

Original Article

Effects of butylphthalide combined with Naofukang on cognitive function and the expression of serum neurotrophic factor in patients with vascular dementia

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Abstract: Objective: This study aimed to determine the effect of butylphthalide combined with Naofukang on cognitive function and serum neurotrophic factor level in patients with vascular dementia. Methods: A total of 172 patients with vascular dementia were randomly enrolled and equally divided into an observation group and a control group. Patients in the control group received Naofukang orally besides routine treatment, while the observation group was treated with Butylphthalide Soft Capsules in addition to Naofukang for 12 weeks. Simple Mental State Examination (MMSE) and Clinical Dementia Degree Scale (CDR) were used to assess cognitive function. Activity of Daily Life Scale (ADL) and Barthel Index were used to evaluate the self-care ability of patients. Adverse drug reactions were recorded while serum neurotrophic factor (BDNF) level was detected by ELISA. Results: The therapeutic effect of the observation group (86.05%) was significantly higher than that of the control group (70.93%) ($P < 0.05$). MMSE and CDR scores showed no significant difference between two groups before treatment ($P > 0.05$). After treatment, the MMSE score in observation group was significantly increased and the CDR score was statistically reduced compared to those in the control group ($P < 0.05$). In addition, MMSE scores were significantly elevated and CDR scores were markedly decreased after treatment ($P < 0.05$). Butylphthalide combined with Naofukang significantly improved the Barthel index score, whereas it markedly decreased the ADL score compared to single use of Naofukang ($P < 0.05$). After treatment, serum BDNF level was significantly increased in both groups and it was further up regulated in the observation group compared to control group ($P < 0.05$). Adverse reaction rates showed no significant difference between the two groups ($P > 0.05$). Conclusion: Butylphthalide combined with Naofukang can improve cognitive function and patients' self-care ability, which maybe implicated to the changes in BDNF levels.

Keywords: Butylphthalide, Naofukang, vascular dementia, cognitive function, serum neurotrophic factor

Introduction

Vascular dementia (VaD) is caused by brain damage owing to cerebral hypoperfusion and lesions such as multiple stroke or chronic cerebral ischemia [1-3]. Cognitive dysfunction is an important clinical symptom of VaD patients. The patients frequently present with dysfunction of language, memory, orientation, behavior and so on, which seriously affects cognitive function, social activities and quality of life. The disease even causes great psychological pressure and economic burden to patients and their families [4]. VaD is one of the impor-

tant types of Alzheimer's related diseases. In recent years, as the aging population increases, the prevalence of VaD is rising rapidly. The incidence of VaD in males is clearly higher than that in females [5]. At present, the treatment of VaD mostly depends on drug intervention, but the clinical effects vary in multiple types of medicine. Therefore, how to effectively improve the cognitive impairment and patient's quality life remains to be settled [6].

Butylphthalide is a new kind of lipid-soluble drug, which can directly exert anti-cerebral ischemia functions and protect neurons against

injury through the blood-brain barrier [7, 8]. Therefore, in 2002, butylphthalide was officially approved by China's Food and Drug Administration as a new drug for the treatment of acute ischemic stroke. Many animal experiments and pharmacodynamic studies have confirmed that butylphthalide has protective effects on nerve cells, and its structure as well as clinical safety has been determined [9, 10]. Naofukang, also known as piracetam, is a cyclic derivative of alpha-aminolevulinic acid. It has the function of activating, protecting and repairing neurons and can be used for the treatment of memory impairment and cognitive dysfunction caused by cerebrovascular diseases, brain trauma and various toxic encephalopathy [11, 12]. This study explored the therapeutic effect of Butylphthalide combined with Naofukang on VaD and analyzed their effects on brain-derived neurotrophic factor (BDNF), which is expected to provide ideas and evidence for the clinical treatment of VaD.

Materials and methods

General information

A total of 172 patients with VaD from June 2017 to June 2018 were enrolled. The diagnosis was performed according to the diagnostic criteria of VaD based on American Psychiatric Association's Diagnostic and Statistical Manual for Psychiatry (DSMIV) 5th edition.

