Stellate ganglion block improves postoperative cognitive dysfunction in aged rats by SIRT1-mediated white matter lesion repair

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Research Article

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Abstract

Background

Postoperative cognitive dysfunction is a common complication of the central nervous system after surgery, especially in elderly patients. White matter lesions cause cognitive impairment. Although stellate ganglion block (SGB) is an effective intervention for postoperative cognitive dysfunction, the exact mechanism remains unclear. The SIRT1 signaling pathway is involved in the process that SGB alleviates postoperative cognitive dysfunction. However, the underlying mechanisms that SGB improves postoperative cognitive dysfunction through SIRT1 in aged rats and its association with white matter lesion repair are yet to be elucidated.

Methods

To simulate a surgery-induced cognitive dysfunction-like condition, 20-month-old aged male Sprague-Dawley rat was subjected to splenectomy to induce cognitive impairment. Cognitive function was assessed by Morris water maze test. Western blotting was used to determine SIRT1 and NF-κB expression levels in the hippocampus and white matter under different circumstances. Immunohistochemistry was used to examine SIRT1 expression in white matter. The morphology of neural cells in white matter was observed by HE staining. NF-κB activity was measured by EMSA. The serum and white matter TNF-α, IL-6 and IL-10 levels were determined by ELISA.

Results

After splenectomy, the expression of SIRT1 in the rat hippocampus and white matter was dramatically decreased, NF-κB activity was enhanced, the levels of TNF-α, IL-6 and IL-10 in serum and white matter were increased, and rat showed significant cognitive impairment. After treatment with stellate ganglion block, activation of SIRT1 mediated by the SIRT1/NF-κB signaling pathway alleviated neuroinflammation, reversed white matter injury, and ameliorated surgery-induced cognitive dysfunction.

Conclusion

In conclusion, we provide strong evidence to demonstrate that postoperative cognitive dysfunction in elderly patients is associated with white matter injury, and stellate ganglion block can improve postoperative cognitive impairment in the older by reversing white matter lesion by activating SIRT1.

Introduction

Postoperative cognitive dysfunction (POCD) is a common complication of the central nervous system following surgery and is characterized by an impairment of cognitive function, including mental

confusion, nervousness, personality changes, and memory problems[1, 2]. POCD can present at any age, especially in elderly patients[3]. As the aging of society, the number of surgical procedures among the elderly and the incidence of POCD is significant increasing[4]. POCD is associated with poorer recovery, lower health-related quality of life and higher mortality, especially the risk of death in the first year after surgery[5, 6]. Thus, POCD has gained much attention from the public. Although the pathophysiological mechanisms of POCD remain to be elucidated, neuroinflammation is initiated by elevated peripheral inflammatory cytokines, which disrupted blood-brain barrier integrity, and has been widely implicated as a major factor[7]. Moreover, related studies have shown that stellate ganglion block (SGB) can reduce inflammation and improve cognitive impairment[8, 9].

Stellate ganglion block (SGB), which has been used since the beginning of the last century, is a common and safe clinical block technique[10, 11]. The technique temporarily reduces overactivity of the sympathetic nervous system and block its function of the innervation area (the head, neck, upper thorax and arms) by injecting local anesthetic in and around the stellate ganglion (located at the base of the neck)[12, 13]. With the development of modern medicine, some scientists have found that SGB can regulate multisystemic functions in the body and is a treatment not only of the painful disorders, but also of other non-painful disorders such as autoimmune and neurological disorders[14, 15]. The findings from previous clinical studies suggested that SGB may be an effective intervention for POCD[16, 17]. This is most likely related to the reduce in inflammatory responses and the function of the SIRT1 signaling pathway[15, 17, 18]. In addition, growing evidence supports an important role for SIRT1 in the regulation of cognitive function and inflammatory response in the central nervous system[19].

Silent information regulator 1 (SIRT1) is a NAD^+-dependent deacetylase[20]. SIRT1 regulates the function of many transcription factors and cofactors through deacetylation, and participates in the regulation of different biological processes, including inflammation, apoptosis, and metabolism[21]. SIRT1 expression is abundant in hippocampal neurons [22]. The hippocampus is a brain area that is known to be important for learning and memory and is highly susceptible to inflammation[23, 24]. Previous studies suggest that SIRT1 expression is essential for hippocampus-dependent memory formation[25]. This has been demonstrated in vitro and in various animal models[26].

Recently, evidence has demonstrated that white matter lesion (WML) is independent predictors and risk factors for developing dementia in the older, and may even be a direct factor in cognitive dysfunction[27]. Previous research suggested that there are early widespread white matter microstructure abnormalities in POCD patients[28]. Nonetheless, the exact pathophysiologic mechanisms through which white matter impair cognitive functions are unclear. Accordingly, this study aims to shed light on the underlying mechanisms that SGB improves postoperative cognitive dysfunction through SIRT1 in aged rats and investigates its association with the reduction of white matter damage using a variety of in vivo approaches.

Materials And Methods

Animals

A total of 120 healthy male Sprague-Dawley (SD) rats (weighing 300~350 g, 20-month-old) were used in this study. Animals were provided by Nanchang University Laboratory Animal Science (Nanchang, China) (license No.SYXK 2021-0004). The rats were housed in a standard condition under a 12-hour light/dark cycle at constant temperature (22 ± 1 °C) and relative humidity (50 ± 10%). All animals were allowed free access to forage and activity. The animals were acclimated for at least 1 week before the start of the experiments. All procedures were conducted in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals in China. This study was approved by the Animal Ethics Committee of Nanchang University.

Animal Experimental Design

The rats were randomly assigned to four groups (n = 30/group) using the random number table: group C (rats exposed to the sham-operated control group), group S (rats only exposed to splenectomy), group SGB (rats exposed to splenectomy and transection cervical sympathetic trunk and group EX (rats were pretreated with EX527 before surgery). The rats in group EX were injected IP daily with 5 mg/kg of EX527 five minutes prior to surgery, a SIRT1 inhibitor dissolved in 99% sterile saline/1% DMSO, while the remaining mice were injected with equivalent amounts of saline containing 1% DMSO.

POCD Model

In the present study, POCD model in aging rats was made using splenectomy with referring to the method of Bi Y et al [29]. The rats were fasted for 12 hours before surgery. The rats were deeply anesthetized with 2% sodium pentobarbital (40mg/kg) and placed in the supine position on the surgery table. Under aseptic conditions, the abdominal wall was shaved and disinfected. A median abdominal incision (2-3cm) was made to expose the spleen, which was then separated from the surrounding tissue. We ligated splenic vessels securely and removed the spleen, subsequently suturing the peritoneum injecting intraperitoneally with broad-spectrum antibiotics (5% cefoperazone,50 mg/kg) to prevent infection, and stapling the skin at last. The sham-surgery control group (group C) were subject to all anesthetic and surgical manipulations under identical conditions but without removal of the spleen. Except for Sham, spleen was removed by the foregoing approach in the remaining three groups to prepare POCD model. After surgery, 1 ml of saline was given subcutaneously for volume repletion.

SGB Model

SGB model in aging rats was made by transection of the cervical sympathetic trunk based on previous studies[30]. During the establishment of the POCD Model, all groups need to do the following procedures after the abdominal incision was made. After skin sterilization, a median incision (1.5-2cm) was made on the neck. The subcutaneous tissues, fascia and muscles were bluntly detached to expose the right

common carotid artery. The rats in all groups, except for those in the C and S group, also need to do the following surgical operations. Under a surgical microscope, the bifurcation of the right common carotid artery was exposed and the superior cervical ganglia on the dorsal side of it was found. Subsequently, the right cervical sympathetic trunk at 3-mm from the superior cervical ganglia was transected and ligated. The appearance of Horner syndrome (ptosis, miosis, and enophthalmos) was the measure of successful SGB model.

