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Naringenin alleviates cognitive dysfunction in rats with cerebral ischemia/reperfusion injury through up-regulating hippocampal BDNF-TrkB signaling: involving suppression in neuroinflammation and oxidative stress

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Cognitive dysfunction is one of the common complications of cerebral ischemia-reperfusion (CI/R) injury after ischemic stroke. Neuroinflammation and oxidative stress are the core pathological mechanism of CI/R injury. The activation of brain derived neurotrophic factor (BDNF)-tyrosine receptor kinase B (TrkB) signaling antagonize cognitive dysfunction in a series of neuropathy. Naringenin (NAR) improves cognitive function in many diseases, but the role of NAR in CI/R injury-induced cognitive dysfunction remains unexplored. The study aimed to explore the potential protective effects of NAR in CI/R injury-induced cognitive dysfunction and underlying mechanism. The rats were exposed to transient middle cerebral artery occlusion (MCAO) and then treated with distilled water or NAR (50 or 100 mg/kg/day, p.o.) for 30 days. The Y-maze test, Novel object recognition test and Morris water maze test were performed to assess cognitive function. The levels of oxidative stress and inflammatory cytokines were measured by ELISA. The expressions of BDNF/TrkB signaling were detected by Western blot. NAR prevented cognitive impairment in MCAO-induced CI/R injury rats. Moreover, NAR inhibited oxidative stress (reduced levels of malondialdehyde and 4-hydroxynonenal, increased activities of superoxide dismutase and Glutathione peroxidase) and inflammatory cytokines (reduced levels of tumor necrosis factor-α, Interleukin-1β and Interleukin-6), up-regulated the expressions of BDNF and p-TrkB in hippocampus of MCAO-induced CI/R rats. NAR ameliorated cognitive dysfunction of CI/R rats via inhibiting oxidative stress, reducing inflammatory response, and up-regulating BDNF/TrkB signaling pathways in the hippocampus. ***NeuroReport*** **35: 216–224 Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc.**

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Introduction

Ischemic stroke (IS) as a severe neurological disorder that affects millions of people and is the second-leading cause of death world [1,2]. Restoration of blood supply, also known as reperfusion, is the only way to treat cerebral ischemia. However, reperfusion causes massive cell death and tissue destruction in the perfusion territory inevitably, which is recognized as cerebral ischemia-reperfusion (CI/R) injury [3]. Cognitive dysfunction is one of the most common and severe consequences of CI/R injury after IS [4]. It has been confirmed that cerebral ischemia impairs learning and memory in patients with IS [5], and 25–30% of IS survivors suffered from immediate or delayed cognitive impairment or dementia [6]. However, no specific approaches prevent cognitive deficits induced by CI/R injury. It should be noted that the increase of oxidative stress and inflammatory response in the hippocampus are critical event during CI/R-induced cognitive deficits [7,8]. Therefore, therapeutically targeting CI/R-induced hippocampal oxidative stress and inflammation would be a novel strategy for treating cognitive deficits after IS.

Naringenin (NAR) is a plant-derived flavanone that is widely distributed in citrus fruits, which is known for its antioxidant and anti-inflammatory properties. Because NAR cross the blood-brain barrier easily, it has a neuroprotective effect on the brain [9,10]. It has been confirmed that NAR ameliorates cognitive deficits in diabetic rat [11]. Also, NAR was reported to ameliorate cognitive impairment in rats induced by isoflurane anesthesia [12], methylmercury [13] and lipopolysaccharide [14]. These previous research provide evidence that NAR has the function of ameliorating cognitive impairment. Therefore, we exposed rats to middle cerebral artery occlusion (MCAO) for constructing a model of CI/R injury to explore the potential therapeutic function

of NAR on CI/R injury-induced cognitive dysfunction and whether it is involved in the regulation of oxidative stress and inflammation in the hippocampus.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin widely expressed in the central nervous system, which plays a critical role in neuronal survival and differentiation, and synaptic plasticity by activating tyrosine receptor kinase B (TrkB) receptor [15,16]. Accumulating evidence suggests that BDNF-TrkB is essential for learning and memory [17]. It has been demonstrated that downregulation of hippocampal BDNF/TrkB signaling contribute to cognitive dysfunction in many animal models of disease such as Alzheimer's disease (AD) [18], diabetes [19] and schizophrenia [20]; On the contrary, the activation of BDNF/TrkB improve cognitive performance in the above model [18–20]. These observations indicate that the upregulation of hippocampal BDNF/TrkB signaling may be a novel candidate for cognitive dysfunction therapy. Therefore, we further investigated whether the regulation of hippocampal BDNF/TrkB signaling may be an important factor to the protection of NAR against cognitive deficit by CI/R injury.

