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Rehabilitation training improves cognitive disorder after cerebrovascular accident by improving BDNF Bcl-2 and Bax expressions in regulating the JMK pathway

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Abstract. – OBJECTIVE: To explore the effect of rehabilitation training on cognitive impairment after cerebrovascular accident and its potential mechanism.

PATIENTS AND METHODS: 100 patients of cerebrovascular accident treated in our hospital from August 2018 to August 2019 were selected as the subjects, and 50 patients with physical examination were selected as healthy control group. The patients with cerebrovascular accident were randomly divided into control group (50 patients) and research group (50 patients). The patients in the control group were given routine medication, the patients in research group were given rehabilitation training on the basis of routine drug therapy. The blood samples were collected on admission and 6 months after admission to detect the molecular markers related to inflammation, nerve cell nutrition and function and apoptosis in the serum. The cognitive function was evaluated by scales. We established a rat cerebral ischemia model, compared the differences in the evasive latency, serum CRP, BNDF, Bcl-2, BAX, Glu, NE levels and BNDF, TrkB, pTrkB, JNK levels in hippocampus, amygdala, and prefrontal tissue between model rats after rehabilitation training and model rats without rehabilitation training.

RESULTS: On admission, there were no significant differences in the scores of Barthel index (BI), Fugl-Meyer motor function scale (FM), Montreal cognitive assessment scale (MoCA) and mini-mental state examination (MMSE) ($p>0.05$). 6 months later, the above scores and BNDF, Bcl-2, and norepinephrine were significantly higher in the research group ($p<0.05$), while CRP, Bax, 5-HT and glutamate in the research group were significantly lower than those in the control group ($p<0.05$).

CONCLUSIONS: Rehabilitation training can improve the motor function, mental state and cognitive level of patients, reduce the levels of neurotoxic factors, pro-inflammatory factors and pro-apoptotic factors, and improve the levels of inhibiting apoptotic factors, neurotrophic factors and neurotransmitters. In animal experiments, rehabilitation training can increase BDNF and its activated receptors in hippocampus, amygdala and prefrontal lobe of rats, and decrease JNK of apoptotic protein, suggesting that rehabilitation training may regulate the expression of apoptotic proteins Bcl-2 and Bax by upregulating BDNF and its receptors and acting on JNK pathway, thereby inhibiting cell apoptosis and improving cognitive impairment after cerebrovascular accident.

Key Words:
Cerebrovascular accident, Rehabilitation training, Cognitive impairment, BDNF, BcIL-2, Bax.

Introduction

Cerebrovascular accident (CVA), also known as stroke, is a general term for cerebrovascular

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diseases caused by different inducers. It is a cerebrovascular accident caused by internal cerebral artery rupture, occlusion or stenosis. The former includes cerebral embolism, lacunar infarction and cerebral thrombosis, cerebral softening or focal ischemic necrosis due to hypoxic ischemia, and the latter includes subarachnoid hemorrhage and primary cerebral parenchymal hemorrhage. Cerebrovascular accident can lead to permanent brain dysfunction, such as movement disorders, language disorders, cognitive disorders, emotional disorders, etc. Among them, cognitive disorders mainly include executive ability, concentration and attention, logical reasoning ability, language expression ability, body coordination ability and other disorders, which can lead to a sharp decline in patients' life ability and quality of life. Therefore, the reconstruction of patients' cognitive ability is an important part of the treatment.

Early rehabilitation training is beneficial to establish synaptic connection between axons of nerve cells, strengthen collateral circulation, and promote functional reorganization and compensation of healthy lateral brain hemisphere[1]. Katz-Leurer et al[2] have shown that compared with rehabilitation training in 4-15 days after onset, more patients receiving early rehabilitation training within 72 hours after onset can act independently with shorter hospital stay and less rehabilitation treatment again. Therefore, the combination of early rehabilitation training and routine drug therapy can better cure the patients, so as to improve the quality of life.

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophic factor family. Its specific receptor is Tropomyosin receptor kinase B (TrkB). The combination of the two factors can provide nutritional support to nerve cells[3]. El-Tamawi et al[4] have shown that serum BDNF level is positively correlated with the recovery of cognitive level after cerebrovascular accident. Apoptosis refers to the active programmed cell death process initiated by cells under the regulation of genes following the stimulation of external signals[5]. In cerebrovascular accidents, nerve cells in the central part of severe ischemia are necrotic, and the peripheral parts of ischemia and the parts of the brain that are susceptible to ischemia may have apoptosis, such as neurons in the hippocampus and lobe[6]. Animal experiments showed that the cognitive function of rats was positively correlated with Bcl-2 level of anti-apoptotic factors in brain tissue, but negatively correlated with the Bax level of pro-apoptotic factors[7], which shows that apoptosis related protein is an important target to improving cognitive impairment after cerebrovascular accident.

At present, studies on the treatment of cognitive impairment by rehabilitation training after cerebrovascular accident mainly focuses on the use of scales and blood indicators to measure the effect of rehabilitation training and improve the content and form of rehabilitation training. However, there are few studies on the molecular mechanism of rehabilitation training to improve cognitive function. The clinical experiment part of this study compared the performance of the two groups of patients receiving routine drug therapy and rehabilitation on the basis of routine drug therapy in the psychological scale score and serum neurotrophic factor, inflammatory factor, apoptotic factor, neurotransmitter level. We speculated that rehabilitation training may regulate Bcl-2 and Bax levels of JNK pathway, inhibit neuronal apoptosis, reduce inflammatory response and the release of harmful neurotransmitters by upregulating the levels of BDNF and its activated receptors. Then, the cognitive impairment after cerebrovascular accident can be improved, and the molecular mechanism of rehabilitation training was further verified in animal experiments.

Patients and Methods

Treatment of Patients with Cerebrovascular Accidents

Patients

In this study, 100 patients with cerebrovascular accident treated in our hospital from August 2018 to August 2019 were selected as the subjects. 50 patients underwent physical examination were selected as healthy control group. Patients in the study met the diagnostic criteria issued by the 4th National Annual Conference on Cerebrovascular Diseases: 1. Patients who had a cerebrovascular accident for the first time; 2. Patients diagnosed as cerebrovascular accident by MRI or CT of head; 3. Patients with stable vital signs, conscious, educated in primary school and above, be able to receive psychological assessment; 4. Patients with cognitive impairment diagnosed by mini-mental state examination (MMSE). Exclusion criteria: 1. Patients with diseases that affected cognitive outcomes, including psychiatric symptoms, aphasia, loss of recognition, etc.; 2. Patients who did not

receive a full course of treatment; 3. Patients with cognitive-affecting diseases, such as Parkinson's disease, Alzheimer's disease, or a history of alcohol and drug abuse, or patients who have been found to have had cerebrovascular accidents.