Morris water maze test

The Morris water maze (MWM) test is widely used to evaluate the cognitive function[31]. All parameters during the trials were recorded by a computerized video tracking system. The MWM consisted of a circular pool (160 cm diameter, 60 cm high) filled with opaque water (21-22°C) and a round platform (12 cm diameter, 30 cm high) was submerged,1 cm under the surface of the water. The pool was divided into four quadrants, the target quadrant (the one containing the platform), and three non-target quadrants (adjacent and opposite quadrant). On the first experimental session, the rats without treatment were given the training of the place navigation test for four consecutive days (4 trails per day with a 30s rest between each). They were randomly released in the water starting from one of four quadrants to locate the hidden platform in 120s, with every quadrant once per day. If at the 120s mark, the platform was not found, the rats were guided to the platform, kept on it for 30s and then the latency was recorded as 120s. The second stage started 1 day after the end of the first stage. On the second experimental session, the spatial probe tests were performed at day 1, 3 and 7 after treatment to examine spatial learning and memory. At 1, 3 and 7 days after operation, eight rats per group were randomly and blindly selected and each of these was allowed a free 120s swim after removing platform. The latency to traverse and the swimming trajectory during the probe trials were recorded. The number of crossings of the area where the platform used to be were recorded in post-operative day 8.

Preparation of serum and brain tissue samples

Following the MWM test on 1, 3, and 7 days, blood (2ml) were collected via tail vein bleeds from the rats (eight mice in each group) and centrifugated at 4,000 rpm for 10 min. Next, the supernatants were taken and preserved at -20 °C as serum samples. Brain tissue samples were made according to the following procedures. After that, the rats were euthanized by injecting chloral hydrate and removed the cerebral cortex. The segment of the intact hippocampus and white matter was isolated rapidly. The segment of intact hippocampus and white matter, especially corpus callosum, was stored respectively in liquid nitrogen as the hippocampal sample and the white matter sample for further use. Subsequently, three groups of white matter samples were culled randomly, fixed in 4% paraformaldehyde for 24 hours, washed, dehydrated, embedded in paraffin and evenly sectioned to 5 μm in standard procedure as the paraffin sample of white matter.

Enzyme-linked immunosorbent assay

According to manufacturer's instructions, the supernatant of serum and white matter samples were assayed for the concentrations of TNF-α ,IL-6 and IL-10 using Enzyme-linked immunosorbent assay (ELISA) kits.

Immunohistochemistry and hematoxylin-eosin staining

The pathological changes of rat white matter were observed and recorded by HE staining and correlative light microscopic analysis. The expression of SIRT1 in white matter was assessed by immunohistochemistry staining. The previous paraffin sections (n=3/group) were stained with hematoxylin-eosin (HE) staining using standard procedures. The remaining were subjected to immunohistochemical staining by renaturing, blocking, incubating overnight at 4 °C with primary anti-SIRT1 and then incubating with secondary antibody for 1h at room temperature. The stained sections were observed under fluorescent microscopy and then analyzed the expression of SIRT1 with Image J software.

Measurement of Expression of SIRT1 and NF-κB

Western blotting was used to determine the SIRT1 and NF-κB protein expression levels of the hippocampus and white matter. Based on the previous method of our research group[32],the above hippocampal samples were placed in EP tubes and were lysed by a radio-immunoprecipitation assay (RIPA) buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The supernatant (soluble lysate) was collected after centrifugation and the protein concentration was determined using BCA assay. An equal amount of protein was separated by 8% SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane, blocked with 5% non-fat milk and incubated with primary antibodies overnight. The following day, blots were incubated with the secondary antibodies for one hour at room temperature. Finally, blots were detected and recorded by a Bio-Rad Gel imaging system. The grey value of each band was analyzed by Image J and normalized to the grey value of β-actin. At least three independent experiments have been carried out. Antibodies used for western blotting analysis are listed below: Primary antibodies used in the study included rabbit monoclonal anti-SIRT1 (1:1 000), rabbit monoclonal anti-NF-κB p65 (1:1 000), rat monoclonal anti-β-actin (internal standard, 1: 1 000); Secondary antibodies: goat anti-rabbit immunoglobulin G-horseradish peroxidase (IgG-HRP) (1: 1 000), rabbit anti-rat IgG-HRP (1: 1 000).

Measurement of NF-κB activity

NF-κB activity was analyzed using electromobility shift assays (EMSA). According to the manufacturer's instructions, the previous hippocampal and white matter samples were separately taken and used for detection of NF-κB activity by EMSA kit. The EMSA gel obtained by electrophoresis were compressed by a direct compression technique with X-ray film. Autoradiography was performed at -70°C. After 8-12 hours, we used auto developing X ray film machine to develop a film. Afterwards, the results were developed on X-ray film, scanned and recorded. Quantitation and analysis of NF-κB activities was carried out by

densitometry (Bandleader 3.0 software, Magnitec Ltd. Israel), and the background density was normalized by band density.

Statistical analyses

All of the data are presented as means ± S.E.M. All data were first analyzed for normal distribution. Then, differences between two groups were compared using the Student' s t test. Differences among multiple groups were assessed using one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test. Data were analyzed with GraphPad Prism (GraphPad Software, CA, USA). Image analysis was performed with Image J software (NIH) and Bandleader 3.0 software (Magnitec Ltd. Israel). Values of $p < 0.05$ were considered statistically significant.

Results

The postoperative cognitive dysfunction is associated with white matter lesions and inflammatory responses

In this study, we established animal model of POCD by splenectomy, and assessed changes in cognitive function and inflammatory cytokines on post-operative days 1, 3 and 7(Fig. 1). Spatial learning and cognitive flexibility were tested by the Morris water maze test (MWM). On post-operative days 1,3 and 7, both group C (rats exposed to the sham-operated control group) and group S (rats only exposed to splenectomy) could reach the hidden platform (Fig. 2a). However, the rats in group S exhibited more disordered routes, longer escape latency and lesser number of platform crossings when compared to group C (Fig. 2a, c, d). The Student's t tests revealed significant differences between the two groups (Fig. 2c, d). The place navigation test and the spatial probe tests, which reflects hippocampal-dependent learning and memory, was carried out in the test phase of the MWM. These data suggest hippocampal cognitive function is impaired in aged rats of POCD.

The pathological changes of white matter in old rat were observed by HE staining under light microscope. HE staining of the white matter showed that neurons were arranged, no significant necrosis or apoptosis, and cytoplasm and nuclei were clear in group C (Fig. 2b). However, the neuronal arrangement was disrupted, the number of neurons was decreased, and neuronal degeneration and necrosis were observed (Fig. 2b). Neuronal degeneration and necrosis appeared hyperchromatic nuclei, nuclear pyknosis, obvious vacuoles, and astrocyte hyperplasia in group S (Fig. 2b). These represented scattered hypoxic-ischemic white matter injury (Fig. 2b). These data indicate that the surgery cause damage to neuronal cells, and white matter lesions are present in postoperative cognitive dysfunction.

ELISA was applied to compare TNF-α, IL-6, and IL-10 level in the serum and white matter between group C and S. At days 1,3 and 7 after surgery, the concentrations of TNF-α, IL-6, and IL-10 in the serum and white matter were significantly increased in group S compared to group C, which demonstrated inflammatory response in vivo were activated during POCD (Fig. 2e-j). The Student's t tests revealed significant

differences between the two groups (Fig. 2e-j). These results suggest the postoperative cognitive dysfunction is associated with white matter lesions and inflammatory responses, including peripheral and central inflammation.

Decreased SIRT1 expression and activated NF-κB signaling in the hippocampus and white matter induces postoperative cognitive dysfunction

Western blotting was used to detect the SIRT1 and NF-κB protein expression levels of the hippocampus and corpus callosum on post-operative days 1,3 and 7. Compared with group C at the same time points, decreased expression of SIRT1 and increased expression of NF-κB in group S were observed, regardless of in the hippocampus and corpus callosum (Fig. 3a). These suggest SIRT1 activity was inhibited and NF-κB signaling was activated in the aged POCD model.

