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Research on changes in cognitive function, β-amyloid peptide and neurotrophic factor in stroke patients

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Abstract. – OBJECTIVE: To investigate the changes as well as the related mechanism in cognitive function and levels of serum β-amyloid peptide (Aβ) and brain-derived neurotrophic factor (BDNF) in stroke patients.

PATIENTS AND METHODS: A total of 30 patients with acute stroke treated in our hospital from June 2015 to September 2016 were selected as stroke group, while 30 volunteers during the same period were enrolled as control group. Changes in cognitive function of patients were evaluated using the Montreal Cognitive Assessment (MoCA) and mini-mental state examination (MMSE) before and after the treatment. At the same time, the concentrations of serum $A\beta_{1-40}$ and BDNF were detected, and their correlations with the MMSE score were analyzed. Finally, levels of serum cyclic adenosine monophosphate (cAMP) and phosphorylated-cAMP-response element binding protein (p-CREB), and the phosphorylation level of Tau protein were detected by Western blotting.

RESULTS: MoCA and MMSE scores of patients in stroke group were significantly lower than those in control group ($p < 0.01$), and the scores were significantly higher in stroke patients after treatment than those before treatment ($p < 0.01$). Compared with those in control group, the serum $A\beta_{1-40}$ concentration in patients in stroke group was significantly increased ($p < 0.01$), but the BDNF level was significantly decreased ($p < 0.01$). Compared with those before treatment, the serum $A\beta_{1-40}$ concentration in patients was significantly decreased after treatment ($p < 0.01$), but the BDNF concentration was significantly increased ($p < 0.01$). Correlation analysis showed that the MMSE score was negatively correlated with the concentration of $A\beta_{1-40}$ ($r^2 = 0.764$, $p < 0.01$), but positively related to the level of BDNF ($r^2 = 0.827$, $p < 0.01$). Compared with those in control group, the content of serum cAMP and p-CREB in stroke patients was significantly decreased ($p < 0.01$), but the expression of p-Tau was statistically increased ($p < 0.01$).

CONCLUSIONS: The cognitive function in stroke patients is impaired, with the rising content of serum $A\beta_{1-40}$ and reduction of BDNF, the mechanism of which is related to the decrease of cAMP and p-CREB and the increase of p-Tau. This provides a theoretical basis for searching the new therapeutic targets and new drugs for stroke.

Key Words:
Stroke, Cognitive function, β-amyloid peptide, Brain-derived neurotrophic factor.

Introduction

Stroke is a kind of disease of neurological function damage due to local cerebral blood circulation disorder. It is characterized by high incidence, mortality and recurrence rates, seriously affecting the life quality of patients and bringing huge burdens to patients' family and country[1]. Ischemic stroke is the most important type of stroke, in which the energy metabolism disorder, oxidative stress response, and inflammatory response caused by ischemia lead to neuronal apoptosis, and damage the cognitive function of patients[2]. β-amyloid peptide (Aβ) is an important protein causing Alzheimer's disease (AD), and its deposition in the brain tissues can form senile plaques, and trigger a series of pathophysiological reactions, leading to the increase in neuronal apoptosis level[3]. In recent years, a large number of studies have shown that $A\beta_{1-40}$ is highly expressed

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in ischemic tissues of the rat model of stroke, suggesting that the neuronal apoptosis in stroke is related to the aggregation of $A\beta_{1-40}$[4,5]. According to the Tau protein hypothesis, the increased level of phosphorylated (p)-Tau in brain tissues is a key factor leading to the Aβ aggregation[6]. Michalski et al[7] showed that the Aβ content in the blood of AD patients is also significantly increased, providing a basis for the diagnosis and differential diagnosis of AD. Brain-derived neurotrophic factor (BDNF) has an effect of promoting the survival of nerve cells, and it can significantly increase the synaptic plasticity of nerve cells[8]. Rahman et al[9] reported that the BDNF protein expression in brain tissues of AD patients is significantly decreased, thereby reducing the expressions of synapse-associated proteins. BDNF is an important downstream factor of cyclic adenosine monophosphate (cAMP) and cAMP-response element binding protein (CREB), and the cAMP/CREB/BDNF signaling pathway is involved in the regulation of BDNF expression[10]. This study aims to analyze the changes in cognitive function, serum $A\beta_{1-40}$ and BDNF in patients with acute stroke before and after treatment, analyze their correlations, and investigate the possible mechanism.