The aim of the present study was to evaluate the protective effect of NAR on the CI/R injury-induced cognitive dysfunction and the underlying mechanisms. Our experiments found that NAR improved the learning and memory dysfunction, inhibited oxidative stress and inflammatory cytokines in hippocampus, enhanced hippocampal BDNF/TrkB signaling pathway in MCAO-induced CI/R rats. These findings suggested that NAR as a novel therapeutic approach for treatment of cognitive impairments after CI/R injury, which may be related to the regulation of oxidative stress, inflammatory reaction and BDNF/TrkB signaling in hippocampus.

Materials and methods

Reagents

NAR, sodium pentobarbital was supplied by Sigma-Aldrich; Merck KGaA. The BCA protein assay kit was obtained from Dojindo Molecular Technologies, Inc. The assay kits for glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE) measurement were purchased from Wuhan USCN Business Co., Ltd. The ELISA (Elisa) kits for Tumor necrosis factor-alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) were purchased from R&D Systems (R&D Systems, Minneapolis, MN, USA). Specific monoclonal antibodies against BDNF, p-TrkB, β-actin, and Anti-rabbit IgG-HRP-linked Antibody were supplied by Cell Signaling Technology, Inc.

Animals

100 adult male Wistar rats (age, 8–10 weeks; weight, 250–300 g) were supplied by Hunan SJA Labora tory Animal Co., Ltd (Hunan, China). In order to prevent the rats from attacking each other and to fully recover from the wounds after CI/R surgery, the rats were housed in individual cages. All rats were placed in a specific-pathogen-free environment under controlled environmental conditions with temperature (22 ± 2 °C) and humidity (50 ± 10%) under12h light/12 h dark cycle (lights from 08:00 to 20:00), and given standard food and water. The research related to animal use has complied with all the relevant national regulations and institutional policies for the care and use of animals.

Induction of focal cerebral ischemia-reperfusion

The rats were subjected to transient MCAO by inserting a thread into the middle cerebral artery (MCA) for establishing focal CI/R model according using the modified Zea Longa method [21,22]. The steps are as follows: rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (35 mg/kg) and a median incision was made in the neck, fixed on the operating table. The external carotid artery was ligated at the proximal end of the carotid bifurcation, and the internal carotid artery was closed with an artery clip. A small cut was made at the bifurcation of the common carotid artery, and the nylon thread plug with a heparin-coated tip (diameter 0.26 mm, length 2.0 cm) was slowly pushed into the cranial artery through the left common carotid bifurcation. The depth of advancement was approximately 18 mm from the bifurcation of the common carotid artery. The thread was gently moved until a sense of resistance was felt, that is, the threaded plug reached the beginning of the MCA, thereby blocking the blood flow in the MCA and causing focal cerebral ischemia. After 2 hours of ischemia, the nylon thread plug was pulled out and the artery stump was ligated to complete the reperfusion. Rats in the sham group were treated with the same surgical procedures without the insertion nylon monofilament. After operation, the rats were given intramuscular injection of penicillin 200 000 U for anti-infection for 3 consecutive days.

Neurological deficit score

After 24 h of reperfusion, Neurological deficit scores were performed using the Zea Longa method to identify whether the CI/R model was successful. Specific operation: Lift the rat tail to make the limbs of the rats off the ground, and observe the extension of the two forelimbs of the rats. The rats were then placed on a level surface to observe their crawling. The rats were pushed to observe whether there was any difference in the resistance between the two sides. Zea Longa scoring system is 5-grade on a scale of 0 to 4, the following are scoring criteria: 0 point: no any neurological deficit; 1 point: The left forelimb of rats can't entirely stretch, mild neurological deficit; 2 points: rats rotate to the ischemic side while walking, moderate neurological deficit; 3 points: rats dump to the ischemic when standing, severe neurological

deficit; 4 points: rats cannot walk on their own and lose consciousness. Rats with point 0 and 4, seizures, or cerebral hemorrhage were excluded from the study; remaining rats were assigned to each group as required. All evaluations were performed by researchers who were blinded to treatment groups.

Experimental protocol

After 1 weeks of acclimatization, rats were randomly assigned to five groups (20 rats in each group): (i) Sham group: received same surgery except for the MCAO; (ii) CI/R group: subjected to MCAO and then treated with vehicle (distilled water, 1.5 mL, p.o.) for 30 days; (iii) CI/R + low-dose NAR group: subjected to MCAO and then treated with Nar (50 mg/kg/day, diluted in distilled water, p.o.) for 30 days; (iv) CI/R + high-dose NAR group: subjected to MCAO and then treated with Nar (100 mg/kg/day, diluted in distilled water, p.o.) for 30 days;high-dose NAR alone group: the rats received NAR (100 mg/kg/day, diluted in distilled water, p.o.) for 30 days. On the second day after the completion of the NAR or vehicle intervention, behavioral tests were performed. For better understanding, schematic diagram of the experimental schedule is illustrated in Fig. 1.