To provide further evidence of the alteration of SIRT1 and NF-κB in the hippocampus and corpus callosum, we performed two determinations. The NF-κB activity was determined with EMSA, while the relative SIRT1 protein expression was analyzed with densitometric analysis of western blotting. In the hippocampus and corpus callosum, the SIRT1 activity was markedly decreased and NF-κB activity was significantly increased in the rats of group S compared with group C at the same time points (Fig. 3b-e). The Student's t tests revealed significant differences between the two groups (Fig. 3b-e). To summarize, our results suggest that inhibited SIRT1 expression and activated NF-κB signaling in both the hippocampus and white matter induces POCD.

SGB attenuates inflammatory responses by upregulating SIRT1 and inhibiting NF-κB signaling

To determine whether SGB has a potential role in POCD, we established animal model of SGB by transection of the cervical sympathetic trunk (Fig. 1). We have previously demonstrated that decreased SIRT1 expression in the hippocampus causes postoperative cognitive dysfunction, however it's unclear whether SIRT1 can help SGB improve cognitive function. Therefore, we designed group EX (rats were pretreated with EX527, a SIRT1 inhibitor, before surgery) for comparison purposes (Fig. 1).

On days 1,3 and 7 after treatment, blot images of western blotting analyses showed that, with the exception of group C, the relative SIRT1 expression of the hippocampus decreased in all other groups, but that of group SGB was higher than that of group S and EX; with the exception of group C, the NF-κB activity of the hippocampus increased in all other groups, but that of group SGB was lower than that of group S and EX (Fig. 4a). This finding was also supported by EMSA and densitometry analysis (Fig. 4b, c). There were no significant differences in the expression of SIRT1 and NF-κB between the group S and EX from one-way ANOVA followed by Tukey Kramer multiple comparisons test ($p > 0.05$) (Fig. 4b, c). Our data indicates that SGB could upregulate SIRT1 and inhibit NF-κB signaling during POCD.

The ELISA results showed that serum concentrations of TNF-α, IL-6, and IL-10 were significantly higher in the remaining groups compared to group C at 1, 3, and 7 days postoperatively, with group SGB having

lower serum TNF-α and IL-6 concentrations and higher serum IL-10 concentrations than group S and EX (Fig. 4d-f). There were no significant differences in the serum concentrations of TNF-α, IL-6, and IL-10 between the group S and EX from one-way ANOVA followed by Tukey Kramer multiple comparisons test ($p > 0.05$) (Fig. 4d-f). The tendency towards changes of the concentrations of TNF-α, IL-6, and IL-10 in white matter was similar to those of in the hippocampus (Fig. 4g-i). This demonstrates that SGB attenuates peripheral and central inflammatory responses during POCD.

SGB activates SIRT1 to reverse white matter damage reduce postoperative cognitive dysfunction

To investigate the effect of SGB on white matter in POCD and a possible functional role of SIRT1, histopathology changes in white matter were first observed using HE staining. The necrosis of neural cells was used to assess damage to the white matter of the brain. The next three groups all demonstrated dispersed ischemia alterations (i.e., a decrease in the number of normal neurons, degenerative necrosis, and astrocyte hyperplasia) compared to group C, but the neuronal necrosis of white matter in group SGB was significantly improved compared with group S and EX (Fig. 5a). These findings imply that SGB can improve white matter damage in postoperative cognitive dysfunction.

Next, the expression of SIRT1 in white matter was assessed by immunohistochemistry and western blotting, and neuronal necrosis was detected by HE staining. The positive expression of SIRT1 in neural cells was mostly noted in the nuclear with occasional cytoplasm expression, appearing as brownish or tan in immunohistochemistry. Representative samples of group S and EX revealed a markedly weakened decreased SIRT1 expression in white matter in comparison with group C; SIRT1 expression in group SGB was weaker than that of group C but much stronger than that of group S and EX (Fig. 5b). The tendency of SIRT1 expression in white matter was further confirmed by western blotting, EMSA and densitometry analysis (Fig. 5c, d). Meanwhile, the NF-κB expression in white matter exhibited an opposite trend to that of SIRT1 (Fig. 5c, e). There were no significant differences in the expression of SIRT1 and NF-κB between the group S and EX from one-way ANOVA followed by Tukey Kramer multiple comparisons test ($p > 0.05$) (Fig. 5d, e). These findings imply that SGB can reverse white matter damage in POCD by activating SIRT1.

SGB ameliorates postoperative cognitive dysfunction through activating SIRT1

To explore the effect of SGB on postoperative cognitive dysfunction, we selected to conduct a behavioral assessment on days 1, 3, and 7 after surgery. The representative trajectories in spatial probe tests of MWM is shown above (Fig. 6a). Compared with group C, the rats in group S and EX showed more disorganized routes, longer escape latency and lower number of platform crossings (Fig. 6a-c). Among them, the rats in group EX were unable to find the hidden platform (Fig. 6a). This implies that the deficiency of SIRT1 cause cognitive impairment. In addition, Although the general trend of group SGB in the above indicators was similar to that of group S and EX, it was slighter (Fig. 6a-c). However, the rats in

group SGB had a more direct routes, a shorter escape latency, and higher platform crossing times than group S and EX, suggesting that SGB can reverse the cognitive impairment by activating SIRT1 (Fig. 6a-c). There were no significant differences in the escape latency and platform crossing times between the group S and EX from one-way ANOVA followed by Tukey Kramer multiple comparisons test ($p > 0.05$) (Fig. 6b, c). There were no significant differences in terms of the escape latency on days 3 and 7 after treatment between the group SGB and C from one-way ANOVA followed by Tukey Kramer multiple comparisons test ($p > 0.05$) (Fig. 6b).

According to these findings, SIRT1 activity was inhibited and white matter was destroyed during POCD. However, SGB restored white matter damage, reduced neuroinflammation, and improved cognitive function in POCD by activating SIRT1/NF-κB signaling pathway.

Discussion

In this study, we identified SIRT1 as a key molecular determinant of SGB's effects on white matter lesion repair and hippocampus-dependent memory using a combination of molecular biology, cellular morphology, ethology, and light microscopy. The operation decreased SIRT1 expression in the hippocampus and white matter, and the reduced SIRT1 levels could further trigger or exacerbate neuroinflammation, caused white matter damage and eventually lead to cognitive impairments in old rats. It's worth noting that EX527, a SIRT1 inhibitor, caused increased neuroinflammation, aggravated white matter injury, and worsened cognitive impairment. SGB could ease SIRT1-mediated neuroinflammation, correct white matter injury, and diminish cognitive impairments in old rat. These results imply that SIRT1-mediated white matter damage repair may play a key role in the mechanism that SGB improved POCD in aged rats (Fig. 7).

Postoperative cognitive dysfunction (POCD) is a universal central nervous system complications defined by a cognitive disturbance after surgery[33]. POCD was originally reported in 1955 is a hot topic in anesthesiology and neurology[34]. POCD causes worse recovery, lower quality of life and higher mortality, particularly in the first year following surgery[5, 6]. Therefore, POCD has gained widespread attention from the public. Multiple studies were used for exploring POCD pathogenesis and establishing the means of diagnosis and treatment. The etiology of POCD is multifactorial. Previous studies have indicated that it involved a complex interaction between patient, environmental, and iatrogenic factors, and the main is advancing age[35, 36]. A large body of research has demonstrated that patients over 65 years of age who have undergone operation or critical illness are at a higher risk for it[37]. Thus, we adapted 20-month-old male rats for establishing animal model. POCD present in 25.8% of patients at seven days after surgery and 9.9% at three months; in some cases, it may be persist in the long run[38]. So, we selected the samples on postoperative day 1, 3, and 7 for detecting molecular, cellular morphology and cognitive function changes. Many studies have revealed that several potential mechanisms, including hippocampal neuroapoptosis with the correlations in SIRT1, central autophagy, and oxidative stress neuroinflammation, would induce cerebral neuron damage and may underlie the pathogenesis of

POCD[36, 39]. Although the exact pathophysiological mechanisms underlying POCD remain to be clarified, neuroinflammation and blood-brain barrier damage has been confirmed as the major factors[7, 40]. Habbas et al. (2015) have found that increased levels of the cytokines tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6) have been linked to the emergence of cognitive impairments following inflammation in the central nervous system[41, 42]. In the present study, to confirm that inflammatory responses were affecting POCD in animal model, we examined inflammatory factors in aged rats. We found that peripheral and central concentrations of the inflammatory chemokines, including TNF-α, IL-6 and IL-10, was elevated (Fig. 2e-j). This is consistent with the prior studies. An excessive increased peripheral proinflammatory cytokines may cause endothelial injury and increased blood-brain barrier permeability, resulting in neuroinflammation and cognitive dysfunction (Fig. 2a).

