increased hippocampal expression of IL-1β and downregulated the expression of hippocampal p-GSK-3β (Ser9), and correspondingly these changes could be inhibited by preoperative lithium treatment too. Furthermore, we found that the surgery activated Pi3K/Akt/mTORC2 signaling pathway could also be inhibited by lithium, and Pi3K activation induced downstream changes in GSK-3β (Ser9) phosphorylation and IL-1β expression. These functional and molecular changes after different interventions highlight the role of surgeries induced "Pi3K/GSK-3β/IL-1β" activation in contributing to the surgery-associated POCD, and give evidence that these changes can be alleviated by the preoperative treatment with lithium.

GSK-3β is a multifunctional serine/threonine kinase found in all eukaryotes (Lei et al., 2011; Medina and Wandosell, 2011). Two highly homologous mammalian isoforms of GSK-3, GSK-3α and GSK-3β exist. GSK-3β is a key regulator of numerous signaling pathways, and involves in a wide range of cellular processes ranging from glycogen metabolism to the regulation of cell survival and neuronal polarity (Gao et al., 2010; Song et al., 2010; Yokota et al., 2010). GSK-3β plays an important role in regulating central neuroinflammatory reaction and memory (Ramirez et al., 2010). Data also showed that GSK-3β inhibits LTP and then decreases the spatial learning ability (Hernández et al., 2002; Hooper et al., 2007; Yuskaitis and Jope, 2009). Given that GSK-3β exerts function by dephosphorylation (Lei et al., 2011; Medina and Wandosell, 2011), we detected the content of p-GSK-3β (Ser9) and found lithium pretreatment upregulated the expression of p-GSK-3β (Ser9), which suggests lithium can suppress the activity of GSK-3β. Therefore, lithium may protect memory impairment by alleviating hippocampal neuroinflammation due to peripheral surgical trauma. Further, these increased contents in p-GSK-3β (Ser9) are associated with the improved performance of spatial learning. Therefore, prophylactic lithium treatment may process potential benefits for memory in aged rats after surgical interference by targeting GSK-3β phosphorylation.

To date, the real risk factors of POCD remain largely unknown. However, types of surgery and advanced age have been noted as two major risk factors of POCD (Sauër et al., 2009). The numbers of neurons and neuronal dendrites and axons as well as the production of neurotransmitters were declined with age, especially over 60 years old (Croisy and Culley, 2003). Meanwhile, the balance between anti- and pro-inflammatory responses is broken, which makes the aged brain more vulnerable to surgeries and stress (Frank et al., 2006), and studies suggest that activation of the central innate immune system leads to exacerbated neuroinflammatory stress and prolonged sickness behaviors in aged mice (Chen et al., 2008; Godbout et al., 2005). Finally such inflammatory responses have given new insight into the pathogenesis of POCD (Krenk et al., 2010). One interesting finding in adult mice was that although no signs of neuroinflammation were found following surgery, aged mice had significantly increased level of IL-1β in the hippocampus 24 h after the surgical procedures (Coburn et al., 2010; Ramaiah and Lam, 2009). Elevated IL-1β in the hippocampus has been shown involving the impairment of synaptic plasticity which finally leads to deficits of long-term potentiation (LTP) that is associated with learning and memory (Pickering and O’Connor, 2007). Oitzl et al. (1993) reported that IL-1β could impair the spatial learning in rats. In addition, the overexpressed IL-1β induced synaptic enhancement of GABA neurons, decrease in the excitability of neurons in the CA1 (Hellstrom et al., 2005), and IL-1β could reduce the expression of brain-derived neurotrophic factor mRNA in the CA1 and CA2 areas, which indirectly inhibits LTP by suppressing the cyclic adenosine monophosphate response element-binding protein/extracellular signal-regulated kinases1/2 pathway resulting in learning disability (Barrientos et al., 2004). Moreover, neuroinflammation and memory dysfunction which resulted from surgeries could be mitigated in mice pretreated with IL-1 receptor antagonist or in IL-1R knocked-out mice (Cibelli et al., 2010). In this study we found that IL-1β in the aged hippocampus was markedly upregulated after surgical procedures, and such change could be significantly blocked by preoperative treatment with lithium.

Surgical stress results in neuroinflammation in aged brain (Sparkman and Johnson, 2008), but how quick this response is triggered and how long it can last after surgeries is not detailed (Hu et al., 2010). In this study, we choose the first 5 days as the time window to observe whether the cognition function at the early postoperative period was influenced
the expression of p-GSK-3β in the hippocampus. The results demonstrated a significant change in the spatial memory and the hippocampal expression of the two inflammatory molecules after surgical procedures during the early period of the operation, which indicated that cytokine-related inflammatory responses occurred following surgeries. In consideration of the role of PI3K pathway in regulating the phosphorylation of GSK-3β (Camins et al., 2009), we detected activation of PI3K/AKT/mTORC2, and found that surgical procedures induced upregulation of the expression p-PI3K, p-AKT, mTORC2 and Rictor, but not Raptor, which suggests that PI3K/AKT/mTORC2 activation involves in the occurrence of POCD. Besides, administration of PI-103 and GSK-3β inhibitor VII, inhibitors of PI3K and GSK-3β respectively, demonstrates that the change in GSK-3β phosphorylation and IL-1β expression in the hippocampus after surgeries needs activation of upstream PI3K/AKT/mTORC2 pathway.

Before concluding the results, the limitations of this study should be acknowledged. First, although investigation on the levels of cytokines and activation of GSK-3β in the blood sample will help to decipher the relationship between peripheral and the CNS inflammation, we did not measure these changes in blood in this study. Further, we observed changes in the hippocampal IL-1β and GSK-3β expression that displayed parallel change in the spatial memory in these tested animals, and then we remarked these two molecules are involved in the impairment of POCD. To this point, we should say further studies are needed to clarify the precise relationship between memory and IL-1β and GSK-3β expression. Besides, the relationship between IL-1β and GSK-3β also needs to be investigated further. Third, our study merely presented a phenomenon regarding the surgery-associated POCD and the possible role of IL-1β and GSK-3β in this pathological process. Whether or not there are any in-depth mechanisms of this observation needs to be known. Finally, UTP recordings are the standard characteristic of the hippocampal memory alteration, but we here only gave a behavioral change in spatial memory, which significantly affected the conclusions drawn from the results. Future studies should focus on this via electrophysiological means.

In summary, our results demonstrated at the preliminary level, at least in part, that the decrease in phosphorylation of GSK-3β (Ser9) and the increase in IL-1β in the hippocampus plays critical roles in the POCD in aged rats after exploratory laparotomy, and the prophylactic treatment with lithium increased the hippocampal contents of p-GSK-3β, and decreased IL-1β level resulted from the inhibition of the PI3K/AKT/mTORC2 signaling pathway which contributes to the improvement of the postoperative spatial memory (see schematic flow of lithium’s role in attenuating POCD in Fig. 4).

References