Y-Maze test

The working memory was measured using the Y-maze test [23]. The apparatus consisted of three identical arms (90 × 90 × 70 cm; A, B, C) positioned 120 apart and made of black Plexiglas. First, the rats were habituated in the Y-maze recording room for 30 min. In test phase, the rat was placed at the intersection of three arms and was given 5 min trial to move freely throughout the maze. Arm entry sessions were recorded when the hind paws of the rats were completely into the arm. Animal movement was recorded using an analogue camera connected to the video tracking software ANY-Maze 5.1. Consecutive entries into a new arm before returning to the two previously visited arms was defined as successful alternation. The spontaneous alternation (%) [(number of correct alternations/total number of arm entries – 2) × 100] were used as a working memory index.

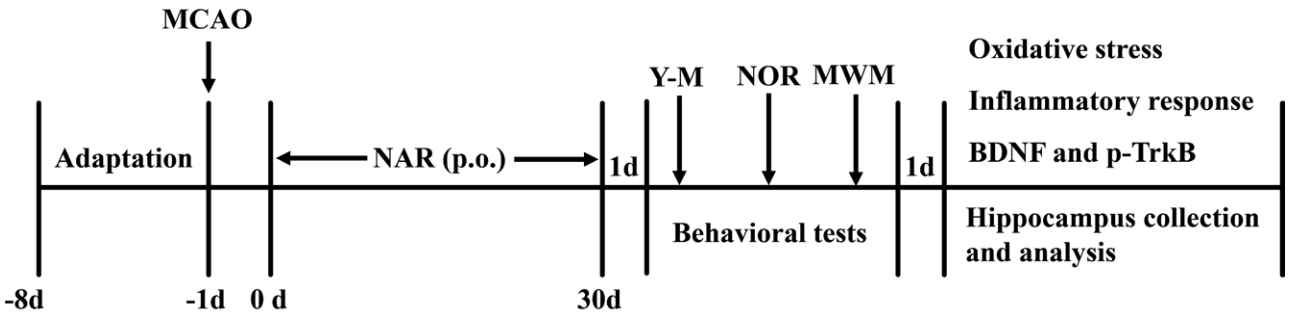
Novel object recognition test

The novel object recognition (NOR) test was conducted as previously described [24] to assess the ability of rats to recognize a novel object in a familiar environment. We performed a NOR test for 3 days including a habituation phase (10 min for 1 day), a training phase (5 min for 1 day) and a test phase (5 min for 1 day) in each rat. During the habituation phase, the rats were habituated in an opaque test box (around 40 cm × 40 cm × 40 cm) and allowed to freely explore without objects for 10 min. On training phase, the rat was placed into the testing box for 5 min to explore two different objects on opposite sides of the arena; During testing sessions, one familiar object and one novel object of similar size were placed into the same places as in the training phase, and the animals were permitted to explore for 5 min. Finally, the total approach time for exploring each object were recorded by an experienced researcher blind to treatments. The following acts were considered as the exploration of the object: touching to the object with the head of animal, sniffing to the object and keeping the distance from nose of rat to the objects less than 2 cm. Climbing or sitting on the object was not defined as exploration. To control the odor cues, the testing box and the objects were cleaned with 70% alcohol at the end of each experiment for every rat. The cognitive function of the rats was judged by the discriminative index (DI) = [time spent with novel object] – [time spent with familiar object]/[time spent with novel object] + [time spent with familiar object]. At the same time, we analyzed the total exploring time of each group during the test period to exclude the interference of animals' motor ability and emotion on the experimental results.

Morris water maze test

The spatial learning and memory of rats was evaluated by using Morris water maze (MWM) as described earlier [25].

Fig. 1



Schematic diagram of the experimental schedule. MCAO, middle cerebral artery occlusion; NAR, Naringenin; p.o., peros; NOR, novel objects recognition test; Y-M, Y-maze test; MWM, Morris water maze test.

The MWM system (Shanghai DOiT Industrial Co., Ltd.) consists of water maze device, water maze image automatic collection and software analysis system. Pictures of rat swimming (analog signal) were collected via the camera was introduced to the computer for analog-to-digital conversion to get the relevant data through digital image analysis. Water maze device mainly consists of a circular pool containing water (diameter of 180 cm, height 60 cm) which was rendered opaque by adding milk powder and a circular acrylic platform (height 38 cm, 12.5 cm diameter). The pool was divided into 4 quadrants, and platform was place in the first quadrant (target quadrant) and submerged 2.0 cm below the water surface during positioning navigation training test. The MWM test mainly includes three parts: positioning navigation training test, space exploration test and visible platform test.

Positioning navigation training experiment: the rats were trained four times a day and were placed in water from each of the four quadrants of the pool with an intertrial interval of 60 s for 5 days, and the time required to find the platform within 120 s (latency) was recorded. If the rat is not found within 120 s, it is directed to the platform and stays for 20 s. The average value of four training results was recorded as the latency of the day. After 5 days of training, the space exploration experiment began on the sixth day: The platform was removed and each rat had 120 s time for free swimming, placed the rat in the pool from the opposite side of the original platform quadrant. The time that rats spent in the target quadrant (where the platform was located during positioning navigation training) and the number of times the rats crossed where the platform had been located were measured and calculated. Finally, to excluded the influence of vision and motor ability on the above results, we carried out visible platform test: placed the platform in the contralateral quadrant during the positioning navigation training and raise it 2 cm above the pool. The rats entered the pool from the contralateral quadrant of the platform, and the latency reaching the platform and average swimming speed were recorded.