Cerebral white matter is the place where nerve fibers and neuroglial cells gather inside the brain, and the white color of the subcortical tissue comes from myelinated nerve fibers[43]. With the widespread availability of magnetic resonance imaging (MRI), white matter hyperintensity (WMH), also known as leukoaraiosis, is a radiologic appearance detected by MRI in over 50% of the elderly[44]. WMH, which is the non-specific areas of increased signal seen on T2-weighted images in brain scans of older patients, are the main manifestation of white matter lesion (WML)[45, 46]. Generally, human cognitive processing abilities are determined by the integrity of white matter structures[47]. Therefore, WML could contribute to cognitive impairment, especially in the elderly[48]. Lambert et al. have observed that WMH is the earliest and consistent MRI change prior to the ischemic and cognitive symptoms onset[46]. Previous research has established that the severity of WMLs correlates with the extent of cognitive impairment[49]. WML has been associated with cerebral hypoperfusion or blood-brain barrier disruption. Because white matter components (e.g., oligodendrocytes) are highly sensitive to ischemia-induced oxidative stress and excitotoxicity[44, 48]. The overexpression of peripheral and central inflammatory chemicals and WML in POCD was validated in our research (Fig. 2). Studies showed that blood-brain barrier compromise may explain the correlation between the peripheral inflammation and the neuroinflammation[50]. One possibility is that overproduction of inflammatory cytokines after surgery leads to blood-brain barrier damage. When the blood-brain barrier is disrupted, inflammatory cells and other serum substances including fibrinogens and complement components are transferred into the central nervous system, where they release a range of cytokines, which in turn stimulate inflammatory responses and induce white matter damage[45].

The hippocampus is a brain area that plays a vital role in learning and memory, many of the experimental evidences relating cognitive decline thus comes from studies of the hippocampus[51]. Increasing evidence supports SIRT1 plays a significant role in the cognitive dysfunction and inflammatory reaction[19]. SIRT1 is a NAD+-dependent deacetylase[20]. NF-κB is an important transcription factor and is a downstream regulatory protein of SIRT1[52]. NF-κB, a heterodimer formed by p65 (RelA) and p50 (NF-κB1), can regulate inflammation and form the hippocampus-dependent memory[53–55]. Therefore, SIRT1 can inhibits NF-κB activity by deacetylating its subunit RelA/p65, remits inflammation responses and decreases neurons

lesions[56]. Meanwhile, the hippocampus highly express proinflammatory cytokine receptors, specifically receptors for TNF-α and IL-6, and more vulnerable to the deleterious effects of pro-inflammatory molecules, which have been proved in various animal models [26]. To further confirm the relationship between SIRT1 and WML during POCD, we evaluated white matter damage by HE staining, detected the expression of SIRT1 in white matter by immunohistochemistry, and tested the expression of SIRT1 and NF-κB in hippocampus and white matter by western blot. According to our findings, downregulated SIRT1 expression in white matter and hippocampus enhanced NF-κB signaling, induced inflammation, caused neuronal damage in white matter, and eventually leaded to cognitive dysfunction (Fig. 2, 3, 5). These agree with earlier research.

Related research have shown that stellate ganglion block (SGB) can improve POCD[8, 17]. The pathophysiologic mechanisms were linked to the reduce in inflammatory responses and the function of the SIRT1 signaling pathway[15, 18]. SGB model in aging rats was established by transection of the cervical sympathetic trunk based on previous studies. In order to further elaborate the impact of SGB on white matter lesion in POCD and its relationship with SIRT1, we set group EX that rats were pre-treated with EX527 (a SIRT1 inhibitor). Group EX was adopted as a comparison with the other groups. It is suggested that the neuroprotection of POCD and the reversion of WML by SGB may be SIRT1-dependent by observing the expression of SIRT1 and NF-κB, white matter lesion and cognitive function in different experimental groups at days 1, 3, 7 after treatment(Fig. 4–6). IL-10 is known to be an anti-inflammatory factor, IL-6 and TNF-α is a pro-inflammatory factor[57]. It was found that SGB stimulated the secretion of anti-inflammatory and pro-inflammatory chemicals at the same time, and the imbalance between anti-inflammatory and pro-inflammatory mediators reduced the post-operative stress inflammatory response and improved POCD, in accordance with the results of inflammatory molecules levels in serum and white matter(Fig. 4d-i). That is the same as related studies. These findings suggested that SIRT1-mediated white matter lesion repair was involved in the process that SGB improve POCD in the older. The utility of SIRT1 in SGB reversing WML and POCD provides a new direction for future diagnosis and treatment of POCD in the aged population.

There are several limitations to this study. First of all, in addition to morphology and molecular biology, more methods should be used for the assessment of white matter injury. Secondly, our study analysis was based on the analysis of different groups at the same time point after surgery, and there was no comparison between the same group at different time points, which is worthy of further study. Finally, the effects of cerebral perfusion on neuroinflammation and cognitive dysfunction were not considered in this study, which is worthy of further study.

Conclusions

In conclusion, we provide strong evidence to prove that postoperative cognitive dysfunction in elderly patients is associated with white matter injury, and reveal that stellate ganglion block can improve postoperative cognitive impairment in the older by reversing white matter lesion by activating SIRT1.

Among them, neuroinflammation are a key hallmark of neurodegenerative diseases, including POCD. SIRT1 acts as a link between white matter damages and cognitive impairments after surgery. Our study sheds new light on the pathophysiology of POCD and clarifies that SIRT1 and white matter could be a new therapeutic target in neurodegenerative illnesses.

Abbreviations

POCD
Postoperative cognitive dysfunction
SGB
Stellate ganglion block
WMH
White matter hyperintensity
WML
White matter lesion
SIRT1
Silent information regulator 1
NF-κB
Nuclear factor- kappa B
MRI
Magnetic resonance imaging
TNF-α
Tumor necrosis factor-alpha
IL-6
Interleukin-6
IL-10
Interleukin-10
SD rats
Sprague-Dawley rats
MWM
Morris water maze test
ELISA
Enzyme-linked immunosorbent assay
DMSO
Dimethyl sulfoxide
HE
Hematoxylin-eosin
IgG-HRP
Immunoglobulin G-horseradish peroxidase
RIPA

radio-immunoprecipitation assay
PMSF
phenylmethylsulfonyl fluoride
PVDF
Polyvinylidene difluoride
EMSA
Electromobility shift assays
ANOVA
Analysis of variance.

Declarations

Author contributions:

Study conception and design and animal study:Jun zhang, Yang Liu,Hejian Li; data acquisition and analysis:Qin Liu,Yanhui Hu,Shuchun Yu; manuscript draft: Jun zhang; manuscript revision:Yong Chen. All authors read and agreed to the final manuscript of the manuscript.

Data Availability

All relevant data are available in the main text or from the authors.

Competing interests

All authors declare that they have no competing interests.