Subjects in the control group met the following conditions: 1. MMSE score was in normal range, with no cognitive impairment; 2. No neurological abnormality after examination; 3. No family history of mental illness or mental illness, and no history of alcohol or drug dependence; 4. No cerebral vascular accident, brain trauma and other central nervous system history; 5. Volunteer to join the study and sign the informed consent.

All enrolled patients and subjects in control group signed written informed consent. The experimental method in this study was approved by the Ethics Committee of The Second Affiliated Hospital of Qiqhar Medical University.

Random number table method was used to divide 100 patients into research group (Re+RT) and control group (RT), with 50 people in each group. There was no significant difference in clinical data between research group and control group, as shown in Table I.

Treatment Methods

Patients in the study group and control group were treated with conventional drugs, including the maintenance of blood pressure and blood glucose stability, nutritional nerve, antioxidant free radicals, prevention of bedsores and infection. Patients with cerebral infarction were treated to improve blood circulation to the brain and anticoagulants. Patients with cerebral hemorrhage were treated to reduce intracranial pressure by dehydration. The content of rehabilitation nursing for patients in the two groups included: 1. Position the healthy limb; 2. Passive movement of joints; 3. Massage the muscles; 4. Change position every two hours; 5. Engage in early bed exercise; 6. Instruct the patient to sit up alone; 7. Train the patient to complete standing movement and maintain standing balance from the sitting position; 8. Train the patient to walk and go up and down stairs; 9. Cultivate the patient's daily life ability, including washing, dressing, eating and drinking, going to the toilet, etc. once a day for half an hour.

Patients with cerebral infarction and cerebral hemorrhage in research group were given rehabilitation treatment respectively within 72 h and 6 days after onset, mainly including ability training for patients with cognitive disorders in attention, thinking ability, memory ability, orientation ability, perceptual ability, etc. The treatments were conducted once a day for half an hour each time, and each course of treatment included 8 weeks. During the training, the therapist alone counseled the patient for training, and at other times, the companion or nurse counseled the patient for training. The main contents included:

1. Rehabilitation training for attention, such as selecting pictures and videos that the patient likes as the learning materials, letting the patient describe the content of the material after watching; Telling the patient about poetry or stories, asking the patient to retell after listening, and so on. The length and complexity of the content increased with the deepening of training.
2. Training for thinking ability, including sorting and solving practical problems, such as, encouraging the patient to decide on the type and quantity of daily meals, going to shops near the hospital to select and purchase items and pay, negotiating with salespeople, etc.
3. Rehabilitation training for memory, such as helping the patient identify colors, shapes and names with cards, building blocks, pictures, alarm clocks, schedules, diaries, etc., and remembering the time.

Table I. Comparison of clinical data between research group and control group.

Groups	Number of Subjects	Age	Sex (M)	Gender (F)	Infarction	Bleeding	MMSE
Research Group (Re+RT)	50	65.3 ± 7.4	32	18	27	23	20.56 ± 1.90
Control Group (RT)	50	66.5 ± 8.1	30	20	29	21	19.90 ± 1.89
Healthy Control Group	50	64.2 ± 9.6	26	24	--	--	28.49 ± 1.26
	$p^a > 0.05$ $p^b > 0.05$ $p^c > 0.05$	$p^a > 0.05$ $p^b > 0.05$ $p^c > 0.05$	$p^a > 0.05$ $p^b > 0.05$ $p^c > 0.05$	$p^a > 0.05$	$p^a > 0.05$	$p^a > 0.05$ $p^b > 0.05$ $p^c > 0.05$	

p^a: Comparison between research group and control group. p^b: Comparison between research group and health control group. p^c: Comparison between control group and healthy control group.

4. Rehabilitation training for orientation ability, such as requiring the patient to remember the route between ward and treatment room, reminding the patient of the time of daily life events, etc.
5. Rehabilitation training for perceptual ability, such as requiring the patient with misrecognition to remember the names of related characters by watching photos, such as therapists, doctors, family members, etc.; training the patient's ability to distinguish the sensation of touch, color, shape, etc.

Assessment of Psychometric Scales

Mini-mental state examination (MMSE) was used for the assessment of patients' cognitive function[8]. There were 30 questions with 1 point per question for a total of 30 points. The assessment of the degree of cognitive impairment is related to education, patients with a score of 20 or less for primary education and 24 or less for secondary education were classified as cognitively impaired. Barthel index (BI) was used to assess the patient's daily living standard[9]. Fugl-Meyer motor function scale (FM) was used to evaluate the motor function of patients[10], the more athletic, the higher the score. Montreal Cognitive Assessment Scale (MoCA) was used to assess patients' cognitive abilities[11], with a full score of 30 points, including visual space (5 points) and execution capability (5 points), language capability (4 points), attention (4 points), memory (4 points), location orientation ability (4 points) and time orientation ability (4 points), the more cognitive, the higher the score.

Detection of Peripheral Blood Indicators

5 ml of elbow vein blood was collected from each patients in the morning after fasting for 12 hours. The blood was transferred to a centrifuge tube for natural coagulation, and the serum was taken after 2 h and placed in a refrigerator at -80°C. Chemiluminescence immunoassay was used to test CRP in the serum, the kit used was purchased from Wuhan Mingde Biotechnology Co., Ltd., the instruments used were Getein 1600 automatic fluorescence immune quantitative analyzers. Double-antibody sandwich ELISA were used to test BDNF, Bcl-2 and Bax in the serum. The kits used were purchased from Shanghai Guangrui Biological Technology Co., Ltd., Shanghai Zhenke Biological Technology Co., Ltd., and Nanjing Haickel Biological Technology Co., Ltd., respectively. All the instruments used were HBS-1096C enzymatic analyzers. Enzyme-Linked Immunoassay (ELISA) was used to test the contents of glutamate, noradrenaline and 5-HT in the serum. The reagents used were purchased from Shanghai Ruiban Biotechnology Co., Ltd., Shanghai Gudo Biotechnology Co., Ltd., and Nanjing Semberga Biotechnology Co., Ltd., respectively. All the instruments used were HBS-1096C ELISA analyzers.