Sample collection

Twenty-four hours after the MWM, the animals were anesthetized with pentobarbital sodium (i.p.), killed by decapitation, and then the brains were quickly removed. The brain tissue is placed on ice, rinsed, then separated from the hippocampus with a glass minute hand, and dried with filter paper. Finally, hippocampus was stored in a refrigerator at –80 °C for later use.

Western blot analysis

The protein expressions of BDNF and p-TrkB were measured by Western blot. The entire hippocampus of rats was removed and homogenized in ice-cold (4 °C) homogenizing buffer. After centrifugation at 12 000*g* for 30 min at 4 °C, the supernatant was collected and the protein concentration was analyzed using a BCA protein assay kit (Beyotime Institute of Biotechnology). Approximately 10 μg of protein per group were separated on 8–12% SDS-PAGE and electrotransferred to PVDF membranes. After blocking the membranes with TBST containing 5% non-fat milk for 2 h at room temperature, membranes were incubated at 4°C with the primary antibody (Anti-BDNF, 1:1000; Anti-p-TrkB, 1:1000; β-actin 1:2000). Then the blots were washed three times with TBST buffer and incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:5000) for 2 h at 4 °C. After washing, the protein bands were added with electrogenerated chemiluminescence reaction solution and visualized using a Tanon-5600 Imaging System (Tanon Science and Technology Co., Ltd.). The signal of the blots was analyzed using Image J software and expression values were normalized to that of β-actin.

Oxidative stress quantification

After sacrifice, the hippocampal tissue of rats was homogenized (10% w/v) in 0.1 mol/l PBS and centrifuged at 12 000*g* for 10 min at 4°C. Subsequently, the supernatant was collected, and the protein concentration was quantified using the aforementioned BCA protein assay. According to the manufacturer's instructions, the activities of GSH-Px and SOD, the levels of MDA and 4-HNE in the supernatant were determined by spectrophotometry using the aforementioned commercially available kits.

Elisa for inflammatory cytokines

As described above, the tissue samples were homogenized in ice-cold PBS and centrifuged (12 000*g*, 10 min, 4 °C). As previously described [26], the supernatant was aspirated to measure the levels of TNF-α, IL-1β and IL-6 by using aforementioned Elisa kits. The results were expressed as pg/mg (per protein).

Statistical analysis

All the data were analyzed performed using SPSS 20.0 software (IBM Corp.) and presented as the mean±SEM. The intergroup significance of escape latency during 5 days of positioning navigation training in MWM test were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by Tukey's post-hoc test, other data were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Two-tailed $P < 0.05$ was considered to indicate a statistically significant difference.

Results

NAR ameliorates the working memory impairment of MCAO-induced CI/R rats in Y-maze test

The result of one-way ANOVA of the spontaneous alternation showed significant effect of NAR ($n = 10$, $F_{4,45} = 17.233$, $P < 0.001$). The analyzed by Tukey's post hoc test showed that the CI/R rats had the lower spontaneous alternation compared with sham group, whereas

NAR (50, 100 mg/kg) obviously increased the spontaneous alternation in CI/R rats (Fig. 2a). In addition, one-way ANOVA showed no significant difference in total arm entries among each group, excluding the influence of gap in activity ability on the above results (n = 10, $F_{4,45} = 0.512$, $P > 0.05$; Fig. 2b). These findings indicated that NAR reversed the working memory dysfunction of MCAO-induced CI/R rats.

NAR ameliorates the cognitive impairment of MCAO-induced CI/R rats in NOR test

The result of one-way ANOVA of the discrimination index showed significant effect of NAR (n = 10, $F_{4,45} = 74.418$, $P < 0.001$). The analyzed by Tukey's post hoc test showed CI/R rats had the lower discrimination index compared to sham group, while NAR (50, 100 mg/kg) significantly increased the discrimination index of CI/R rats (Fig. 2c). Furthermore, one-way ANOVA showed no significant discrepancy of the total exploration time between all groups, excluding the interference of motor ability and emotion to the above results (n = 10, $F_{4,45} = 0.276$, $P > 0.05$; Fig. 2d). These findings revealed that NAR attenuated the cognitive dysfunction of MCAO-induced CI/R rats.