Patients and Methods

Patients

A total of 30 patients with acute stroke treated in our hospital from June 2015 to September 2016 and meeting the criteria in this experiment were designated as stroke group. Inclusion criteria are as follows: (1) patient with the first-onset stroke, and diagnosed with acute stroke by the director of department based on the guidelines for the diagnosis of stroke via magnetic resonance imaging (MRI) or computed tomography (CT), (2) patient who had no serious visual and hearing disorder, and could complete the assessment of various scales, (3) patients without severe motor dysfunction, and (4) patients without serious systemic complications (malignant tumor, hepatic and renal dysfunction, blood system diseases, etc.). Exclusion criteria are as follows: (1) subjects who quitted midway, (2) subjects receiving emergency thrombolysis or interventional therapy, (3) patients who were unable to complete the experiment due to the deterioration of disease, (4) subjects suffering from Alzheimer's disease. In this experiment, 30 healthy volunteers during the same period were selected as control group. Venous blood was drawn from all acute stroke patients before and after admission, which was used for molecular biology experiments. All the patients have signed the informed consent, and this experimental protocol was approved by the Ethics Committee of our hospital.

Evaluation of Cognitive Function of Patients

Changes in cognitive function of patients were evaluated using the Montreal Cognitive Assessment (MoCA) and mini-mental state examination (MMSE), and the cognitive function of patients was determined by the same trained physician in a quiet and undisturbed environment. There are a total of 30 points in the MoCA scale, including the executive function, language, memory, visual structure function, calculation and orientation, abstract thinking, attention and concentration. The score $\geq$ 26 points indicates the normal cognitive function in patients, while that < 26 points indicates the cognitive impairment. There are 30 items and a total of 30 points in the MMSE scale, including the language skill, immediate memory, short-term memory, and time and place orientation. The score $\geq$ 27 points indicates the normal cognitive function in patients, while that < 27 points indicates the cognitive impairment.

Detection of Serum $A\beta_{1-40}$, cAMP and BDNF Concentrations

The concentrations of serum $A\beta_{1-40}$, cAMP and BDNF were detected via enzyme-linked immunosorbent assay (ELISA) in strict accordance with the instructions of the ELISA kit (Wuhan Boster Biological Technology Co., Ltd., Wuhan, Hubei, China). The venous blood was drawn from patients, and centrifuged at 4000× g and 4°C for 10 min. Then the serum was taken for ELISA. After the standard material was prepared, it was added together with the sample into the low-density lipoprotein-coated ELISA plate. The plate was sealed with the membrane, followed by incubation at 37°C for 90 min. After the liquid was poured, the plate was patted dry, and the biotin-labeled human $A\beta_{1-40}$ or cAMP or BDNF antibody was added. Next, the plate was sealed and incubated at 37°C for 60 min. After the liquid was poured, the plate was patted dry, washed with the washing liquid for 3 times, and added with the avidin-peroxidase complex. The plate was sealed with the sealing membrane, followed by reaction at 37°C for 30 min. Next, the developing solution was added for reaction for 30 min, and the

stop buffer was used. The optical density (OD) value of sample in each group was detected at a wavelength of 450 nm using a microplate reader (Omega Bio-tek, Norcross, GA, USA). According to the standard curve, the protein concentration of sample in each sample was calculated.