Animal Modelling and Experimentation

Rat Model of Local Cerebral Ischemia Prepared by Bolt Line Method

This experimental method has been submitted to the Ethics Committee of The Second Affiliated Hospital of Qiqihar Medical University, China for review and approval. Forty 4-week-old healthy male SPF-grade SD rats with body weighted 250-300 g were selected. They were operated with modeling surgery after 2 weeks of adaptive feeding, the Animal Approval Number was SYXK (black)2016-001. A dose of 3% pentobarbital sodium was injected into the model group and the rehabilitation group at a 1 m/kg dose. The fully anesthetized rats (no righting reflex, no contractive reflex at the end of acupuncture) were placed in supine position on the operating table protected by circulating hot water pad. The rat was cut from the middle of the neck, for the separation of the right internal carotid artery, external carotid and common carotid arteries. The external carotid, internal carotid and pterygomaxillary arteries of the rats were ligated. A small incision was made at the branches of the internal carotid artery and the common carotid artery in the rats. After heat treatment, 4-0 nylon filament was inserted into the head and ligated and sutured from the internal carotid artery to the middle cerebral artery. After surgery, the rats were placed in a constant temperature static chamber at 27°C to awake anesthesia, and the neurological symptoms of the rats were evaluated within 24h according to the Kuluz neurological defect scoring standard. If the time was 3 minutes, the modeling was successful[12]. In the sham surgery group, only the artery was isolated and not ligated to the rats. The rats in control group were reared normally without treatment. In order to prevent postoperative infection, each rat was intramuscularly injected with penicillin 400,000 units per day one week after surgery. During the modeling surgery, no

rats died in sham surgery group (10 rats). In the model group (10 rats), 1 rat died during surgery due to subarachnoid hemorrhage. Postoperative death occurred in 1 rat due to asphyxia. In rehabilitation group (10 rats), 1 rat died during surgery due to subarachnoid hemorrhage.

Rehabilitation Training of Rats

The rats in the control group, model group, rehabilitation group and sham surgery group were kept in ordinary cages to avoid animal fighting and injury. The rats in the rehabilitation group received screen grip training[13], rod rotation training[14], balance beam walking training[15], roller mesh trainer rotation training[16] for 1 hour in total, for 4 weeks.

Rat Morris Water Maze Test

Water Maze System (Beijing ZhongshiDichuang Technology Development Co., Ltd., with the model of ZS001, and Art. No. of 1056001) was used to test the cognitive ability of rats[17]. The device is a round vessel made of resin, with a diameter of 1.5 m, a height of 0.6 m, a water depth of 0.25 m and a water temperature of 22°C. Milk powder is added to the water to give it an opaque appearance. A glass platform with a diameter of 0.2 m should be placed in the center of any quadrant of the water maze, with the height of 5 cm lower below the surface.

Rats in the 4 groups were trained with Morris water maze on the 24th day after surgery. The rats were placed in a water maze with their backs to the platform. The time from getting into the water maze to find hidden underwater platform was recorded as escape latency. The next day, the rats were placed in a water maze in a clockwise quadrant on the first day, and the rest was done in the same manner. The platform for testing and record the evasive latency was removed on the 28th day.

Test of Serum indicators of Rats

On the 28th day, all the rats in 4 groups were anesthetized by injection at 1m/kg of 3% pentobarbital sodium. The fully anesthetized rats (no positive reflex, no contractile reflex at the tail) were placed on the operating table of circulating hot water pad in supine position. The abdominal cavity was opened, and the abdominal aorta was fully exposed for 5 ml. The serum was separated at room temperature for 2 h. The rats were then decapitated, and the hippocampus, amygdala and prefrontal lobe were separated and frozen with liquid nitrogen in the fridge at -80°C.

Enzyme Linked Immunosorbent Assay was used to test the levels of CRP, BDNF, Bcl-2 and Bax in the serum of rats. The kits used were purchased from Nanjing Camilo Bioengineering Co., Ltd. (Art. No. was R-KMLJr30258), Yuanmu Biotechnology Co., Ltd. (Art. No. was YM-S1745), Wuhan Huamei Bioengineering Co., Ltd. (Art. No. was CSB-E13604r), Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (Art. No. was DECO2425). Enzyme Linked Immunosorbent Assay was used to test glutamate, norepinephrine, and 5-HT in the serum of rats. The reagents were purchased from Shanghai Renjie Biotechnology Co., Ltd. (Art. No. RJ15294), Nanjing Senbega Biotechnology Co., Ltd. (Art. No. was SBJ-R0128), Nanjing Senbega Biotechnology Co., Ltd. (Art. No. SBJ-R0128). All the instruments used in the above experiments were HBS-1096C enzyme scale analyzers.

Western Blot of Rat Tissue Experiment

The total protein in hippocampus, amygdala and prefrontal lobe of rat samples was extracted by tissue protein extraction kit (purchased from Beijing Pulley Gene Technology Co., Ltd.). Quantitative analysis was carried out with a kit based on modified Coomassie brilliant blue method (purchased from Tiangen Biochemistry Technology Co., Ltd., Beijing, China).

50 μg of protein was separated by electrophoresis and transferred to polyethylene difluoride (PVDF) membrane. BDNF and Bcl-2 were purchased from Emmett Technology Limited, Art. No. ALO-ANT-010-0.2 and 3033-100; Bax primary antibody was purchased from Qingdao Jishikang Biotechnology Co., Ltd., Art. No. PA001732; JNK primary antibody was purchased from Emmett Technology Limited, Art. No. AS16-4011; TrkB primary antibody was purchased from Emmett Technology Limited, Art. No. was 3593-100; pTrkB primary antibody was purchased from Qingdao Jishikang Biotechnology Co., Ltd., Art. No. was PA001804. After sealing at room temperature for 2 h, target protein primary antibody (1:1000) and β-actin primary antibody (1:2000, purchased from APPLYGEN Company, Art. No. was C1845) which were incubated overnight at 4°C. After rinsing three times with TBST, they were added secondary antibody (1:5000, purchased from Abcam Company, Art. No. was ab54481) and incubated at room temperature for 2 hours. The excess antibodies were rinsed with TBST and then ECL color rendering kit was used to show the color. Image-J software

was used to analyze the gray scale of the scanned bands, and compared with the β-actin band gray, the ratio of the two was the result.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, USA) was used to analyze all the data of this experiment, *t*-test method was used to test the level of $\alpha=0.05$, and $p<0.05$ shows that the difference was statistically significant.