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Figures

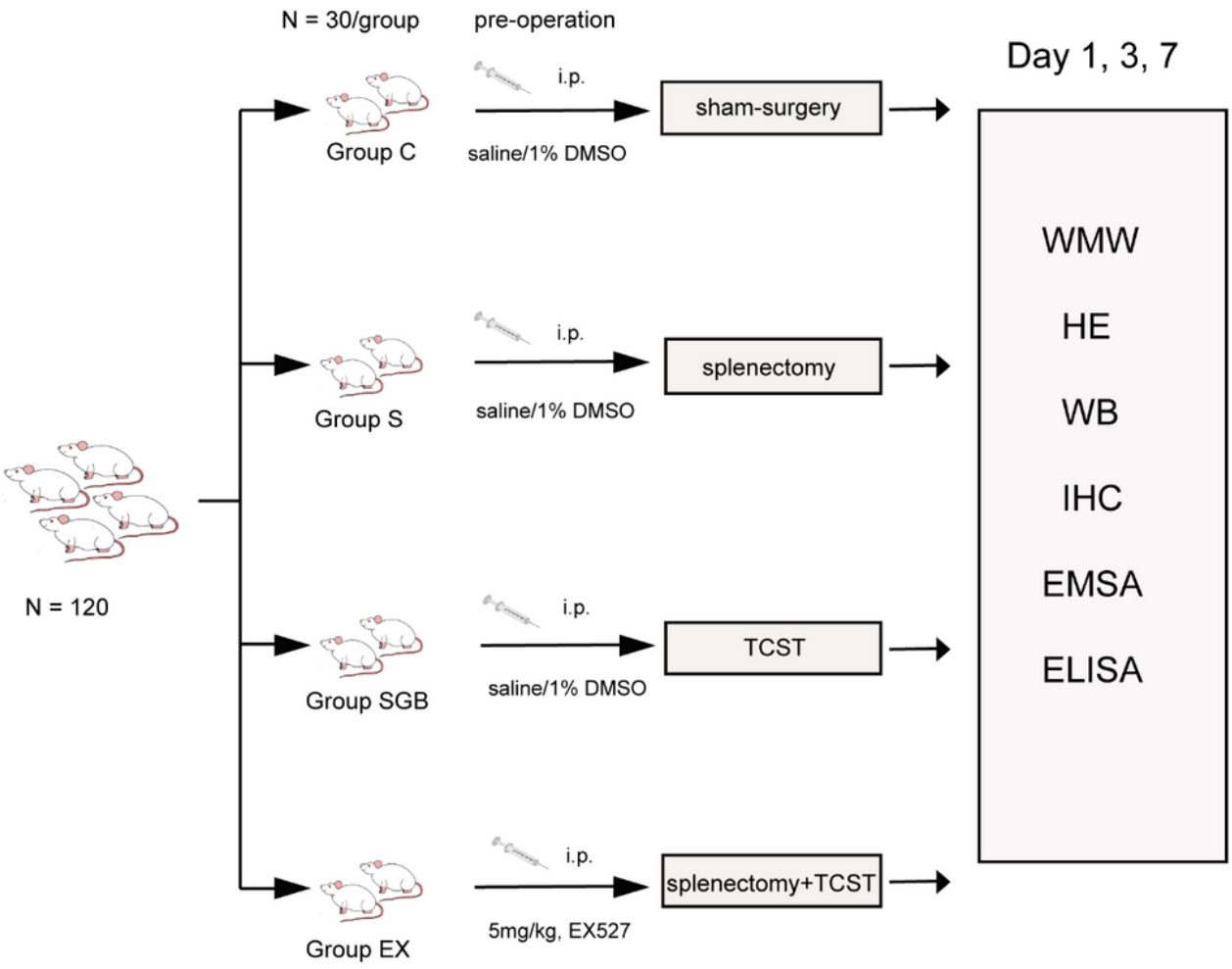

Figure 1

The flowchart of animal experimental design. TCST represent transection of the cervical sympathetic trunk.

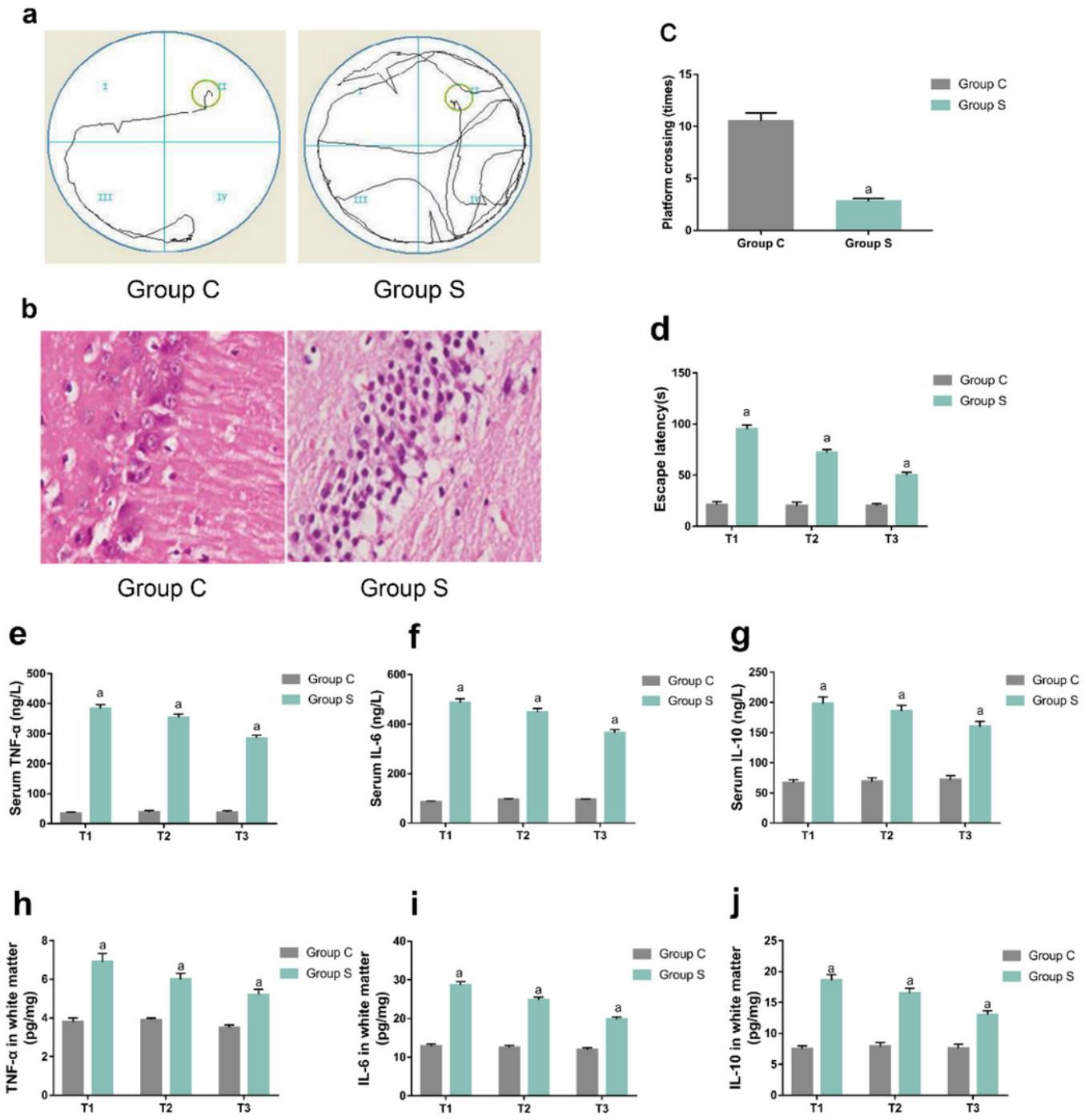

Figure 2

White matter lesions and inflammatory responses in aged rats of POCD. (**a**) The representative trajectories of rats in the sham-operated control (group C) and splenectomy (group S) group during Morris water maze test (MWM). Pool Quadrants: II=target quadrant that contained the platform; I=quadrant to the left of the target quadrant; III=quadrant opposite the target quadrant; IV=quadrant below the target quadrant. Rats were placed into the water from the center of quadrant III in the same physical location at

the same time, given a 120-second probe time, observed and noted. (**b**) Representative histopathological image of the white matter in rats in the group C and S by HE staining under light microscope (× 400). (**c**) The number of times the rats crossed the platform in the group C and S in post-operative day 8. (**d**) Escape latencies of rats in the group C and S in MWM during post-operative days 1, 3 and 7. (**e–g**) Serum TNF-α, IL-6, and IL-10 levels in the group C and S during post-operative day 1, 3 and 7. (**h–j**) The concentration of TNF-α, IL-6, and IL-10 in white matter during post-operative day 1, 3 and 7 in the group C and S. All data are mean ± S.E.M., N=8/group. Statistical significance was performed by Student's t-test. aP<0.05 vs group C. T1 represent post-operative day 1, T2 represent post-operative day 3 and T3 represent post-operative day 7.

