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Original Article

Effects of butylphthalide combined with folic acid and vitamin B on factors and cognitive function of patients with ischemic stroke

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Abstract: *Objective*: To explore the effect of butylphthalide combined with folic acid and B vitamins on NSE, S100 protein, homocysteine, inflammatory factors and cognitive function in patients with ischemic stroke. *Methods*: 132 patients with ischemic stroke admitted to department of neurology from February 2017 to October 2019 were divided into an observation group and a control group according to random principles. All patients were treated to stabilize blood pressure, blood lipids, blood sugar, along with nutritional nerve treatment. Patients were treated with folic acid and vitamin B6 in control group and butylphthalide soft capsules, folic acid and vitamin B6 in observation group. After 1-month treatment, venous blood was collected to detect the levels of NSE, S100 protein, Hcy, IL-6, C-reactive protein (CRP), and TNF-α. The evaluation scale (MoCA) evaluated the patients' cognitive function. *Results*: No significant differences regarding sex, age, BMI, previous medical history (hypertension, diabetes, hyperlipidemia), smoking history, alcohol abuse history, residence, infarct location and infarct size were found between two groups. Compared with control group, observation group had significantly higher treatment effective rate ($P<0.05$). After treatment, the levels of NSE, S100 protein, Hcy, IL-6, CRP and TNF-α were significantly decreased and the MoCA score was improved in two groups ($P<0.05$) with more significant changes in observation group ($P<0.05$). *Conclusion*: Butylphthalide combined with folic acid and B vitamins has a significant treatment effect on patients with ischemic stroke and can improve patients' cognitive function.

Keywords: Butylphthalide, ischemic stroke, cognitive function

Introduction

Ischemic stroke is one of the most common cerebrovascular diseases that threaten human physical and mental health in modern society. This disease is usually caused by a blood clot that blocks or plugs a blood vessel in the brain, which stops blood from flowing to the brain. Within minutes, brain cells begin to die. Another cause is stenosis, or narrowing of the artery [1]. If circulation isn't restored quickly, brain damage can be permanent. With the development of society and changes in people's living habits and working conditions, ischemic stroke shows a high incidence and disability rate. It is currently characterized by high morbility, mortality, and recurrence rate. Although, the incidence of ischemic stroke increases with age, in recent years the disease has shown a trend of rejuvenation [2]. Ischemic stroke accounts for 75-80% of strokes and is one of the most important cerebrovascular diseases. If the treatment is delayed, it may easily cause sequelae such as cognitive impairment, limb dysfunction, and even death [3, 4]. The brain tissue of patients with ischemic stroke is in the state of ischemia and hypoxia, which will produce a large number of inflammatory factors and free radicals. It is clinically significant to treat strokes as quickly as possible. Blood thinners may be used to stop a stroke while it is happening by quickly dissolving the blood clot. Post-stroke rehabilitation can help people overcome disabilities caused by stroke damage [5]. The purpose of the treatment of ischemic stroke is to reduce the cerebral ischemia and hypoxia effectively and reduce brain tissue damage [6].

Butylphthalide is a racemic n-butylphthalide, which is self-synthesized domestically and has the same composition as natural L-celery A.

Experiments have shown that it can effectively block the pathological process of brain damage caused by cerebral ischemia [7]. Homocysteine (Hcy) is considered to be an independent risk factor for cerebrovascular disease [8]. The folic acid and B vitamins are the main drugs to improve Hcy level [8]. In this study, butylph thalide combined with folic acid and B vitamins was used to treat patients with ischemic stroke to analyze and explore the changes of neurological function, inflammatory factors and cognitive function.

Materials and methods

Patients

This study was approved by the medical ethics committee of our hospital, and 132 patients with ischemic stroke admitted to the department of neurology from February 2017 to October 2019 were randomly divided into an observation group and a control group according to random principles. There were 67 patients in the observation group, including 41 males and 26 females, aged 45-72 years, with an average age of 56.4±9.3 years. The cerebral ischemic sites were: 31 cases of basal ganglia, 17 frontal lobes, 11 parietal lobes, and 8 cases of temporal lobes. There were a total of 65 patients in the control group, including 39 males and 26 females, aged 44-73 years, with an average of 55.9±10.3 years old. The cerebral ischemic sites were: basal ganglia in 32 cases, frontal lobe in 15 cases, parietal lobe in 12 cases and temporal lobe in 6 cases. Inclusion criteria: ① according to the diagnostic criteria of Chinese Acute Ischemic Stroke Diagnosis and Treatment 2010 [9]; ② the first occurrence of ischemic stroke; ③ admission 24 hours after onset; ④ the necessary imaging and blood test results of head CT or MRI, electroencephalogram; ⑤ complete case statistics. Exclusion criteria: ① suffering from mental illness; ② not the first incidence; ③ accompanying diseases of important organs such as heart, liver or kidney; ④ history of severe brain trauma; ⑤ history of cerebral diseases such as cerebral hemorrhage; ⑥ pregnancy or breastfeeding women; ⑦ in recent years have participated in other relevant clinical trials or those who cannot cooperate with the treatment. All enrolled patients signed an informed consent form for medical clinical trials on a voluntary basis.