Results

Serum Indicators of Patients

The serum CRP, BNDF, BCL-2, Bax, 5-HT, glutamate and norepinephrine levels in control group and research group on admission were shown in Figure 1 ($p>0.05$). 6 month later, the serum levels of BNDF ($p=0.002$), Bcl-2 ($p=0.004$) and norepinephrine ($p=0.002$) in research group were significantly higher than those in control group, and the serum levels of CRP ($p=0.0003$), Bax ($p=0.001$), 5-HT ($p=0.006$) and glutamate ($p=0.0005$) were significantly lower than those in control group.

Assessment of Patient Mental Scale

As shown in Figure 2, on admission, there was no significant difference in the scores of Barthel index (BI), Fugl-Meyer motor function scale (FM), Montreal cognitive assessment scale (MoCA), mini-mental state examination (MMSE) between control group and research group ($p>0.05$). 6 month later, the scores of Barthel index (BI) ($p=0.001$), Fugl-Meyer motor function scale (FM) ($p=0.002$), Montreal cognitive assessment scale (MoCA) ($p=0.007$) and mini-mental state examination (MMSE) ($p=0.0002$) were significantly higher in the research group than those in the control group.

Rat Morris Water Maze Test

As shown in Figure 3, compared with control group and sham surgery group, the escape latency of model group was significantly prolonged ($p<0.05$) during the whole process of the test, indicating that cerebral ischemia seriously affected the learning and memory levels of rats. The escape latency of rehabilitation group was significantly lower than that in the model group ($p<0.05$), suggesting that rehabilitation training can significantly improve the learning and memory levels of cerebral ischemic rats.

Serum Indicators in Rats

As shown in Figure 4, four weeks after modeling surgery, serum CRP ($p=0.0006$), Bax ($p=0.006$), 5-HT ($p=0.03$), glutamate ($p=0.0002$) and NE ($p=0.0006$) in rehabilitation group were significantly lower than those in model group, and serum BDNF ($p=0.0006$) and BCL-2($p=0.02$) were significantly higher than those in the model group.

Western Blot of Hippocampus, Amygdala and Prefrontal Lobe of Rats

As shown in Figure 5, four weeks after modeling, JNK of prefrontal lobe ($p=0.009$), amygdala ($p=0.007$) and hippocampal ($p=0.0002$) in rehabilitation group were significantly lower than those in the model group. BDNF of hippocampus ($p=0.006$), amygdala ($p=0.006$), prefrontal lobe ($p=0.001$), and pTrkB/TrkB of hippocampus ($p=0.028$), amygdala ($p=0.002$), and prefrontal lobe ($p=0.0003$) were significantly higher than those in model group.

Discussion

In recent years, study has shown that after cerebrovascular accident, the brain still retains complete recombination ability and strong plasticity[18], which lays a theoretical foundation for patients with cerebrovascular accident to rebuild their living ability through rehabilitation training. Rehabilitation training has a positive effect on nerve cell regeneration and the reconstruction of nerve cell connection. It can strengthen the nutrition supply of the brain, accelerate the recovery of the damaged part of the lobe, and enhance the activity level and nervous system excitability of the cerebral lobe, to promote the reconstruction of brain function after cerebrovascular accident[19].

BDNF belongs to the NTFs family, which is a class of alkaline proteins synthesized by nerve cells with a molecular weight of 14kD, containing 119 amino acids[20]. BDNF is widely distributed in the peripheral and central nervous systems by binding specific receptors TrkB. They were induced to form dimeric and phosphorylated specific sites to initiate signal transduction pathways, regulate intracellular Ca^{2+}, and inhibit neuronal cell apoptosis. It can also promote the division and differentiation of neural stem cells and the growth of axons, improve synaptic plasticity, regulate neurotransmitter secretion, and antagonize neurotoxic substances. Thus, it can protect and promote