Western Blotting

After the peripheral serum was obtained from patients in each group, radioimmunoprecipitation assay (RIPA) lysis solution (Beyotime Biotechnology, Beijing, China) was added at a volume ratio of 1:1, and the serum was disrupted using an ultrasonic cell disruptor and centrifuged at 12000× g and 4°C for 10 min. After the total protein in the supernatant was quantified using the bicinchoninic acid (BCA) protein assay kit Peprotech (Rocky Hill, NJ, USA), the loading system in an equal concentration was prepared. After the 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared, 20 μL samples were added into each well, followed by electrophoresis at 80 V until all samples reached the bottom of the gel. The protein was transferred onto a polyvinylidene difluoride (PVDF) membrane (IPVH00010, Millipore, Billerica, MA, USA) under the constant current of 260 mA. After 90 min, the membrane was blocked with 5% skim milk powder prepared by Tris-buffered saline with Tween-20 (TBST) for 2 h, and the target band was cut and incubated with CREB, p-CREB, Tau, p-Tau, BDNF and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primary antibodies (Cell Signaling Technology, Danvers, MA, USA, diluted at 1:1000) at 4°C overnight. Then, the band was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Beyotime Biotechnology, Shanghai, China) at room temperature for 1 h, and washed with TBST for 3 times. Enhanced chemiluminescence (ECL) solution was added for image development using a developing machine, and quantitative analysis was performed using the Image J 6.0 software. The relative expression level of each protein was calculated using p-CREB, p-Tau/Tau and BDNF/GAPDH, respectively.

Statistical Analysis

In this study, data were presented as mean ± standard deviation, and analyzed using Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA). x^2-test was used for enumeration data, analysis of variance was used for the comparison among groups, and Pearson analysis was used for the correlation among factors. $p < 0.05$ suggested that the difference was statistically significant.

Results

General Data

General data of patients in each group were collected after admission. General data of patients in stroke group and subjects in control group showed that there were no statistically significant differences in gender, age, years of education and body mass index (BMI) between stroke group and control group ($p > 0.05$) (Table I).

Changes in Cognitive Function

The cognitive function of patients in each group was evaluated before and after treatment, and healthy volunteers during the same period were taken as controls. Results showed that MoCA and MMSE scores of patients in stroke group before and after treatment were significantly lower than those in control group ($p < 0.01$). In addition, the scores were significantly higher in patients after treatment than those before treatment ($p < 0.01$) (Figure 1).

Changes in Serum $A\beta_{1\text{-}40}$ and BDNF Concentrations

The concentrations of serum $A\beta_{1\text{-}40}$ and BDNF were measured by using the ELISA kit. Results showed that compared with those in control group, the serum $A\beta_{1\text{-}40}$ content in patients in

Table I. General data of patients in each group ($\bar{x} \pm s$).

Group	Gender (male/female)	Age (years old)	Years of education (years)	BMI (kg/m^2)
Stroke group	(17/13)	65.9 ± 8.7	6.8 ± 4.5	24.6 ± 1.3
Control group	(16/14)	66.2 ± 4.1	6.3 ± 5.1	24.2 ± 0.7
p	> 0.05	> 0.05	> 0.05	> 0.05
t	0.668	0.882	0.725	0.763

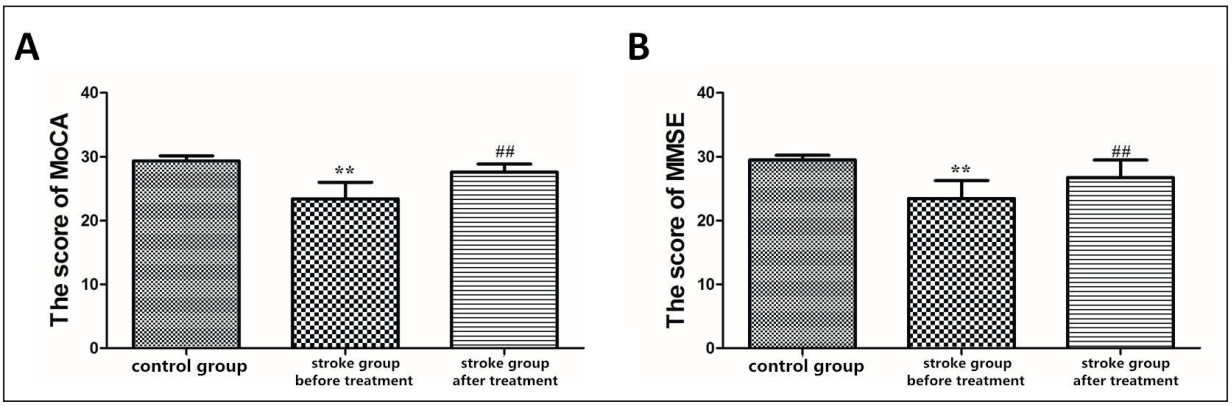

Figure 1. Evaluation of cognitive function, A) MoCA score, B) MMSE score. MoCA and MMSE scores of patients in stroke group before and after treatment were significantly lower than those in control group. Scores were significantly higher in patients after treatment than those before treatment. $^{**}p < 0.01$ *vs.* control group, $^{\#\#}p < 0.01$ *vs.* before treatment.