Grouping and treatment

Both groups of patients were given routine medical treatments to stabilize blood pressure, blood lipid, and blood sugar, along with nutritional nerve treatment, such as methylcobalamin tablets [Eisai (China) Pharmaceutical Co., Ltd., specification: 0.5 mg, production batch number: National Pharmaceutical Standard Word H20030812, 3 times a day, 0.5 mg each time], the control group was given folic acid tablets at the same time except the basic treatment (Tianjin Lisheng Pharmaceutical Co., Ltd., specification: 5 mg, production batch number: Guoyao Zhunzi H12020215, 3 times a day, 5 mg each time), vitamin B6 (Tianhua Traditional Chinese Medicine Co., Ltd., specification: 10 mg, production batch number: National Pharmaceutical Standard H42020613, 3 times a day, 10 mg each time) treatment. The observation group was given styrene at the same time of basic treatment of Phthalophthalate soft capsules (Stone Pharmaceutical Group Enbi Pharmaceutical Co., Ltd., specifications: 0.1 g, production batch number: National Pharmaceutical Standard H20050299, 3 times a day, 0.2 g each time), folic acid, vitamin B6 treatment. Knowledge Assessment Scale (MoCA) was used to assess the patients' cognitive function.

Detection of factors via enzyme-linked immunosorbent assay (ELISA)

Aseptic venous blood (8 ml) was collected and further centrifuged at 3,000 g and 4°C for 10 min. The serum was then separated and placed in a refrigerator at -80°C. The standard solution was prepared to make the standard curve, and the standard curve was drawn using CurveExpert 1.4 software for sample quantification. The 96-well plate coated with the corresponding antibodies was taken, added with the standard solution or sample solution, and affixed with the sealing membrane, followed by incubation at 37°C for 90 min. After the liquid in the plate was patted dry, the biotin-labeled antibodies [anti-NSE, S100, Hcy, interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α)] were added, and the plate

Table 1. Clinical data of patients (mean ± SD)

Factors	Observation group (n=67)	Control group (n=65)	t/χ^2 value	*P* value
Sex (male/female)	48/19	45/10	1.726	0.1889
Age (years)	56.4±9.3	55.9±10.3	0.2929	0.7701
BMI (kg/m^2)	24.2±1.9	23.8±2.1	1.148	0.253
Previous medical history				
Hypertension	32	31	6.275e-5	0.9937
Diabetes	23	23	0.0162	0.8987
Hyperlipidemia	12	11	0.0224	0.8811
Smoking	25	19	0.9699	0.3247
Alcohol abuse	18	13	0.8655	0.3522
Residence Urban	52	49	0.0911	0.7628
Infarct location			0.588	0.9991
Frontal lobe	13	12		
Temporal lobe	10	9		
Parietal lobe	8	10		
Occipital lobe	7	7		
Basal ganglion	9	8		
Thalamus	8	7		
Cerebellum	7	8		
Brainstem	5	4		
Infarct size			0.138	0.9333
Lacunar infarction	37	36		
Medium infarction	19	17		
Massive infarction	11	12		

Table 2. Comparison of the total efficiency of the two groups

Group	n	Basic recovery	Significant progress	Progress	Ineffective	Total effective rate (%)
Control	65	9	14	30	12	81.5%
Observation	67	16	19	27	5	92.5%
χ^2	-	-	-	-	-	84.52
P						0.031

was sealed with the sealing membrane, followed by incubation at 37°C for 60 min. After the plate was washed with washing solution for 4 times (3 min each time), ABC working solution was added, and the plate was sealed, followed by incubation at 37°C for 30 min. After the plate was washed again with washing solution for 4 times, tetramethyl benzidine developing solution was added, and the plate was sealed, followed by incubation in a dark place at 37°C for 20 min. Then TMB stop buffer was added and mixed evenly. The absorbance value at 450 nm was detected using a microplate reader, and substituted into the standard curve to calculate the concentrations of NSE, S100 protein, Hcy, IL-6, CRP and TNF-α in blood in each group.

Statistical analysis

SPSS 22.0 statistical software was applied to analyze data. The measurement data were expressed by mean ± standard deviation (SD) and compared by two independent sample t tests. The count data were expressed as a percentage and assessed by chi-square test. P<0.05 indicates a statistically significant difference.