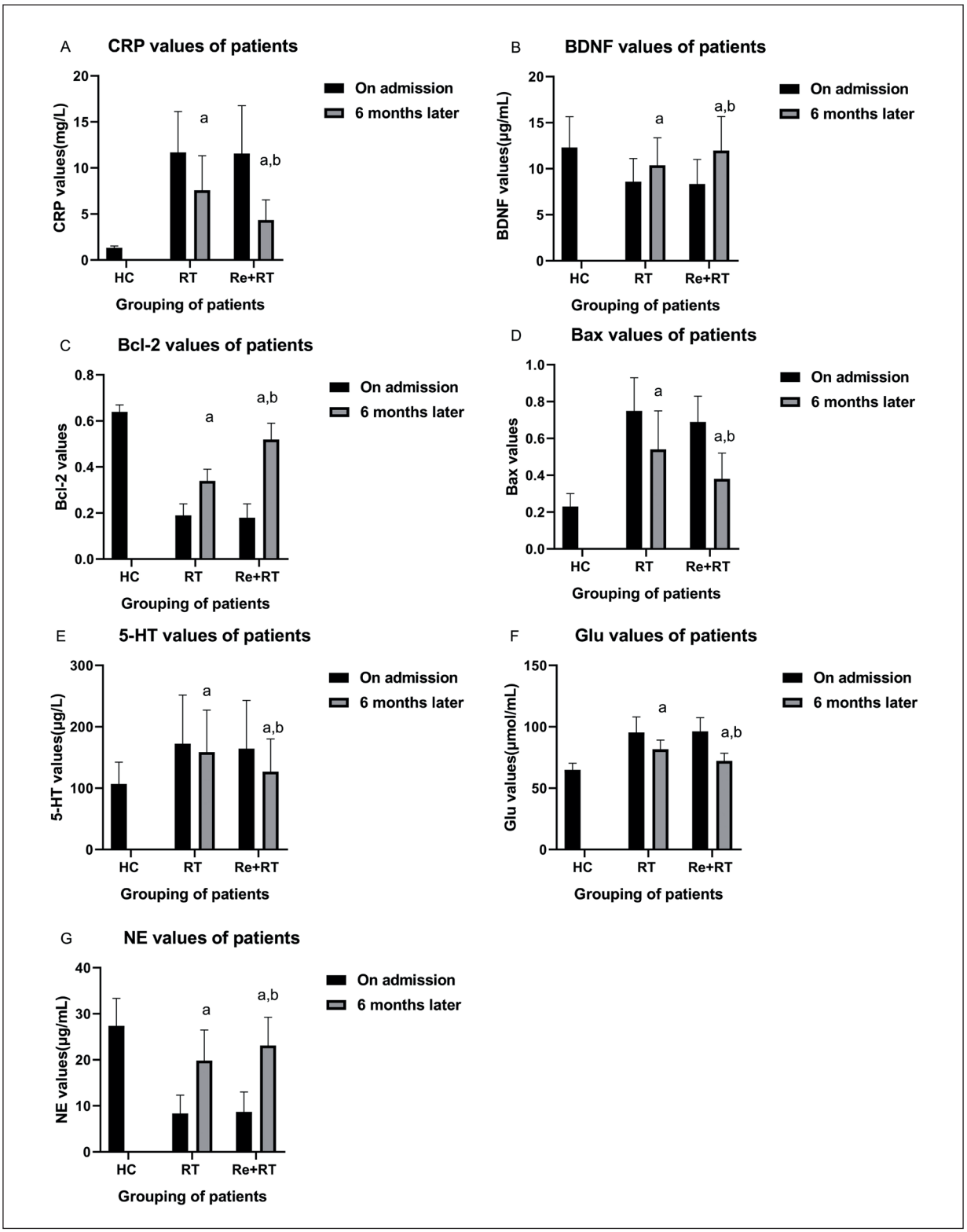

Figure 1. Serum indicators in research group and control group on admission and 6 month later. **A,** The difference was significant at 6 months compared with admission. **B,** CRP values of patients; (**B**) BDNF values of patients; (**C**) Bcl-2values of patients; (**D**) Bax values of patients; (**E**) 5- HT values of patients; (**F**) Glu values of patients; (**G**) NE values of patients.b: The difference between the study group and the control group was significant. RT: Control group (routine treatment). Re+RT: Research group (rehabilitation + routine treatment).

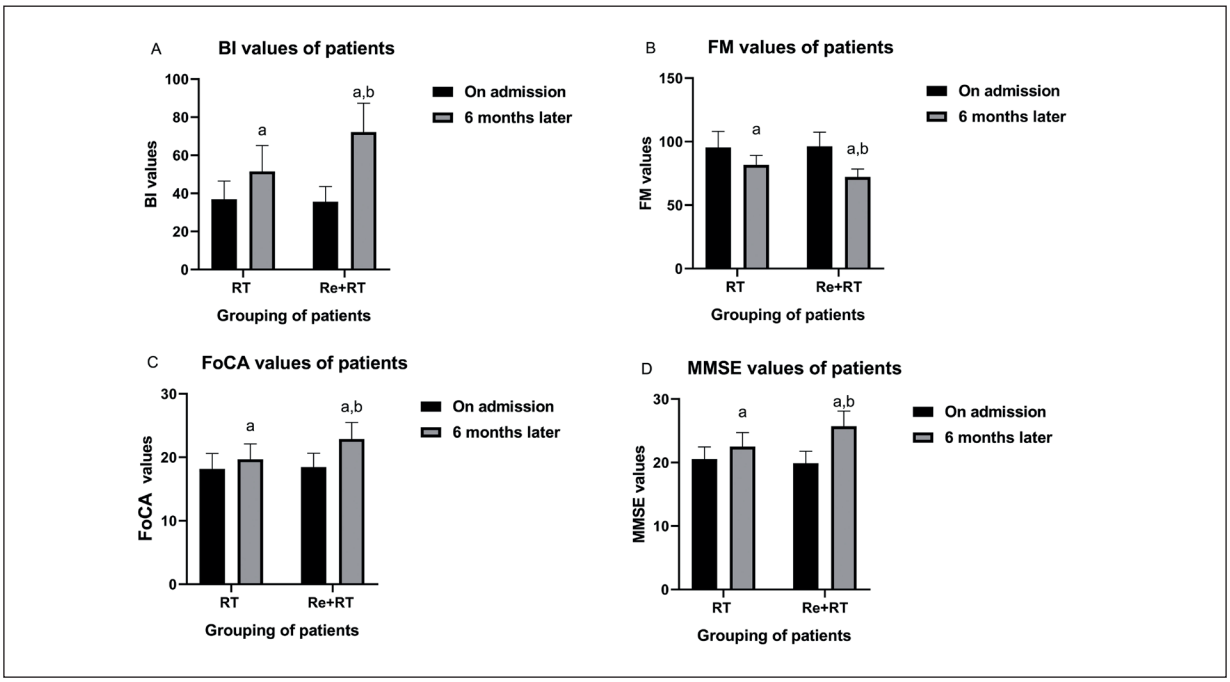

Figure 2. Score of psychological scale in research group and control group at admission and 6 month later. (**A**) BI values of patients; (**B**) FM values of patients; (**C**)FoCA values of patients; (**D**) MMSE values of patients. a: The difference was significant at 6 months compared with admission. b: The difference between research group and control group was significant. RT: Control group (routine treatment) Re+RT: Research group (rehabilitation + routine treatment).

the recovery of cerebral ischemia[21-23]. Studies[24,25] have shown that the expression of BDNF-TrkB in rats with intracranial hemorrhage was significantly increased after rehabilitation training. Exercise

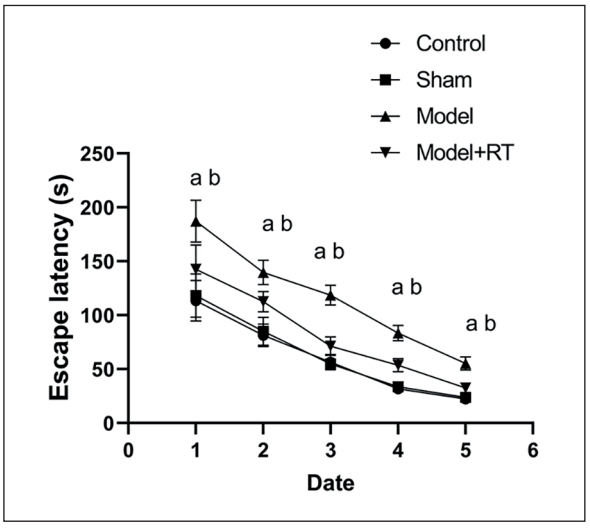

Figure 3. Rat escape latency in Morris water maze. **A,** The difference between model group and control group was significant. **B,** The difference between rehabilitation group and model group was significant.