stroke group was significantly elevated ($p < 0.01$), but the BDNF level was significantly reduced ($p < 0.01$). Compared with those before treatment, the serum $A\beta_{1\text{-}40}$ concentration in patients was significantly decreased after treatment ($p < 0.01$), but the BDNF concentration was significantly up regulated ($p < 0.01$) (Figure 2).

Correlation Analyses of MMSE Score of Patients With Serum $A\beta_{1\text{-}40}$ and BDNF Concentrations

The correlations between MMSE score in stroke patients and levels of serum $A\beta_{1\text{-}40}$ and BDNF were studied. Results revealed that the MMSE score was negatively related to the concentration of $A\beta_{1\text{-}40}$ ($r^2 = 0.764$, $p < 0.01$), but positively correlated with the expression of BDNF ($r^2 = 0.827$, $p < 0.01$) (Figure 3).

Changes in Serum cAMP Concentration

The concentration of serum cAMP in patients was detected using the ELISA kit. We found that compared with that in control group, the content of serum cAMP in stroke patients was significantly decreased ($p < 0.01$), and the serum cAMP concentration in patients after treatment was significantly higher than that before treatment ($p < 0.01$) (Figure 4).

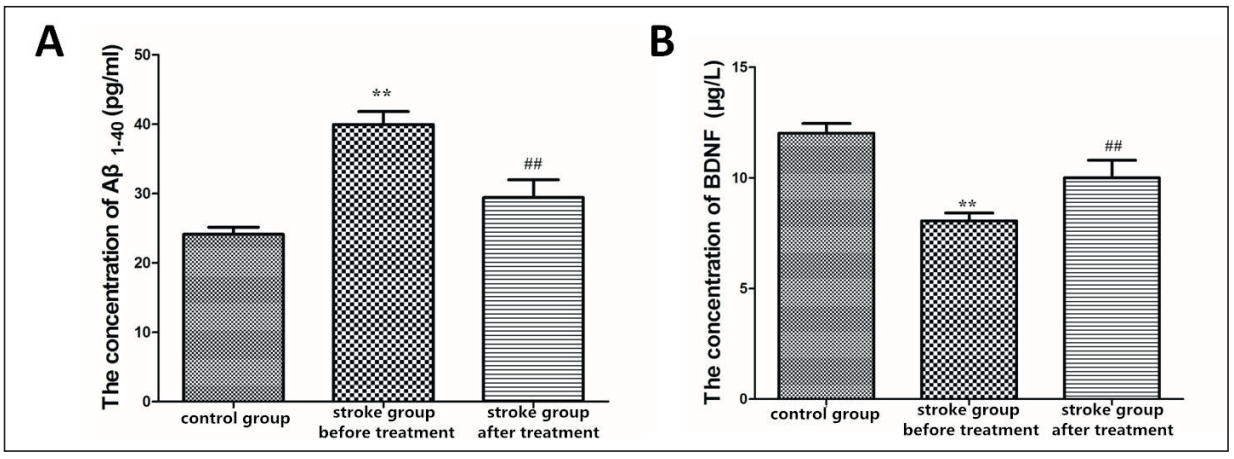

Figure 2. Concentrations of serum $A\beta_{1\text{-}40}$ and BDNF, A) the concentration of serum $A\beta_{1\text{-}40}$, B) the concentration of BDNF. The serum $A\beta_{1\text{-}40}$ concentration in stroke patients was significantly higher than that in control group, but the BDNF level was significantly lower than that in control group. The serum $A\beta_{1\text{-}40}$ content in stroke patients after treatment was significantly lower than that before treatment, but the BDNF expression was significantly higher than that before treatment. $^{**}p < 0.01$ *vs.* control group, $^{\#\#}p < 0.01$ *vs.* before treatment.