NAR ameliorates the spatial learning and memory impairment of MCAO-induced CI/R rats in MWM test

In the positioning navigation training test, the result of two-way repeated measures ANOVA of the escape latency showed significant effects of treatment with NAR (n = 10, $F_{4,45} = 8.359$, $P < 0.001$) and days (n = 10, $F_{4,42} = 92.7$, $P < 0.001$). However, there were no significant effect of NAR × day interaction (n = 10, $F_{16,180} = 0.724$, $P > 0.05$) for escape latency in all training days. Tukey's post hoc test analysis showed that the escape latency of CI/R rats from day 2 to day 5 was significantly prolonged compared with the sham group, but NAR (50, 100 mg/kg) shortened the escape latency in CI/R rats from training day 2 onward, indicating that NAR improved the spatial learning of CI/R rats (Fig. 2e). In the space exploration test, the result of one-way ANOVA of the percentage of time spend on the target quadrant (n = 10, $F_{4,45} = 29.751$, $P < 0.001$) and the times across platform (n = 10, $F_{4,45} = 12.357$, $P < 0.001$) showed significant effects of NAR. Tukey's post hoc test analysis showed that CI/R rats had lowered the percentage of time spend on the target quadrant and the times across platform compared to sham rats, while NAR (50, 100 mg/kg) obviously enhanced the percentage of time spend on the target quadrant and the times across platform in CI/R rats (Fig. 2f and g), indicating that NAR improved the spatial memory of CI/R rats. In the visible platform test, one-way ANOVA showed no significant differences in the latency to platform (n = 10, $F_{4,45} = 0.238$, $P > 0.05$) and the average swimming speed (n = 10, $F_{4,45} = 0.190$, $P > 0.05$) of the rats in each group (Fig. 2h and i), excluding the interference of visual perception and swimming capability of rat to the above results. Taken together, these data suggested that NAR apparently attenuated the spatial learning and memory dysfunction of MCAO-induced CI/R rats in MWM test.

NAR inhibits the hippocampal inflammatory cytokines of MCAO-induced CI/R rats

The result of one-way ANOVA of the TNF-α (n = 5, $F_{4,20} = 49.955$, $P < 0.001$), IL-1β (n = 5, $F_{4,20} = 95.760$, $P < 0.001$) and IL-6 (n = 5, $F_{4,20} = 115.282$, $P < 0.001$) showed significant effects of NAR. Tukey's post hoc test analysis showed that treatment of NAR (50, 100 mg/kg) dramatically reversed up-regulations of TNF-α, IL-1β and IL-6 of hippocampus in CI/R rats (Fig. 3a–c). These data indicated that NAR inhibited hippocampal inflammatory cytokines in MCAO-induced CI/R rats.

NAR inhibits the hippocampal oxidative stress of MCAO-induced CI/R rats

The result of one-way ANOVA of the SOD (n = 5, $F_{4,20} = 47.771$, $P < 0.001$), GSH-Px (n = 5, $F_{4,20} = 68.036$, $P < 0.001$), MDA (n = 5, $F_{4,20} = 53.908$, $P < 0.001$) and 4-HNE (n = 5, $F_{4,20} = 77.228$, $P < 0.001$) showed significant effects of NAR. Tukey's post hoc test analysis showed that treatment of the CI/R rats with NAR (50, 100 mg/kg) significantly increased the activities of SOD and GSH-Px, reduced the content of MDA and 4-HNE in hippocampus (Fig. 3d–g). These data indicated that NAR inhibited hippocampal oxidative stress in MCAO-induced CI/R rats.

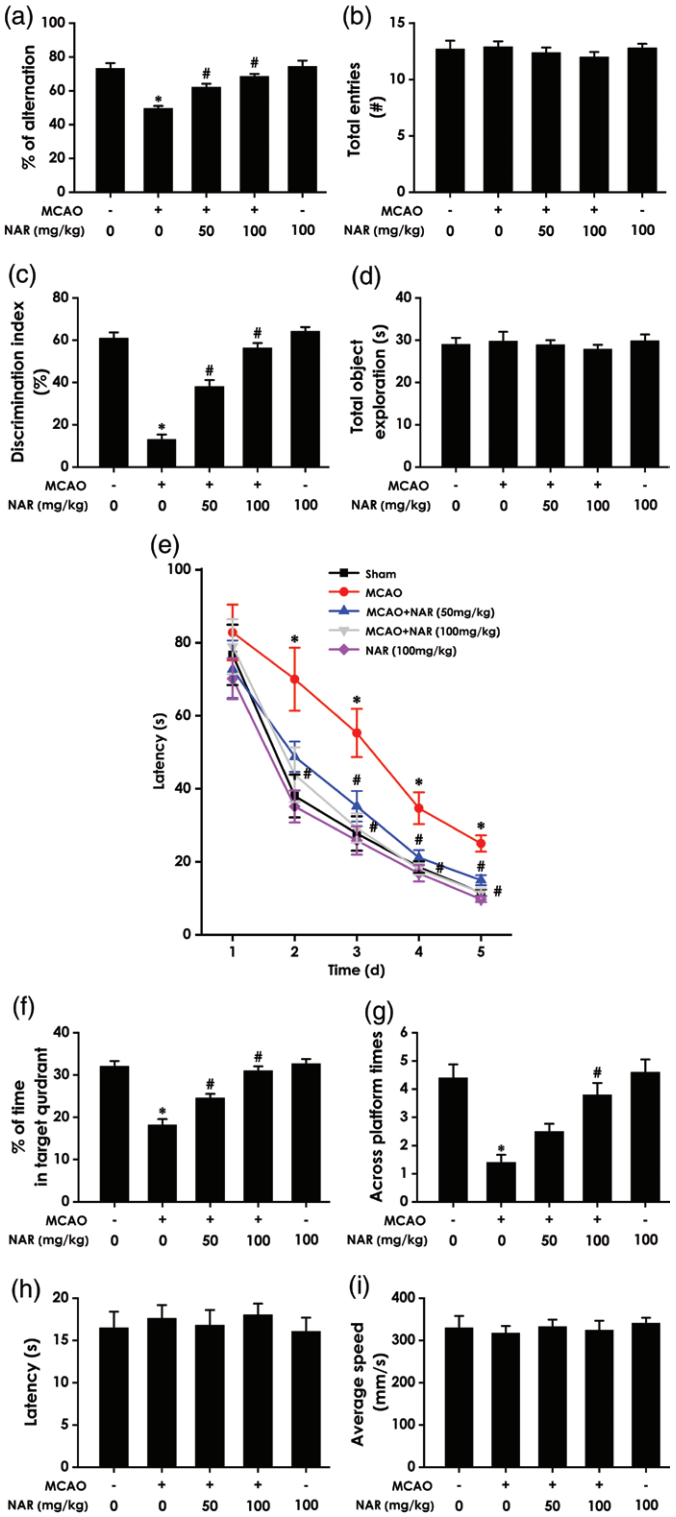
NAR up-regulates hippocampal BDNF-TrkB signaling of MCAO-induced CI/R rats