rehabilitation therapy can alleviate cognitive impairment after brain injury by stimulating brain BDNF-TrkB signaling pathway. After treatment with salvianolate injection, the recovery of cognitive function in rats was accompanied by increased BDNF-TrkB expression and activation of pathways[26], indicating that BDNF-TrkB plays a crucial role in the recovery of the nervous system after stroke. In the clinical trials of this study, the serum BDNF level of research group and control group was significantly lower than that in healthy group, and the BDNF level of the patients after treatment was significantly improved, while research group was significantly higher than that in control group. At the same time, research group was significantly better than control group in terms of cognitive ability. Similar positive correlation between serum BDNF level and learning and memory ability was also found in animal experiments, which was consistent with the results of previous studies. It is generally believed that the hippocampus is related to learning and memory, which is mainly involved in the generation of new memory and the coding and extraction of recalled plot memory. Patients with hippocampus damaged can show anterograde amnesia, that is,

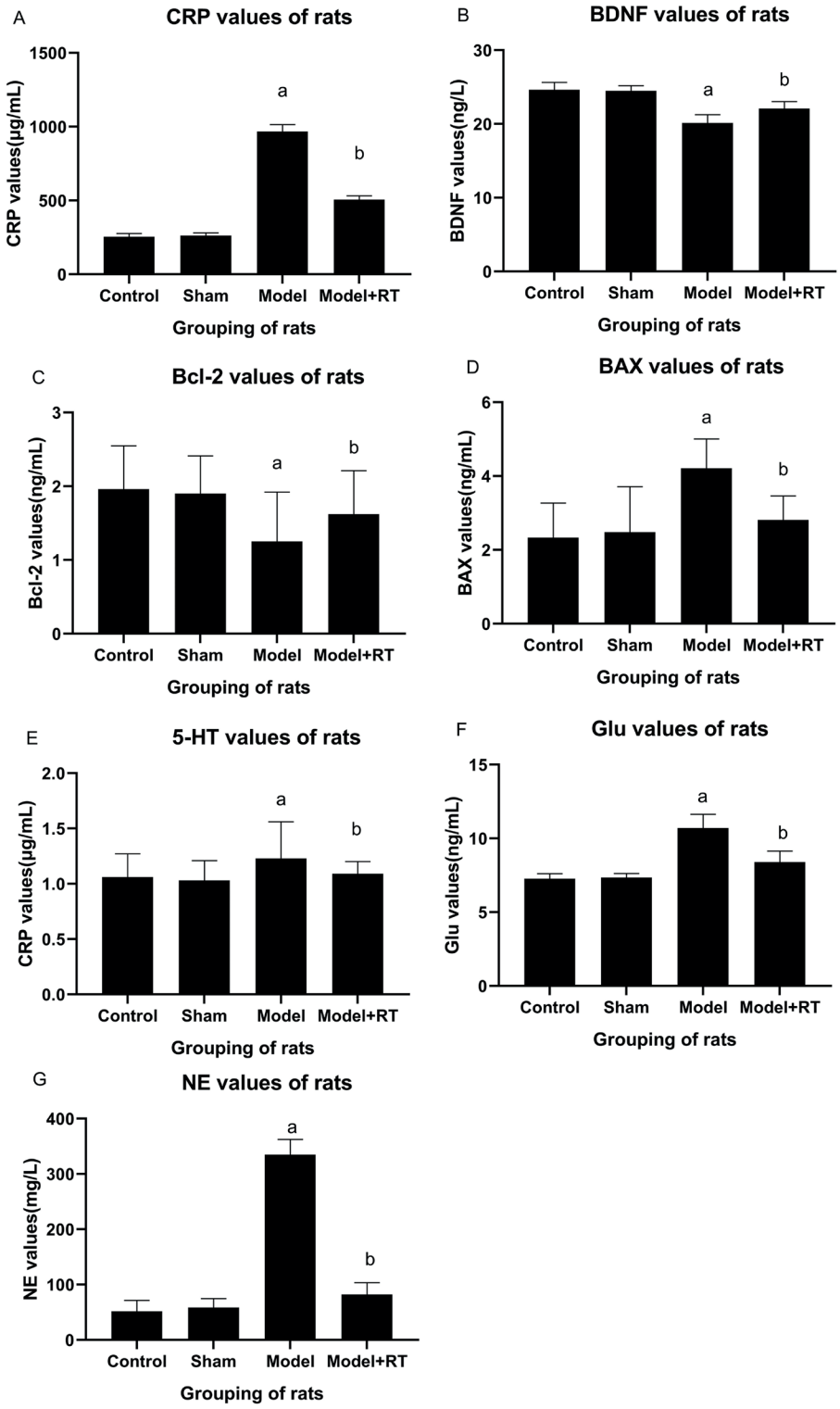

Figure 4. Serum indexes of rats. (**A**)CRP values of rats; (**B**) BDNF values of rats; (**C**)Bcl-2values of rats; (**D**)Bax values of rats; (**E**) 5- HT values of rats; (**F**) Glu values of rats; (**G**) NE values of rats.a: The difference was significant compared with control group. b: The difference between research group and control group was significant.