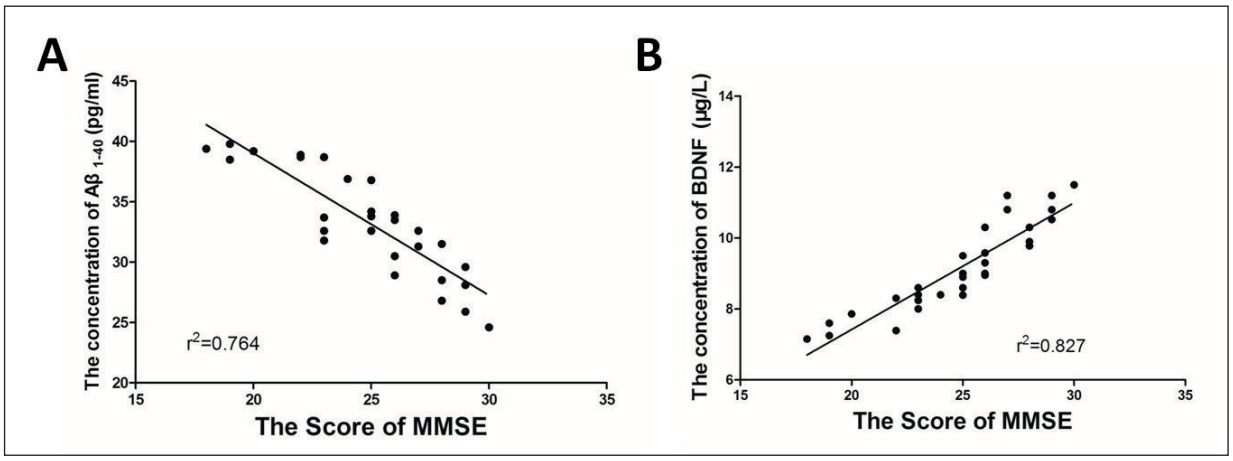

Figure 3. Correlations of MMSE score with concentrations of serum $A\beta_{1-40}$ and BDNF. The MMSE score was negatively correlated with the concentration of $A\beta_{1-40}$, but positively correlated with the concentration of BDNF ($p < 0.01$).

Changes in Relevant Proteins in Serum

The result of Western blotting indicated that compared with those in control group, the expressions of p-CREB and BDNF in serum of stroke patients were significantly decreased ($p < 0.01$), but the p-Tau level was statistically increased ($p < 0.01$). Moreover, after treatment the expressions of p-CREB and BDNF in serum of patients were higher than those before treatment ($p < 0.01$), but the p-Tau expression was significantly lower than that before treatment ($p < 0.01$) (Figure 5).

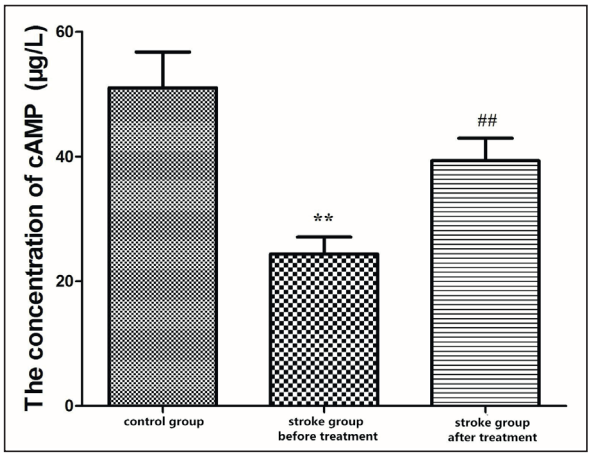

Figure 4. Serum cAMP concentration. The content of serum cAMP in stroke patients was significantly lower than that in control group, and the serum cAMP concentration in patients after treatment was significantly higher than that before treatment. $^{**}p < 0.01$ *vs.* control group, $^{\#\#}p < 0.01$ *vs.* before treatment.