The result of one-way ANOVA of the expressions of BDNF (n = 3, $F_{4,10} = 38.769$, $P < 0.001$) and p-TrkB (n = 3, $F_{4,10} = 25.563$, $P < 0.001$) showed significant effects of NAR. Tukey's post hoc test analysis showed that NAR (50, 100 mg/kg) obviously reversed the down-regulations of expression levels of BDNF and p-TrkB in the hippocampus of CI/R rats (Fig. 4a and b), indicating the up-regulatory role of NAR in the hippocampal BDNF-TrkB signaling.

Discussion

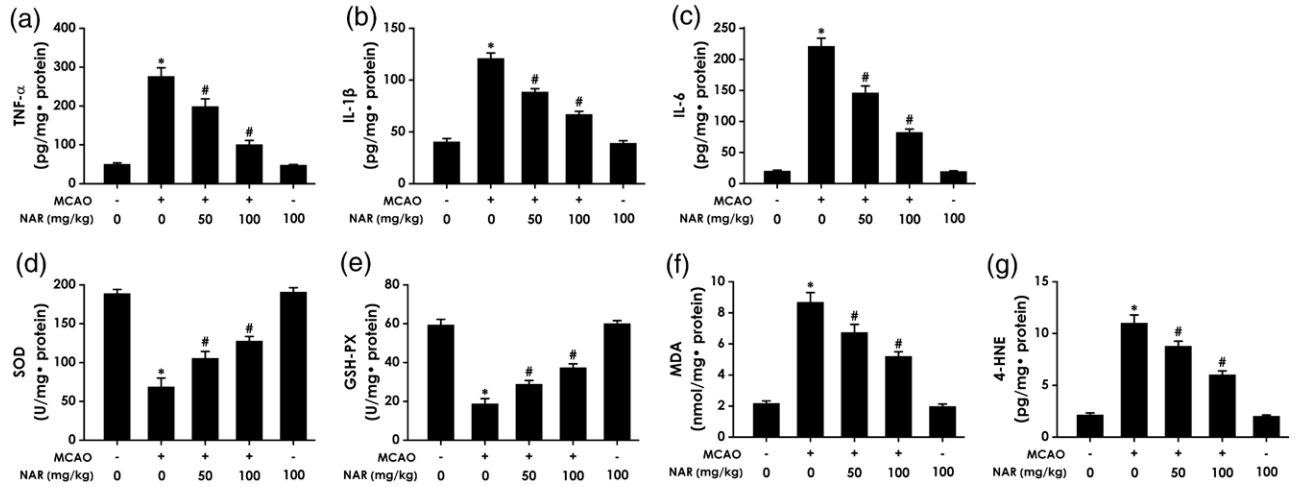
Although NAR can ameliorate cognitive loss caused by certain diseases or adverse factors in animal models [11–14], its role in cognitive impairment during CI/R has not been elucidated. Also, NAR has powerful anti-inflammatory and antioxidant effects in the brain [27]. Activation of hippocampal BDNF/TrkB signaling alleviates cognitive deficits [18–20]. The present study used MCAO-induced CI/R rats for exploring the role of NAR on the CI/R-injury-induced cognitive deficits and the underlying mechanisms. Our findings revealed that NAR ameliorates cognitive deficits of CI/R rat model via