Results

General data of the two groups

The clinical data from observation and control groups were collected and compared. The results showed no significant difference in sex, age, BMI, previous medical history (hypertension, diabetes, hyperlipidemia), smoking history, alcohol abuse history, residence, infarct location and infarct size (P>0.05) (**Table 1**).

Comparison of the treatment effectiveness

After 1-month treatment, compared with control group, the total effective rate in the observation group was significantly higher (P<0.05) (**Table 2**).

Comparison of NSE and S100 protein levels before and after treatment

After 1-month treatment, the NSE and S100 protein levels in the two groups of patients were decreased significantly compared with those at the time of admission. The decrease was more significant in the observation group and the differences were statistically significant (P<0.05) (**Tables 3** and **4**).

Table 3. Comparison of NSE levels between the two groups before and after treatment

Group	NSE (μg/L)		t	P
	admission	After treat		
Control	20.1±4.3	15.4±2.8	2.543	0.023
Observation	20.1±4.3	10.2±3.1	2.634	0.026
t	2.972	2.851	-	-
P	0.014	0.019	-	-

Table 4. Comparison of S100 protein levels between the two groups before and after treatment

Group	S100 (μg/L)		t	P
	admission	After treat		
Control	0.87±0.23	0.61±0.16	2.943	0.010
Observation	0.89±4.3	0.43±0.13	2.523	0.021
t	2.824	2.651	-	-
P	0.012	0.026	-	-

Table 5. Comparison of Hcy levels before and after treatment between the two groups

Group	Hcy (μmmol/L)		t	P
	Admission	After treat		
Control	17.6±3.3	14.3±2.5	2.343	0.026
Observation	17.5±3.1	10.4±2.3	2.727	0.014
t	2.512	2.924	-	-
P	0.026	0.011	-	-

Comparison of Hcy levels before and after treatment

After 1-month treatment, compared with the time of admission, the Hcy level of the two groups of patients was decreased significantly and the level was decreased more significantly in the observation group (P<0.05) (**Table 5**).

Comparison of IL-6, CRP and TNF-α levels before and after treatment

After 1-month treatment, compared with admission, the levels of IL-6 (**Figure 1**), CRP (**Figure 2**), and TNF-α (**Figure 3**) in the two groups were significantly reduced and the observation group showed more significantly reduced levels (P<0.05).

Comparison of MoCA scores before and after treatment

After 1-month treatment, the MoCA score in the two groups of patients was significantly higher than that of admission and the observation group had more significantly increased score (P<0.05) (**Table 6**).

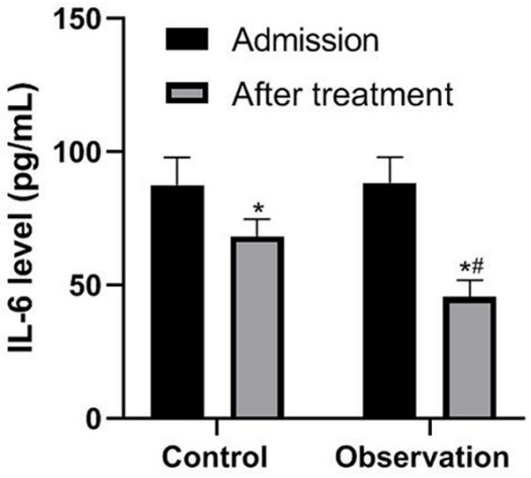

Figure 1. IL-6 level. Compared with admission, *P<0.05; compared with control group after treatment, #P<0.05.

Discussion

With the continuous progress of China's aging society, cerebrovascular disease ranks the first in the cause of death, and ischemic stroke accounts for more than 3/4 of all cerebrovascular diseases. The probability of recurrence of patients with ischemic stroke after treatment is significantly increased, accompanied by various sequelae, with a high disability rate, which seriously affects the quality of life of patients [10]. The pathological process of ischemic stroke is complicated and many factors are involved. In addition, the disease has an acute onset and progresses rapidly. At present, there is no specific treatment [11]. Thrombolytic therapy is currently a better treatment approach for ischemic stroke, but there are still contraindications for thrombolysis and treatment time window [12].