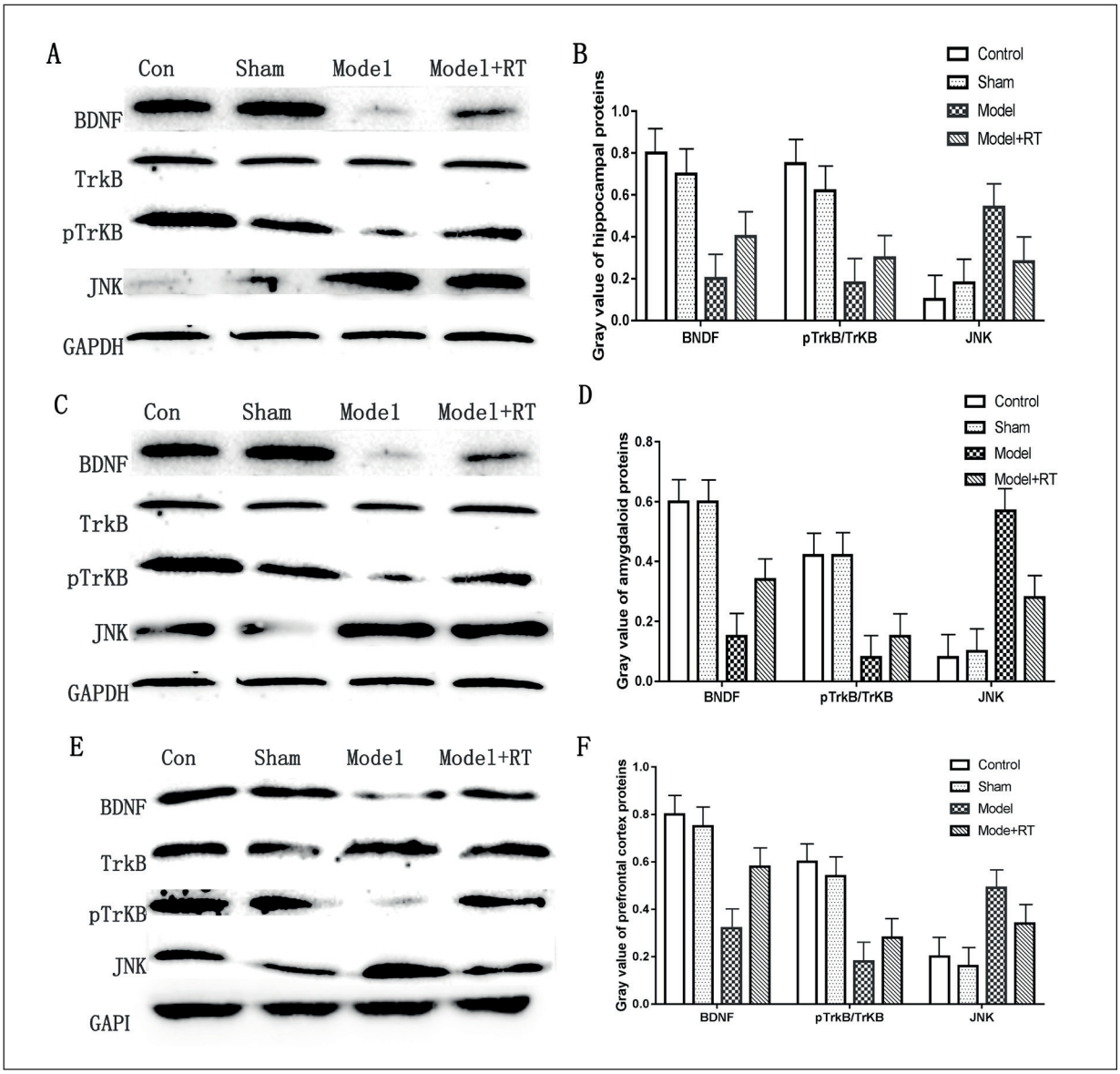

Figure 5. Western blot of rat tissues. **A,** Hippocampus tissue protein expression. **B,** Gray value statistics diagram of hippocampus tissue protein expression. **C,** Amygdala tissue protein expression. **D,** Gray value statistical diagram of amygdala tissue protein expression. **E,** Prefrontal cortex tissue protein expression. **F,**Gray value statistical diagram of prefrontal cortex tissue protein expression.

they cannot form new memory[27]. The amygdala is highly correlated with fear conditioned reflex, which plays an important role in the explicit memory of emotional events and can help identify emotional facial expressions and participate in the processing of sad expressions. So, it is of great significance in social life[28]. The physiological function of prefrontal lobe is mainly related to cognitive control, including the psychological ability of planning, controlling and managing data processing flow, which urges people to complete goal-oriented behavior. Patients with prefrontal injury usually have normal intellectual ability, but the ability to make goal-oriented behavior is impaired[29].

The hippocampus is a key brain area for the formation of spatial learning and memory. Synaptic plasticity is the biological basis of spatial learning and memory, and long-term potentiation (LTP) is the physiological basis of spatial learning and memory[30]. Patients with hippocampal damage can show anterograde amnesia, that is, unable to

form new memories[31]. Cerebral ischemia damage can lead to the loss of synapses of hippocampal neurons, abnormal calcium signal pathways, loss of function and cell death, which can cause obstacles to spatial learning and memory function[32]. A study has shown that in animal models of cerebral ischemia, hippocampal neurons undergo inflammatory and stress damage, the density of synapses is reduced, the morphology and structure are abnormal, and there are abnormal cell discharges in the hippocampal CA1 and CA3 regions, and LTP is inhibited. The spatial learning and memory functions of model animals are impaired[33]. Exercise can lead to increased synaptic density, increased calcium ion concentration and increased LTP, thereby improving spatial learning and memory capabilities[34].

The amygdala and hippocampus are interconnected by synapses, and the front part (Anterior BLA, aBLA) and posterior part (Posterior BLA, bBLA) can be projected to the ventral hippocampus[35]. The amygdala is involved in the formation of working memory, which may be due to difficulties and challenges that will increase the degree of response of the amygdala to goal-related stimuli and enhance the ability to adapt and solve difficult situations by projecting to the motor system and cognitive system[36]. The amygdala also plays an important role in the explicit memory of emotional events. In the process of coding contextual memory, the amygdala can cause subjects to pay more attention to the central details and enhance emotional memory[37]. It has been shown[38] that norepinephrine can regulate the amygdala activation enhancement effect in memory encoding, thereby regulating the processing of memory in the hippocampus, and enhancing the storage and consolidation of memory. This may be one of the molecular mechanisms of the interaction between emotion and memory.

The physiological functions of the prefrontal lobe are mainly related to cognitive control, including the mental ability to plan, control, and manage the flow of information processing, and promote people to complete goal-oriented behaviors and make decisions[39]. Prospective memory (PM) refers to the person's ability to remember what he intends to do in the future. It is related to making plans or setting goals in daily life. It is one of the most important parts of cognitive function. Damage to the prefrontal cortex can lead to reduced PM function, leading to obstacles in decision-making[40].

We found that the BDNF expression and pTrkB/TrkB of hippocampus, amygdala and prefrontal lobe in rehabilitation group were significantly higher than those in the model group, suggesting that rehabilitation training can improve the learning and memory ability, strengthen the fear reflex and emotional memory caused by water entry, enhance the target-oriented behavior and accelerate the recovery and reconstruction of nervous system function.