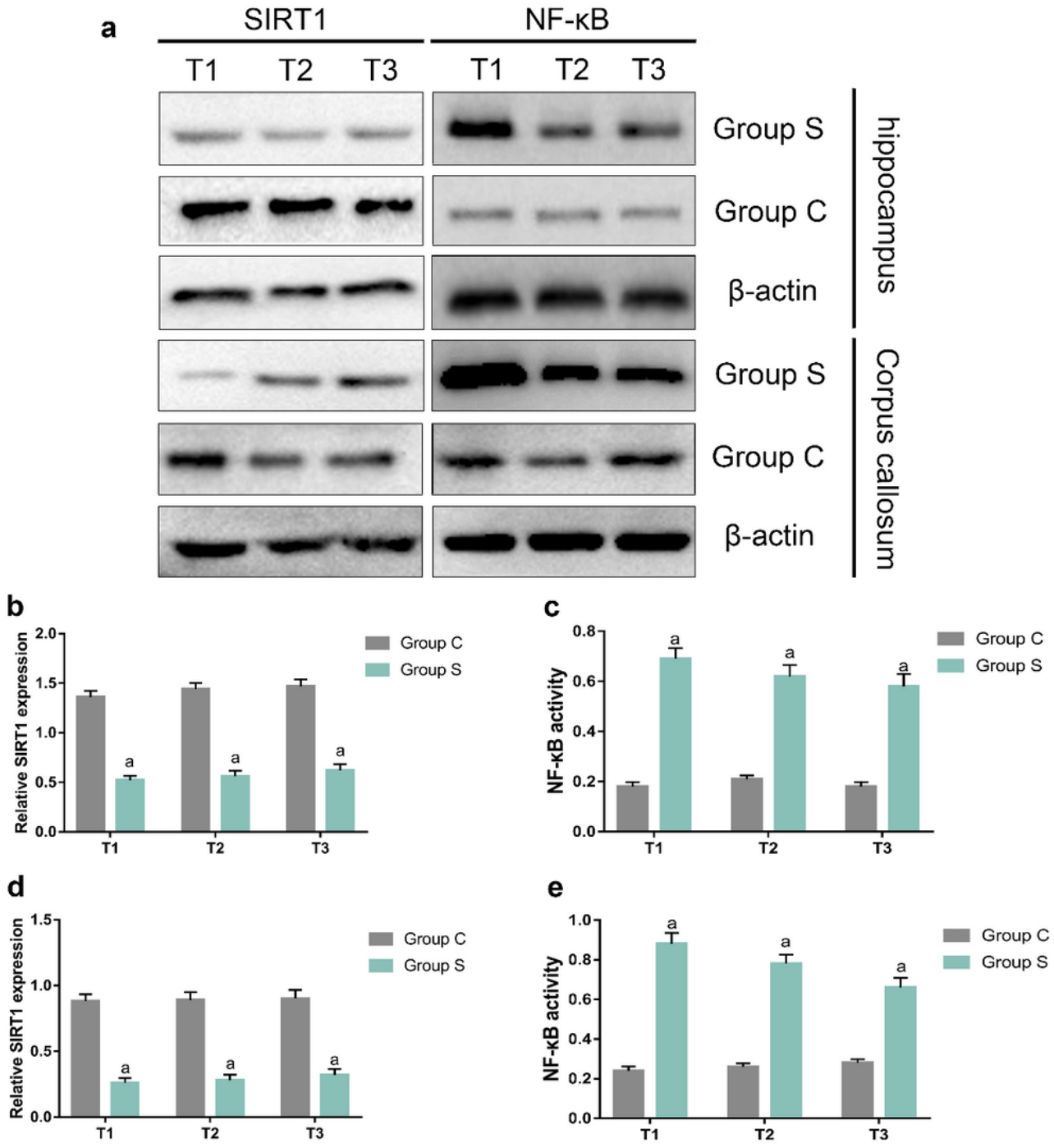

Figure 3

Decreased SIRT1 expression and increased NF-κB activity in the hippocampus and corpus callosum of the POCD model. (**a**) Representative western blotting bands of SIRT1 and NF-κB protein expression in the hippocampus of the group C and S during post-operative days 1, 3 and 7. β-actin was used as an internal control. (**b**) Semi-quantitative analysis of SIRT1 protein expression in the hippocampus of the group C and S during post-operative days 1, 3 and 7 by densitometry. (**c**) Quantification of NF-κB activity in the

hippocampus of the group C and S during post-operative days 1, 3 and 7. NF-κB activity was determined by electromobility shift assays. (**d**) Semi-quantitative analysis of SIRT1 protein expression in the corpus callosum of the group C and S during post-operative days 1, 3 and 7 by densitometry. (**e**) Quantification of NF-κB activity in the corpus callosum of the group C and S during post-operative days 1, 3 and 7. NF-κB activity was determined by electromobility shift assays. All data are mean ± S.E.M., N=8/group. Statistical significance was performed by Student's t-test. aP<0.05 vs group C. T1 represent post-operative day 1, T2 represent post-operative day 3 and T3 represent post-operative day 7.