Inclusion criteria [13]: 1) patients diagnosed with ischemic stroke previously by brain CT or/ or MRI; 2) patients with dementia occurring within 3 months after stroke and lasting for more than 6 months; 3) Hachonski ischemic score ≥ 7 ; 4) MMSE score was between 12-23 points. Exclusion criteria: 1) patients with severe heart, liver, kidney dysfunction or hematological diseases, malignant tumors and cachexia; 2) patients with other nervous system diseases or psychiatric diseases, depression, etc.; 3) patients with dementia before stroke; 4) patients who have incomplete clinical data, or took drugs which affected cognitive function recently, or stopped treatment due to intolerance or severe adverse reactions of drugs in this study. The study was approved by the Ethics Committee of Zhoushan Second People's Hospital. All participants were aware of the contents of this study and signed the informed consent. The 172 patients were randomly and

equally assigned into a control group and an observation group by a double blind method.

Therapeutic methods

Patients from both groups received basic treatment such as routine control of blood pressure, blood sugar, blood lipids, anticoagulation, regulation of cerebral circulation and brain metabolism. The control group was given Naofukang (piracetam tablet, Tianjin Jinshi Pharmaceutical Co., Ltd., the Chinese medicine standard H12020667, specifications: 0.4 g*100 tablets), orally 0.8 g/3 times/d, along with the routine treatment. The observation group received Butylphthalide Soft Capsules (Enbipu, produced by Enbipu Pharmaceutical Co., Ltd. of Stone Pharmaceutical Group, the Chinese medicine standard H20050299, specifications: 0.1 g*24 capsules) orally 0.2 g/3 times/d for 12 weeks.

Observation Indicators

Cognitive function was assessed by MMSE and CDR: Among them, the MMSE scale includes 30 questions with a total score of 30. If the score is lower than 23, a cognitive impairment is implicated. The score is negatively correlated to the severity of cognitive impairment. CDR scale mainly includes memory, orientation, judgment and question-solving ability, work and social communication ability, family life and personal hobbies, and self-care ability for an independent life. Each aspect is assessed by five grades from harmlessness to severe damage. However, the scores of each aspect are not superimposed. Instead, the six aspects are assessed as a total score. The comprehensive results are expressed as 0, 0.5, 1, 2 and 3 points. A higher score indicates more serious cognitive dysfunction.

The ADL and Barthel index were used to assess the self-care ability of the patients: Among them, there are 14 items in the ADL scale, including self-care, exercise, housework and communication. The total score ranges from 14 to 56, and the lowest is 14, which indicates completely normal. When the ADL score is more than 14, it denotes that there are different degrees of functional decline. The critical value is 20. A high score suggests poor prognosis. The Barthel index has 10 items, including eating, bathing, decorating, dressing, defecation control, toilet use, bed and chair trans-

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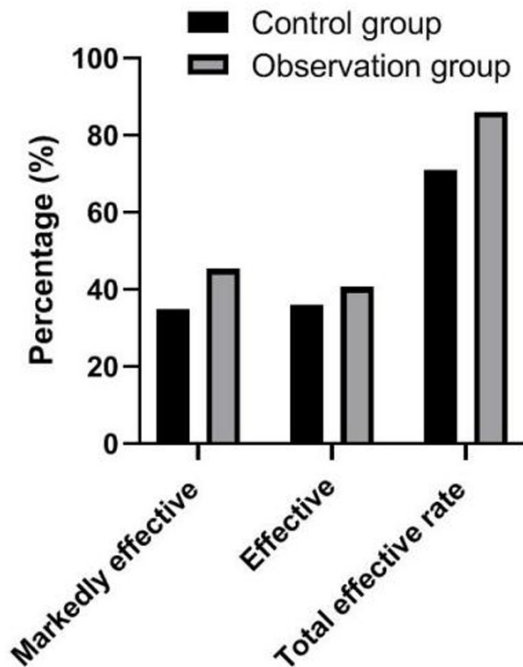


Figure 1. Comparison of efficiency.

fer, walking and stairs up and down. A full score of 100 means that patients have good activities of daily life, while a score below 60 indicates that patients have moderate to severe dysfunction of activities and need care from others in daily life.