As a class of new drugs independently developed by China for the treatment of cerebrovascular diseases, butylphthalide is easy to pass the blood-brain barrier and has direct and effective effects. It has a long therapeutic effect and a small risk of bleeding. In cases where thrombolytic therapy is contraindicated or poor, it can to a great option to improve the patient's condition. Therefore, it was considered to be the first choice for the treatment of ischemic stroke. Butylphthalide has anti-plate-

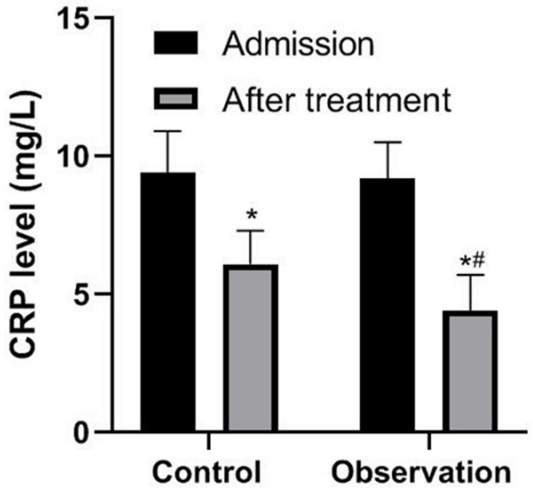

Figure 2. CRP level. Compared with admission, *P<0.05; compared with control group after treatment, #P<0.05.

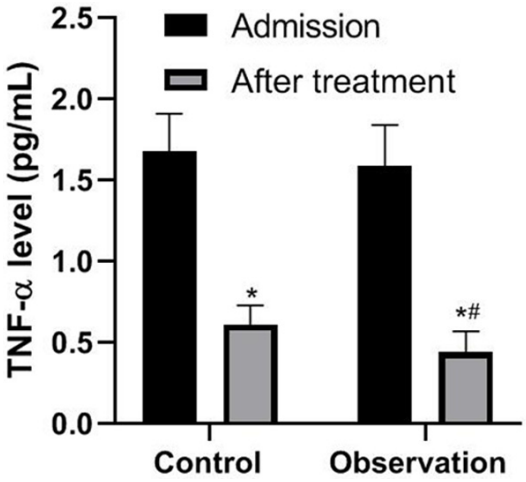

Figure 3. TNF-level. Compared with admission, *P<0.05; compared with control group after treatment, #P<0.05.

let aggregation effect, prevents thrombosis, blocks the cascade of nerve cell apoptosis by inhibiting the expression of cysteine-aspartic protease, and plays a role in inhibiting nerve cell apoptosis and protecting the ischemic brain tissue [7, 13]. This effect has been confirmed by a number of animal experiments [14, 15]. NSE is a kind of enolase dimer isoenzyme. When ischemic stroke occurs, the nerve cells are destroyed, and the NSE that originally existed inside the nerve cells is released in large quantities, causing the level of NSE in the peripheral blood to rise significantly, therefore, the detection of peripheral blood NSE level can

Table 6. Comparison of MoCA scores between the two groups before and after treatment

Group	MoCA score		t	P
	Admission	After treat		
Control	11.3±3.2	16.1±3.0	2.617	0.017
Observation	11.2±3.5	18.3±3.3	2.821	0.020
t	2.527	2.354	-	-
P	0.021	0.025	-	-

be a reliable indicator to assess the degree of brain injury and judge the treatment effect [16, 17]. S-100β protein is an acidic calcium-binding protein that is widely involved in gene expression, cell proliferation, differentiation, and apoptosis. After the occurrence of ischemic stroke, the brain tissue is damaged and the blood-brain barrier is destroyed. The S-100β protein penetrates the blood-brain barrier into the peripheral blood in large amounts, so this protein might be an indicator of glial cell damage [18, 19]. The occurrence of ischemic stroke will cause ischemia and hypoxia in the damaged area of the brain and its surroundings, which in turn will produce an inflammatory cascade reaction and free radicals. During the inflammatory reaction, IL-6, CRP, TNF-α, etc., the main product of the inflammatory response, are indicators of the intensity of the inflammatory response and the therapeutic effect [20]. Hcy is a sulfur-containing amino acid that is prone to oxidation during methionine metabolism. It can produce active superoxidized substances, interfere with lipid anabolism, damage vascular endothelial cells, trigger the body's oxidative response, and impair neuronal cell function [8]. The MoCA scale is developed with reference to the cognitive items and scores of the MMSE scale. It is an evaluation tool for rapid screening of cognitive dysfunction and is widely used in the evaluation of neurocognitive functions [21].

In our study, we showed that after 1-month treatment, compared with the control group, the total effectiveness rate is significantly higher in observation group. Compared with the time of admission, NSE, S100 protein, Hcy, IL-6, CRP and TNF-α levels in the two groups were significantly decreased and the MoCA score was significantly improved. Compared with control group, the changes of the above indexes in the observation group were more significant after 1-month treatment. The above results are

consistent with those of previous studies [22-25]. However, there is still limitation that the clinical effect of combined therapy including butylphthalide, folic acid and B vitamins required validation in a great number of patients, along with the safety assessment. Also, the duration of follow-up after treatment needs to be extended.

In summary, butylphthalide combined with folic acid and B vitamins has a significant treatment effect in patients with ischemic stroke and can improve patients' cognitive function.

Disclosure of conflict of interest

None.

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