Discussion

The clinical manifestations of ischemic stroke, as a frequently-occurring acute cerebrovascular disease in clinic, mainly present as distortion of commissure, hemiplegia, unsmooth speech, obnubilation, sudden weakness on one side of the face, hand or leg, and even sudden fainting and unconsciousness[11]. Cerebrovascular embolism is the leading cause of ischemic stroke, which results in insufficient oxygen supply in brain tissues, brain injury and neuronal apoptosis[12]. At present, the primary treatment strategy for ischemic stroke in clinic is to restore the blood perfusion as soon as possible, making brain tissues regain blood supply and oxygen supply. Samai et al[13] found that stroke patients tend to be accompanied with a certain degree of cognitive impairment, whose possible mechanism is closely related to the neuronal apoptosis and neuroinflammation. Wang et al[14] proposed that ischemia-reperfusion might lead to further aggravation of ischemic brain tissue injury, and deterioration of clinical symptoms. Aβ is a kind of protein closely related to the onset of AD, and previous finding showed that the aggregation of Aβ in the brain is closely related to its metabolic disturbance[15]. Acute stroke leads to microcirculation and energy metabolism disorders by producing a large number of oxygen free radicals and affects the substance metabolism in the brain[16]. Stroke promotes a large amount of $A\beta_{1-40}$ deposit in cerebral vessels, and induces amyloidosis in cerebral vessels. In this study, it was found that