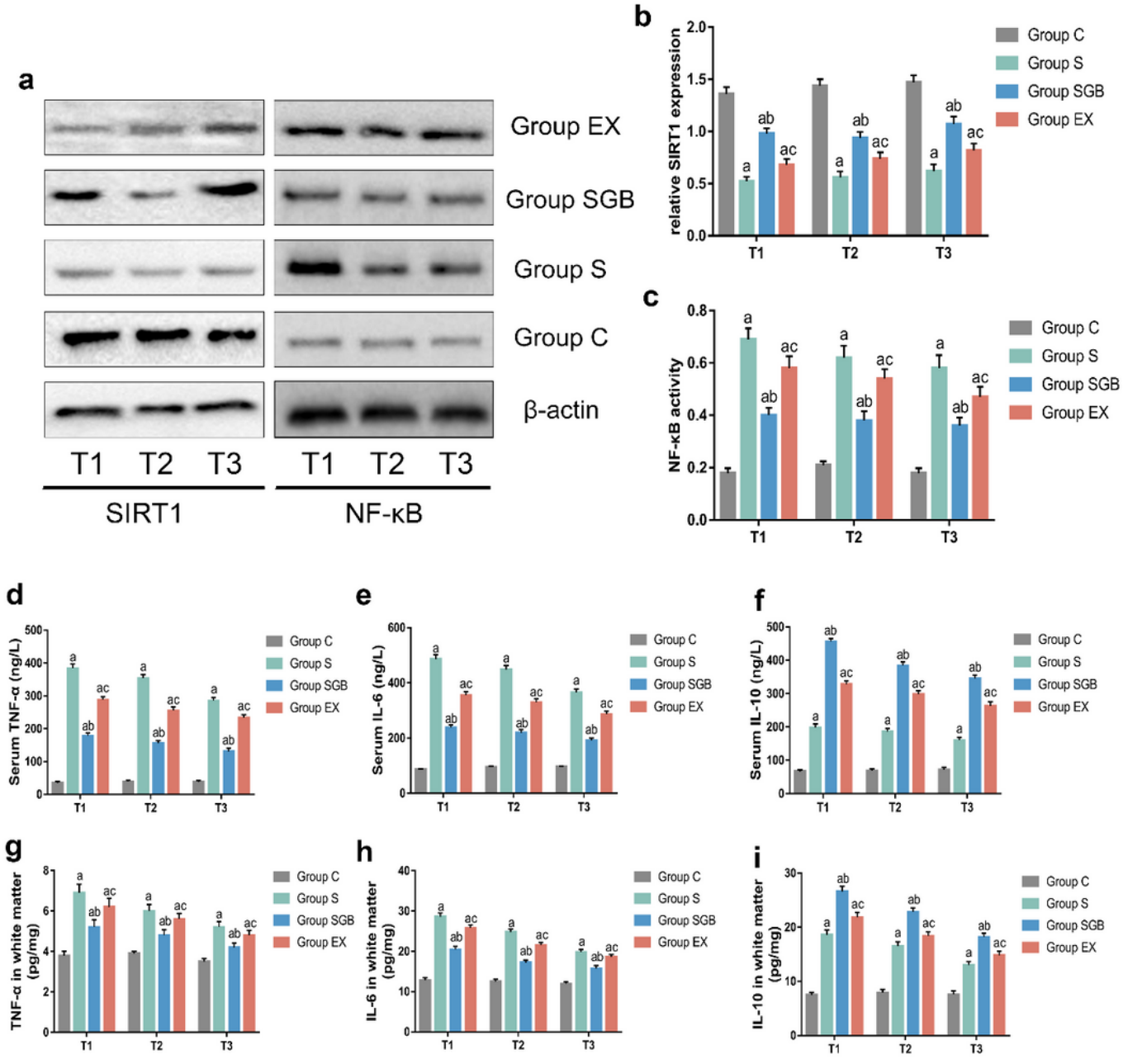

Figure 4

SGB modulates inflammatory responses in aged rats of the POCD model by upregulating SIRT1 and inhibiting NF-κB signaling in the hippocampus. (**a**) Representative western blotting bands of SIRT1 and NF-κB protein expression in the hippocampus. (**b**) Semi-quantitative analysis of SIRT1 protein expression in the hippocampusduring post-operative days 1, 3 and 7 by densitometry. (**c**) Quantification of NF-κB activity in the hippocampus during post-operative days 1, 3 and 7. NF-κB activity was determined by electromobility shift assays. (**d-f**) The concentration of TNF-α, IL-6, and IL-10 in the hippocampus during post-operative days 1, 3 and 7. (**g-i**) The concentration of TNF-α, IL-6, and IL-10 in white matter during post-operative days 1, 3 and 7. All data are mean ± S.E.M., N=8/group. One-way ANOVA and Tukey Kramer multiple comparisons test was used to analyze the significance among groups. aP<0.05 vs group C, bP<0.05 vs group S, cP<0.05 vs group SGB. T1 represent post-operative day 1, T2 represent post-operative day 3 and T3 represent post-operative day 7.

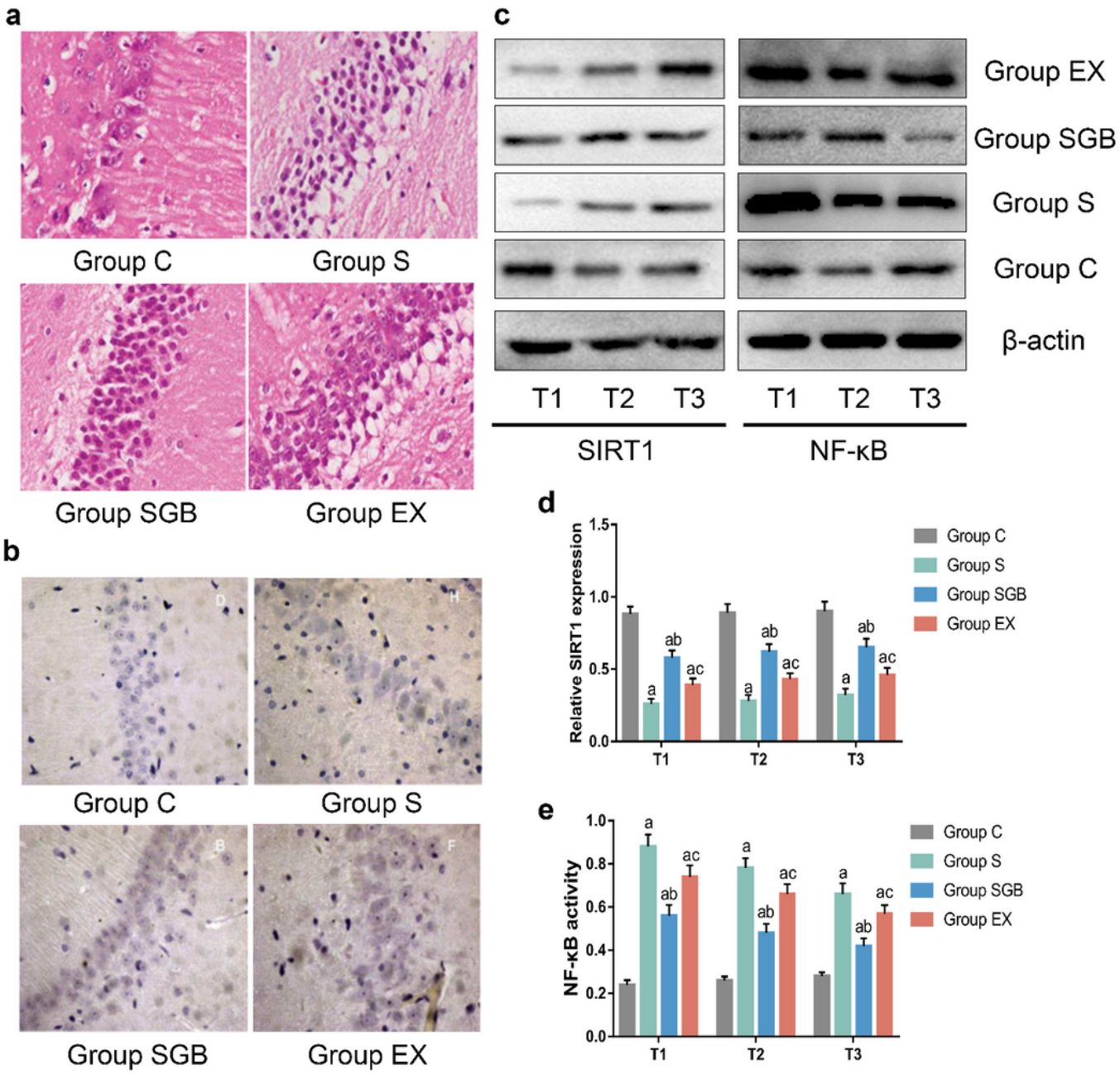

Figure 5

SGB attenuates white matter lesion in aged rats of the POCD model through activating SIRT1. (**a**) Representative histopathological image of the white matter in each group by HE staining under light microscope (× 400) (N=8/group). (**b**) Representative immunohistochemical image of SIRT1 expression in the white matter in each group by immunohistochemical staining under fluorescent microscopy(× 400) (N=8/group). (**c**) Representative western blotting bands of SIRT1 and NF-κB protein expression in the

corpus callosum. (**d**) Semi-quantitative analysis of SIRT1 protein expression in the corpus callosum during post-operative days 1, 3 and 7 by densitometry. (**e**) Quantification of NF-κB activity in the corpus callosum during post-operative days 1, 3 and 7. NF-κB activity was determined by electromobility shift assays. All data are mean ± S.E.M., N=8/group. One-way ANOVA and Tukey Kramer multiple comparisons test was used to analyze the significance among groups. aP<0.05 vs group C, bP<0.05 vs group S, cP<0.05 vs group SGB. T1 represent post-operative day 1, T2 represent post-operative day 3 and T3 represent post-operative day 7.