Detection of the expression of serum neurotrophic factors: In a fasting state, 5 mL of venous blood was extracted into sodium ethylene-diamine-tetra-acetate (EDTA) anticoagulant tubes, and centrifuged at 3000 rpm for 15 min. The plasma was separated and stored at -70°C for further experiments. BDNF level was detected by ELISA according to the instructions of serum BDNF ELISA kit provided by RD Company. Briefly, 50 μl of standard was used to establish a standard curve. A 50 μl sample was added to 96-well plates and then 50 μl enzyme labeling reagent was added followed by addition of 50 μl of developer A, and subsequent addition of 50 μl of developer B. After that, the reaction was stopped by adding 50 μl of stop solution. The blank value was set to zero and the absorbance value (OD value) at 450 nm was detected by a microplate reader.

Incidence of adverse reactions: Adverse reactions during medication were recorded, and the changes of vital signs such as height, weight, blood pressure, heart rate and liver and kidney function were monitored.

Statistical analysis

The data were input into SPSS 15.0 for result analysis and statistical processing. The counting data were displayed as mean \pm standard deviation. The comparison between the two groups and the comparison before and after treatment were analyzed by t-test. The measurement data were displayed as percentage or number of cases, and the comparison between the two groups was analyzed by chi-square test. $P < 0.05$ indicates a significant difference.

Result

Basic characteristics of participants

The control group consisted of 44 males and 42 females, aged 55-74 years (average age: 62.58 ± 4.73), and disease course ranged from 7 to 23 months (average: 5.36 ± 4.08 months). The observation group included 43 males and 43 females, aged 57-75 years (average: 63.42 ± 4.88) with a course of disease of 7-25 months (average: 16.14 ± 4.72 months). General data were comparable and no significant difference was found between the two groups ($P > 0.05$).

Therapeutic effect comparison

The control group had a total effective rate of 70.93% and the observation group had an effective rate of 86.05% with a significant difference between them ($P < 0.05$) (Figure 1).

Comparison of cognitive function scores

There was no significant difference of MMSE and CDR scores between the two groups before treatment ($P > 0.05$). However, after treatment, MMSE scores were significantly increased and CDR scores were markedly reduced, compared to those before treatment. Additionally, MMSE scores was significantly increased in the observation group, with a decreased CDR score, compared to those in the control group ($P < 0.05$) (Figure 2) (Table 1).

Comparison of self-care ability scores

ADL score and Barthel index showed no significant difference before treatment in the two groups ($P > 0.05$). After treatment, ADL score was significantly reduced and Barthel index was markedly increased compared to those

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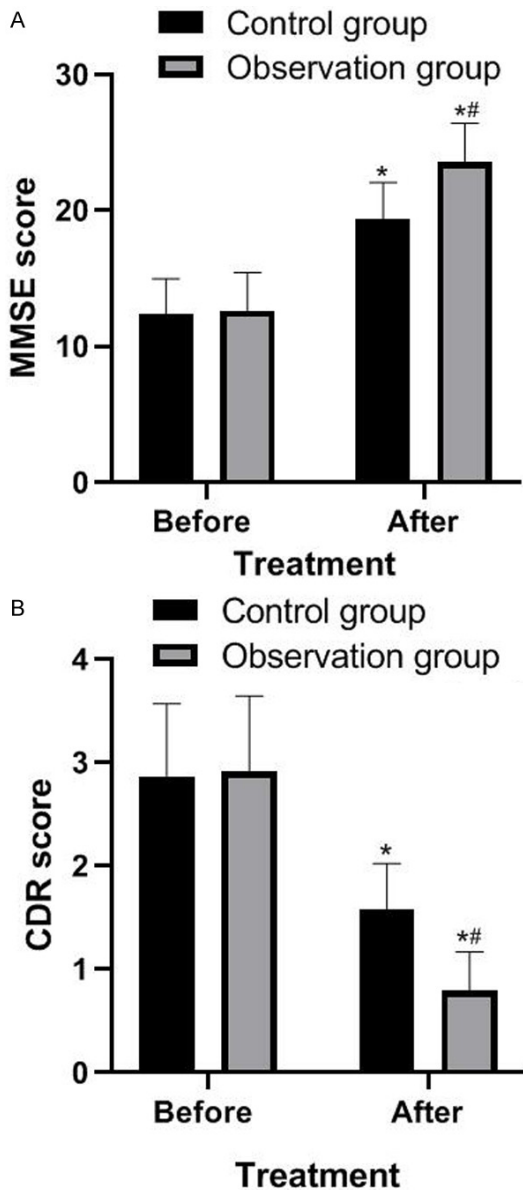