Apoptosis refers to the active programmed death process initiated by cells under gene regulation when stimulated by external signals[41]. Vzdenski[42] has shown that, the main mode of delayed neuronal cell death and penumbra cell death is apoptosis. Bcl-2 family plays an important role in apoptosis. Bcl-2 and Bax are inhibition factors and pro-apoptotic factors, respectively[43]. When the expression of Bcl-2is upregulated, heterodimers of Bcl-2and Bax are formed in the cells, which can inhibit cell apoptosis. When the expression level of Bax is upregulated, the dimer formed is Bax homodimer, which can promote cell apoptosis[44]. Therefore, the proportion of Bcl-2and Bax expression is generally maintained at a dynamic balance, to ensure the normal process of cell apoptosis. The change of the ratio of the two under pathological conditions may indicate the change of apoptosis state[45]. In clinical trials of this study, at 6 months after treatment, serum Bcl-2 and serum Bax in the study group were significantly higher than those in the control group, indicating that the apoptosis of the patients in the study group was inhibited. The scores of Barthel Index (BI), Fugl-Meyer Assessment Scale (FM), Montreal Cognitive Assessment Scale (MoCA) and mini-mental state examination (MMSE) in research group were significantly better than those in control group. There were similar results in animal experiments, i.e., the serum Bcl-2 in the rehabilitation group was significantly higher than that in model group, the serum Bax was significantly lower than that in model group, and rehabilitation group in the water maze test performance was significantly better than model group. It is speculated that rehabilitation training may reduce the level of apoptotic factors, inhibit the apoptosis of central nervous system cells, and promote the recovery and reconstruction of nervous system functions.

JNK is an important type of mammalian mitogen-activated protein kinase, its signal transduction pathway is closely related to cerebral ischemia-reperfusion injury[46]. Benakis et al[47]

have shown that, cerebral ischemia can lead to activation of JNK pathways, which plays an important role in apoptosis and inflammation. After JNK activation, the downstream signal molecule Bad (ser128) combines heterodimers of Bcl-2and Bax and replaces BCL-2, to form Bax homodimer, so that it can further promote apoptosis[48,49]. In this study, the hippocampus in the rehabilitation group, amygdala and prefrontal JNK were significantly lower than those in model group. BDNF expression and pTrkB/TrkB of corresponding tissues were significantly higher than those in model group. Serum Bcl-2in rehabilitation group was significantly higher than that in model group. Serum Bax was significantly lower than that in model group, indicating that rehabilitation training may improve the expression level of BDNF and its activated receptor, leading to the downregulation of JNK expression and the activity of its pathway. By increasing the ratio of BCL-2/Bax, it plays a role in inhibiting cell apoptosis, protecting the nervous system, and promoting the recovery and reconstruction of nervous system functions.

HT is an important neurotransmitter, 5-HT1A receptor mainly distributes in the axons and synapses of cells in the olfactory lobe, septum and hippocampus in the brain. After the receptor binding to 5-HT, the excitability of hippocampal 5-HT neurons is regulated, which affects learning and memory functions[50]. Ban et al[51] have shown that after brain injury by ischemia, the storage and uptake of 5-HT by nerve cells are inhibited and a large amount of 5-HT is released into the peripheral blood. In this work, the serum 5- HT level in research group was significantly lower than that in the control group, while the scores of Barthel index (BI), Fugl-Meyer motor function scale (FM), Montreal cognitive assessment scale (MoCA) and mini-mental state examination (MMSE) were significantly better than those in control group. In animal experiments, the serum 5-HT level of rats in rehabilitation training model group was significantly lower than that in model group, and the learning and memory levels were significantly higher than rats in model group. It suggested that rehabilitation training may improve the cognitive function of rats by repairing nerve cells and promoting new synapse formation.

Glu is an important excitatory neurotransmitter in the brain, which can affect cognitive function by activating specific N-methyl-D-aspartic acid (NMDA) receptors widely distributed in amygdala, striatum, hippocampus, cerebral lobe and other regions[52]. During cerebral ischemia, Glu is released in large quantities and its reuptake is inhibited, resulting in excessive excitability and necrosis of nerve cells[53]. As a result, reducing the Glu level effectively can protect neurons[54]. In this report, serum Glu levels of patients in the research group were significantly lower than those in control group. The scores of Barthel index (BI), Fugl-Meyer motor function scale (FM), Montreal cognitive assessment scale (MoCA) and mini-mental state examination (MMSE) were significantly better than those in the control group. In animal experiments, serum Glu levels of rats in the rehabilitation group were significantly lower than those in model group, and its performance in water maze test was significantly better than those in model group, suggesting that rehabilitation training can effectively reduce serum Glu levels, protect nerve cells, and promote the recovery of nervous system structure and function.

NE is widely distributed in the central nervous system. A good amount of NE can enhance cognitive function, but excessive NE has a negative impact on cognitive function[55]. Studies[56,57] have shown that ischemia and hypoxia can lead to a large number of rapid releases of hippocampal neurons NE, which may play a key role in regulating neuronal apoptosis. In this research, the serum NE levels in research group were significantly lower than those in control group, while the scores of Barthel index (BI), fugl-meyer motor (FM) function scale, Montreal Cognitive Assessment Scale (MoCA), and Mini-Mental State Examination (MMSE) were significantly better than those in control group. In animal experiments, the serum NE level of rats in rehabilitation training model group was significantly lower than that in model group, while the cognitive function reflected in the escape latency of water maze was significantly better than that in model group. It suggested that rehabilitation training can effectively reduce serum NE level and accelerate the recovery of cognitive function.

Conclusions

To sum up, rehabilitation training can effectively improve cognitive impairment in patients with cerebrovascular accidents. Its molecular mechanism may regulate the expression of JNK and the activity of its pathway, increase the ratio of Bcl-2/Bax, inhibit cell apoptosis, and reduce the levels of 5-HT, Glu and NE in serum by improving the

expression and activation of BDNF and TrkB in nerve cells for the protection and remodeling of the functions of the nervous system.

The novelty of this research lies in: we clinically confirmed that rehabilitation training can effectively improve the cognitive impairment of patients with cerebrovascular accidents and used animal modeling experiments to reveal possible molecular biological mechanisms from the level of signal pathways and metabolites, providing reference for the future development of targeted drugs and therapies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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