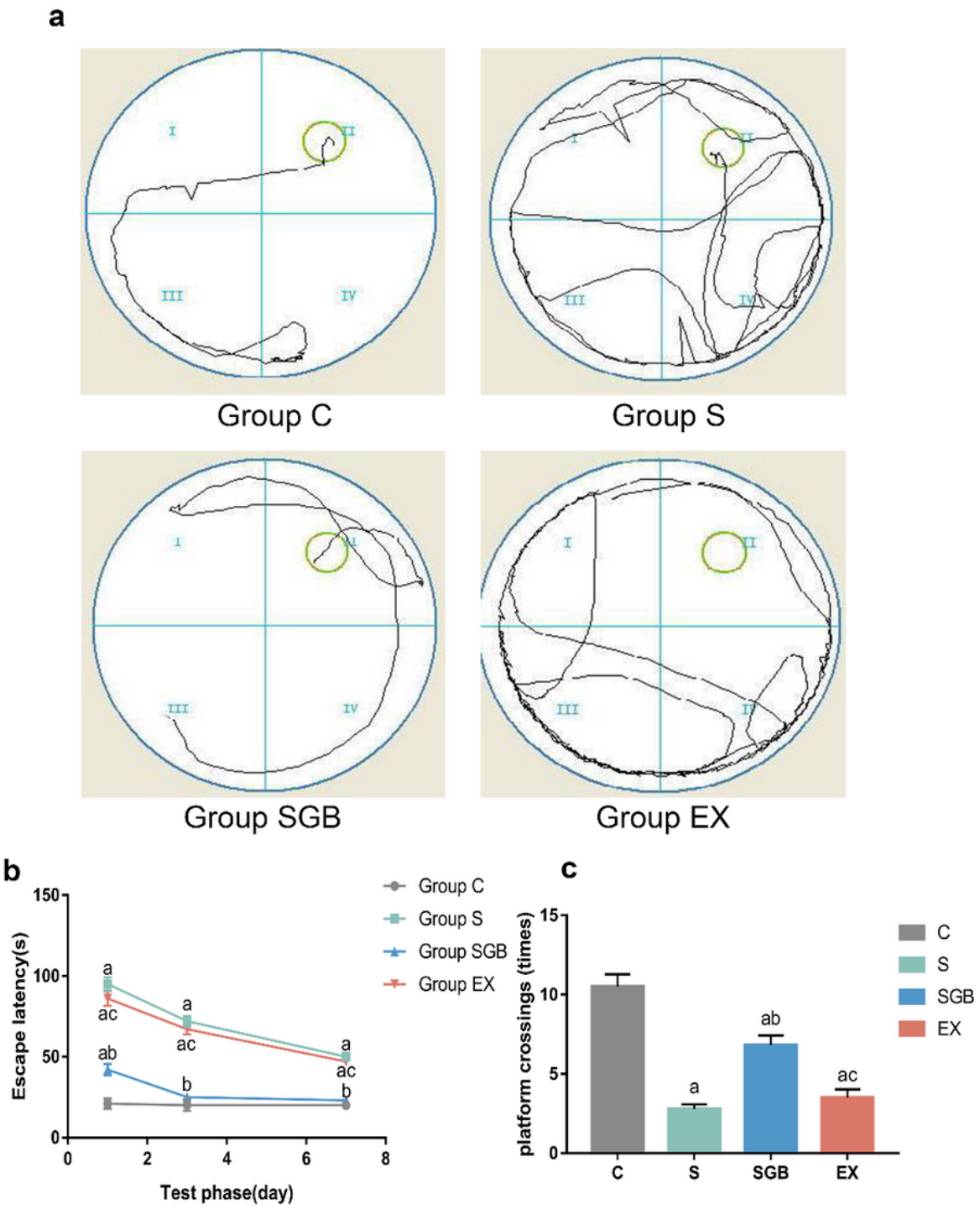

Figure 6

SGB ameliorates cognitive impairment in aged rats of the POCD model through activating SIRT1. (**a**) The representative trajectories of rats in each group during Morris water maze test (MWM). (**b**) Escape latencies of rats in each group of post-operative day 1, 3 and 7 in MWM. (**c**) The number of times the rats crossed the platform in each group of post-operative days 1, 3 and 7 in MWM. All data are mean ± S.E.M., N=8/group. One-way ANOVA and Tukey Kramer multiple comparisons test was used to analyze the significance among groups. aP<0.05 vs group C, bP<0.05 vs group S, cP<0.05 vs group SGB. T1 represent post-operative day 1, T2 represent post-operative day 3 and T3 represent post-operative day 7.

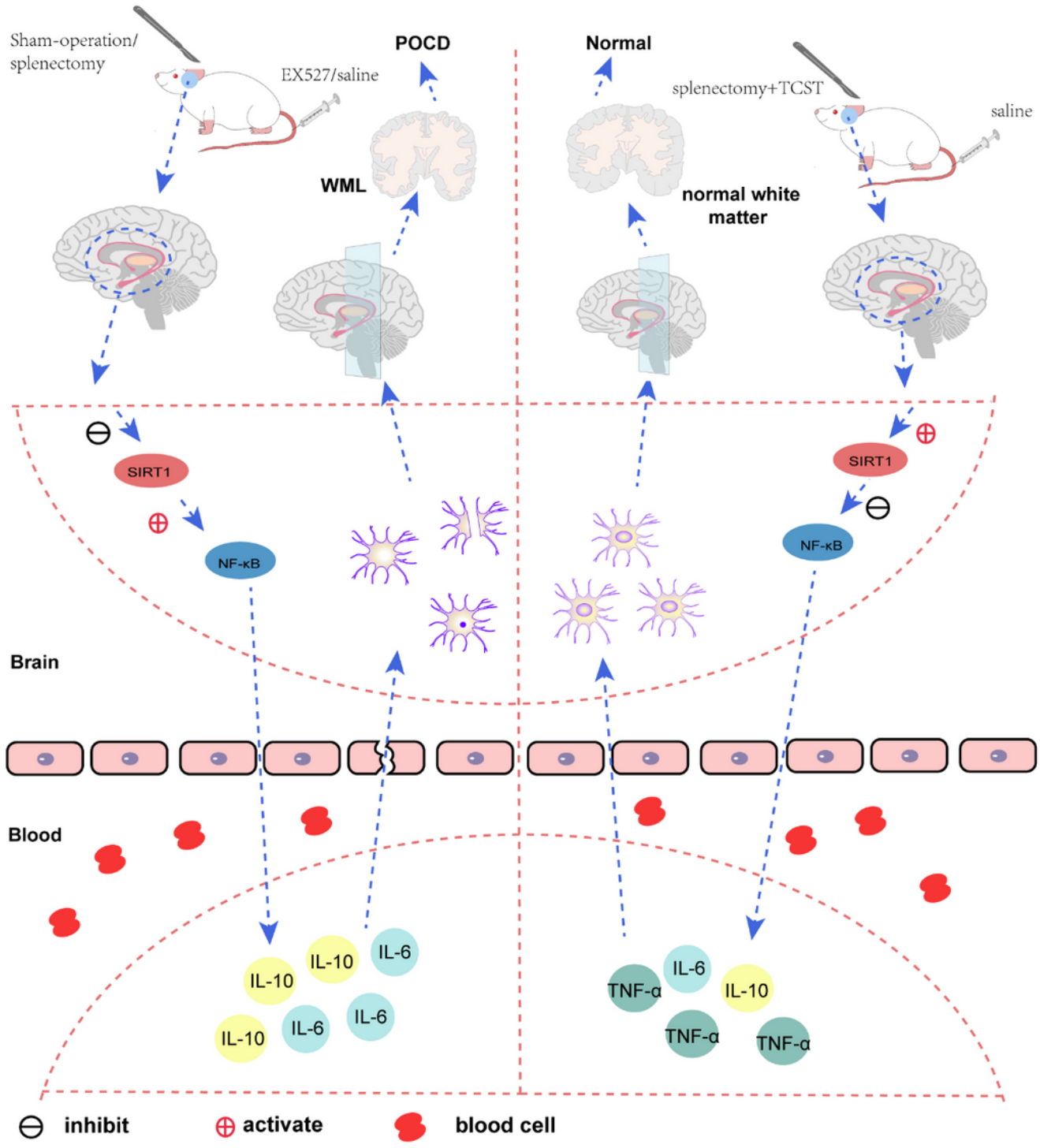

Figure 7

The pathophysiological mechanism that decreased SIRT1 induced white matter lesion in POCD and SGB reversed POCD using SIRT1-mediated white matter damage repair.