Figure 2. Comparison of MMSE (A) and CDR (B) scores before and after treatment in two groups. *, compared to before treatment, $P < 0.05$. #, compared to observation group, $P < 0.05$.

before treatment. ADL score declined and Barthel index rose in the observation group compared to those in control group ($P < 0.05$) (Figure 3) (Table 1).

Comparison of the expression level of serum BDNF

Serum BDNF expression showed no difference before treatment in the two groups ($P > 0.05$). After treatment, BDNF level was increased sig-

nificantly in the observation group compared to that in control group ($P < 0.05$) (Table 1).

Analysis of adverse reactions of two groups of patients

No serious adverse reactions were found in either group and the vital signs were stable during treatment. Also no abnormalities were observed in electrocardiogram, routine blood work and urine results, and liver and kidney function tests. There were 2 cases with nausea, 1 with dizziness and 1 with psychiatric symptoms in the control group, and the total incidence of adverse reactions was 4.65% (4/86). There were 3 cases of nausea, 1 with rash and 1 with psychiatric symptoms in the observation group with an adverse reactions rate of 5.81% (5/86). No significant difference was found regarding adverse reactions in the two groups ($P > 0.05$).

Discussion

VaD is a cognitive impairment syndrome caused by cerebrovascular disease. Its incidence is second only to Alzheimer disease (AD) [14, 15]. The pathogenesis of VaD is complex. At present, it is clinically believed that the pathophysiological basis of VaD is the impairment of brain tissue structure, the toxicity of excitatory amino acids and oxidative stress caused by decreased brain perfusion, abnormal neurotransmitters and brain metabolic rate [16, 17]. VaD patients are accompanied by hypertension, stroke, transient cerebral ischemia and other cerebrovascular diseases. They have been in a state of cerebral ischemia and hypoxia for a long time. Therefore, improving cerebral ischemia and hypoxia is the key for the treatment of VaD [18]. Based on the above theoretical basis, the two drugs, Butylphthalide and Naofukang, have been selected and explored for their therapeutic effects on VaD.

The active ingredient of butylphthalide is synthetic racemate d1-3-n-butylphthalide. It is a new type of drug for stroke therapy and can effectively reduce the damage of cerebral ischemia [19, 20]. Basic research has found that butylphthalide can effectively block multiple pathological links of ischemic brain injury, improve cerebral microcirculation, increase blood flow, relieve microvascular spasm, inhibit platelet aggregation, improve cerebral ischemia

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Table 1. Comparison of the levels of serum BDNF expression between two groups before and after treatment

Group	n	MMSE score		Barthel index		BDNF level	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control	86	12.37±2.61	19.35±2.72*	34.67±3.85	61.07±4.28*	3.14±2.06	3.86±2.54*
Observation	86	12.64±2.80	23.54±2.88*	35.29±3.32	78.93±4.56*	3.01±2.27	4.69±2.49*
t		0.654	9.809	1.131	26.484	2.042	0.043
P		0.514	<0.001	0.260	<0.001	4.624	<0.001

Compared with before treatment, *P<0.05.