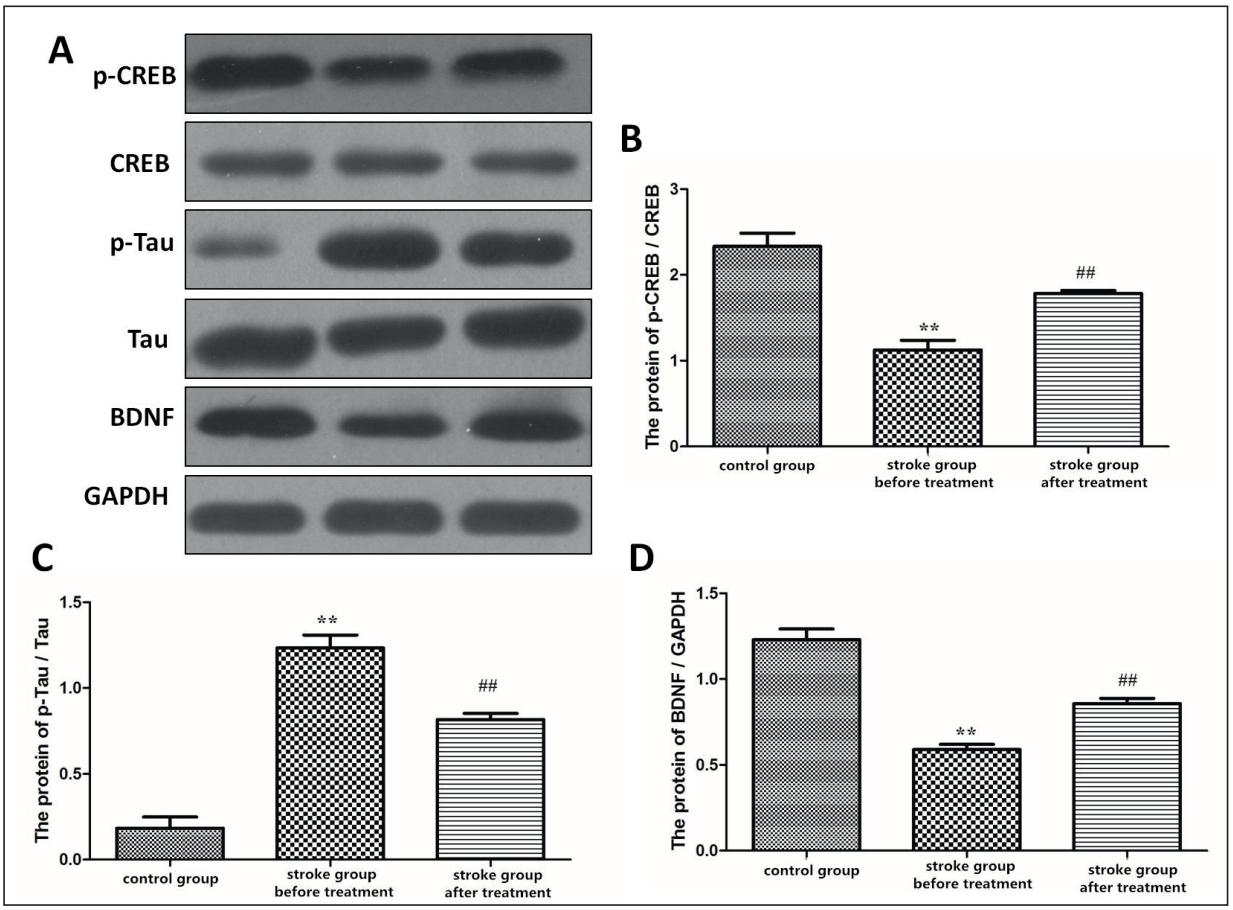

Figure 5. Changes in relevant proteins, ***A)*** protein bands, B) relative expression level of p-CREB, ***C)*** relative expression level of p-Tau protein, ***D)*** relative expression level of BDNF protein. The expressions of p-CREB and BDNF in serum of stroke patients were significantly lower than those in control group, but the p-Tau expression was significantly higher than that in control group. After treatment, the expressions of p-CREB and BDNF in serum of patients were significantly higher than those before treatment, but the p-Tau expression was significantly lower than that before treatment. $^{**}p < 0.01$ *vs.* control group, $^{\#\#}p < 0.01$ *vs.* before treatment.

the level of serum $A\beta_{1\text{-}40}$ in stroke patients was significantly higher than that in healthy people. However, the concentration of serum $A\beta_{1\text{-}40}$ was significantly decreased after healing, and the content of $A\beta_{1\text{-}40}$ was positively correlated with the cognitive impairment in stroke patients. Wen et al[17] validated that the deposition of $A\beta_{1\text{-}40}$ in cerebral vessels further stimulated the secretion of interleukin-6 (IL-6), IL-1, tumor necrosis factor-α (TNF-α) and other pro-inflammatory factors from cerebrovascular endothelial cells, resulting in neuroinflammation. Aggregation of $A\beta_{1\text{-}40}$ causes vascular smooth muscle damage and lumen occlusion, seriously affects the prognosis of patients with stroke[18]. In this study, it was also found that the p-Tau protein expression in peripheral blood of stroke patients was significantly increased, but the level was decreased after treatment. Tau protein is an important microtubule-associated protein in the central nervous system. In neurons and neuroglial cells, hyperphosphorylation and aggregation of Tau protein favor the formation of paired helical filaments (PHFs), which participates in Aβ aggregation. The above results suggest that Aβ deposition in stroke patients is closely related to the high expression of p-Tau protein. It was found in this study that the content of BDNF in peripheral blood of stroke patients was decreased significantly. The expressions of both cAMP and p-CREB were also significantly decreased, and the content of BDNF was negatively correlated with cognitive impairment in patients. CREB is a transcriptional regulatory protein and widely exists in eukaryotic cells. p-CREB can bind to the CRE binding site in

cell deoxyribonucleic acid (DNA) to regulate the transcription of related target genes. BDNF is one of the target genes for transcriptional regulation of CREB[19]. Stroke significantly reduces the cAMP content in the brain, thereby inhibits CREB phosphorylation, reduces BDNF expression in the brain, and inhibits cAMP/CREB/BDNF pathway. Zhang et al[20] demonstrated that the suppression of p-CREB impeded the expression of anti-apoptotic factor B-cell lymphoma 2 (Bcl-2) and promoted the expression of apoptotic factor, thus leading to the increased neuronal apoptosis, which may also be considered as the crucial reason for the cognitive impairment in stroke patients. Recent evidence illustrated that indicators, such as CXCL16, contributed to the development of stroke subtypes[21], which offers the leads for the further validation and combination of serum $A\beta_{1\text{-}40}$ and BDNF, as well as alternative factors in the clinical practice of diagnosis and treatment.

Conclusions

We found that the cognitive impairment in stroke patients is positively correlated with the serum $A\beta_{1\text{-}40}$ content but negatively correlated with the BDNF content, which involved the promotion of p-Tau protein expression and the inhibition of cAMP/CREB/BDNF pathway. This finding may offer fundamental understanding for further therapy of stroke.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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