Fig. 2



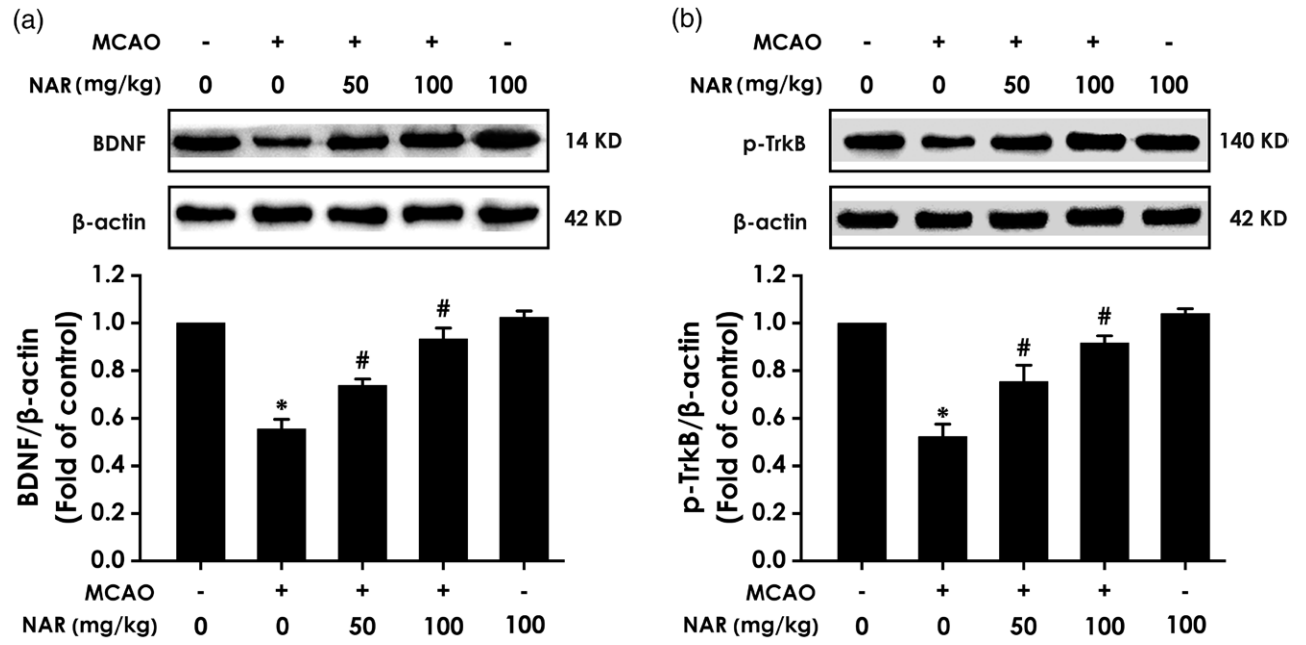
Effect of NAR on the cognitive performance of MCAO-induced CI/R rats in Y-maze test, NOR test and MWM test. Rats were received MCAO and then treated with NAR (50 and 100 mg/kg/d, p.o.) for 30 days. The working memory impairment of rats was test using the Y-maze and the % of alternation (a) and total entries number (b) were recorded. The cognitive impairment of rats was tested using the NOR and Discrimination index (c) and total object exploration (d) in training period were measured. The spatial learning and memory impairment of rats was using the MWM: (e) the escape latency to find platform with four swims per day during 5 days in positioning navigation training phase was recorded. (f and g) The percentage of time expended in the target quadrant (f) and the times across platform (g) of rats in space exploration phase were analyzed. (h and i) The escape latency (H) and average swimming speed (i) of rats in visible platform phase were recorded. The data are expressed as mean ± SEM (n = 10). $^{*}P < 0.05$ vs. sham group; $^{\#}P < 0.05$ vs. CI/R group.

Fig. 3



Effect of NAR on the inflammatory cytokines and oxidative stress in hippocampus of MCAO-induced CI/R rats. Rats were received MCAO and then treated with NAR (50 and 100 mg/kg/d, p.o.) for 30 days. The levels of TNF-α (a), IL-1β (b) and IL-6 (c) in the hippocampus were determined by Elisa; The activities of SOD (d) and GSH-Px (e), as well as the levels of MDA (f) and 4-HNE (g) in the hippocampus were determined by spectrophotometry using commercially available kits. The data are expressed as mean ± SEM (n = 5). $^{*}P < 0.05$ vs. sham group; $^{\#}P < 0.05$ vs. CI/R group.

Fig. 4



Effect of NAR on the BDNF-TrkB signaling in hippocampus of MCAO-induced CI/R rats. Rats were received MCAO and then treated with NAR (50 and 100 mg/kg/d, p.o.) for 30 days. The protein levels of BDNF (a) and p-TrkB (b) in the hippocampus were detected by western blotting. The data are expressed as mean ± SEM (n = 3). $^{*}P < 0.05$ vs. sham group; $^{\#}P < 0.05$ vs. CI/R group.

oxidative stress, inflammatory response and BDNF/TrkB signaling pathway in hippocampus.

Cognitive impairment as one of the complicated complications after IS [28], there is an urgent need to develop neuroprotective medications to prevent and restore. To date, numerous studies suggested the protective role of NAR on cognitive impairment in multiple disease model [11–14]. Thus, we raised the question of whether NAR as potential neuroprotective drug protects against CI/R injury-induced cognitive deficits in patients with IS. We explored the improvement effect of NAR on cognitive deficits of CI/R injury through behavioral experiments

included Y-Maze, NOR and MWM, using MCAO rats as models. The Y-maze test is a special and sensitive test of rodents' working memory in a new environment, taking advantage of their natural ability to explore nature in novel environments [29]. NOR test to detect short-term declarative memory and attention of animals based on their exploration of the differences between familiar and new objects [30]. MWM has been identified as a classic model for the evaluation of spatial learning and memory in rodents and is widely used in studies such as learning and memory, AD, hippocampal/external hippocampal research, intelligence and aging, and drug development [31,32]. The results showed that NAR increased spontaneous alternation on Y-maze test and up-regulated discrimination index on NOR test, in MCAO-induced CI/R rats. In MWM test of CI/R rats, NAR significantly reduced escape latency in positioning navigation training test, as well as increased percentage of time spend on the target quadrant and times across platform in space exploration test. Taken together, these findings indicated that NAR antagonism cognitive impairment in MACO-induced CI/R rats. Therefore, we suggested that NAR is an effective therapeutic drug for CI/R injury-induced cognitive impairment after IS.

There is evidence demonstrating that neuroinflammation and oxidative stress are the core pathological mechanism of CI/R injury after IS [33,34]. The inflammatory reaction after cerebral ischemia is involved in the process of nerve cell death, blood-brain barrier destruction, and ultimately damage to brain tissue and nerve function [33].

Inflammatory mechanisms in the stroke subjects causing an increase in brain damage and poor outcome following ischemic conditions [35]. The importance of the hippocampus for cognitive functions is well established [36]. Previous studies showed that inflammatory cytokines are increased in the hippocampal tissue after ischemia and reperfusion [37]. Excessive ROS production during reperfusion increases the production of lipid peroxide, which greatly exceeds the antioxidant capacity [25,38]; ROS overproduction and decreased the capacity of antioxidant enzymes induce the neuronal loss and brain injury complications such as anxiety, depression and cognitive impairments [39]. Therefore, we speculated that NAR has function as efficient anti-CI/R-induced-cognitive disorder by alleviating hippocampal inflammation and oxidative stress. Our experiments showed that NAR could attenuate the levels of IL-1β, TNF-α and IL-6, enhanced the activities of SOD and GSH-px, decreased the contents of MDA and 4-HNE, in the hippocampus of MCAO-induced CI/R rats. Therefore, the present work revealed that NAR improves the CI/R-induced cognitive dysfunction through inhibiting hippocampal inflammation and oxidative stress.

The present work further investigated the possible mechanism underlying for the protection of NAR against CI/R injury-induced cognitive dysfunction. Hippocampal BDNF/TrkB signaling is considered to play an important role in supporting and regulating learning and memory [40]; increasing studies have shown that activation of hippocampal BDNF/TrkB signaling improves cognitive impairment in a series of neuropathy [18,20,41]. Therefore, we speculated that hippocampal BDNF/TrkB signaling may be involved in NAR-mediated protection against CI/R injury-induced cognitive dysfunction. The present study evaluated the role of NAR on BDNF/ TrkB signaling in hippocampus of MCAO-induced CI/R rats. We revealed that NAR increased the expression levels of BDNF and p-TrkB in hippocampus of MCAO-induced CI/R rats, which suggested that the upregulation of hippocampal BDNF/TrkB signaling contributed to the beneficial effect of NAR on CI/R injury-induced cognitive dysfunction. It has been confirmed that lespedeza bicolor extract containing NAR improves amyloid $Beta_{25-35}$-induced memory impairments by up-regulating hippocampal BDNF in mice [42]. And, antidepressant and neuroprotective effects of NAR via increasing hippocampal BDNF/TrkB signaling in a rat model of chronic unpredictable mild stress [43,44]. These previous findings offer a reasonable explanation for the results obtained in the present study.

Conclusion

Taken together, the present study demonstrated that that treatment of MCAO-induced CI/R rats with NAR improves their cognitive function, as well as inhibits hippocampal oxidative stress and inflammation, up-regulates the hippocampal BDNF/TrkB signaling. These results indicated that NAR ameliorated cognitive dysfunction of CI/R rats via inhibiting oxidative stress, reducing inflammatory response, and up-regulating BDNF/TrkB signaling pathways in the hippocampus. We suggested that NAR may act as a potential preventive agent for CI/R injury-induced cognitive dysfunction after IS.

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Conflicts of interest

There are no conflicts of interest.

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