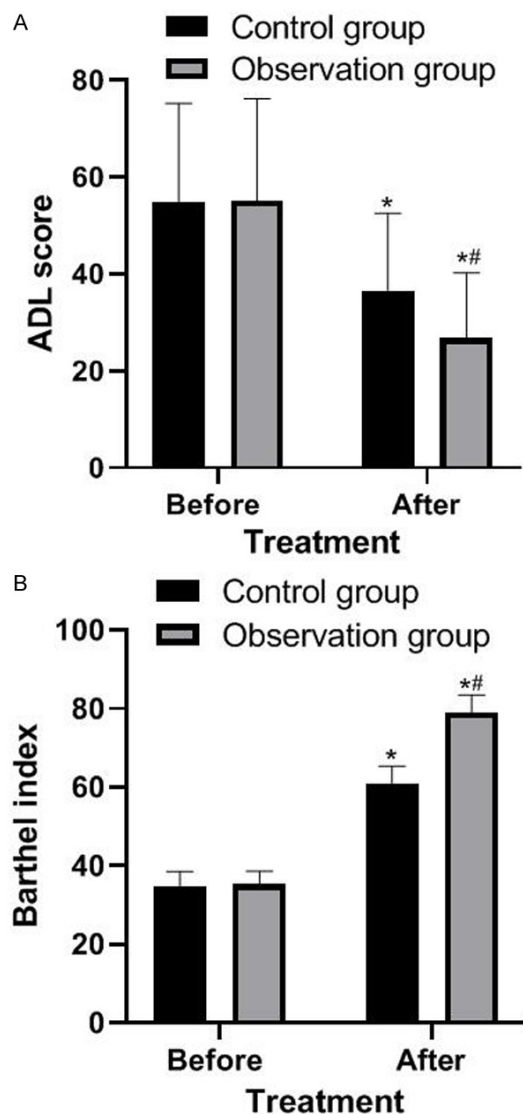


Figure 3. Comparison of ADL score (A) and Barthel index (B). *, compared to before treatment, P<0.05. #, compared to observation group, P<0.05.

and hypoxia, and limit the area of cerebral infarction [9, 21]. Animal experimental studies

have shown that butylphthalide can reduce the production of free radicals and increase the levels of nitric oxide (NO) and prostacyclin in vascular endothelium. It contributes to alleviating brain edema and secondary inflammatory response, inhibiting neuronal apoptosis and protecting neurons in rats with cerebral ischemia and hypoxia [22, 23]. It has been demonstrated that butylphthalide can alleviate VaD induced by chronic cerebral ischemia in a rat model of cerebral ischemia, the mechanism of which is believed to activate Akt/Nrf2 signaling pathway and inhibit the cascade reaction of neuronal apoptosis [24]. It has been found that butylphthalide can enhance cerebral hemodynamics in rats with chronic cerebral insufficiency, and at the same time, improve memory deficits in rats [21]. Several clinical trials have confirmed that butylphthalide exhibited good therapeutic effect on ischemic stroke [25, 26]. A study with rat models found that Naofukang could increase the content of monoamine neurotransmitters in the hippocampus, improve energy metabolism in the brain, reduce brain injury and improve learning and memory ability [27, 28]. In addition, Naofukang can also inhibit platelet aggregation, alleviate the spasm caused by vascular metabolism, restore the blood supply of cerebral ischemia area, and improve the state of cerebral ischemia and hypoxia [28].

In this study, we found that the combined application of butylphthalide and Naofukang had a better therapeutic effect on VaD (86.05%) than single use of Naofukang (70.93%). In addition, MMSE and CDR scales were used to evaluate the cognitive function of patients. It was found that the cognitive function of patients after treatment with both therapeutic strategies was significantly improved, but the effect of the combination of butylphthalide and Naofukang

was significantly better than that of Naofukang alone. The ADL scale and Barthel index were used to evaluate life quality and found improvement in life quality after treatment. Of note, Butylphthalide combined with Naofukang showed significantly higher life quality than that treated with Naofukang singly. Further analysis of the adverse reactions of the two methods showed that there were no serious adverse reactions of the two methods. Our results confirm that both Butylphthalide and Naofukang have better therapeutic effect on VaD, and can improve the cognitive function and patients' life quality, but the combination regime suggests favorable therapeutic effects.

BDNF is abundantly expressed in the hippocampus and involves in the growth, development, differentiation and repair of neurons during the development of the central nervous system. BDNF is shown to be closely associated with cognitive functions such as learning and memory. Previous study established a VaD rat model and treated them with butylphthalide. The results showed that butylphthalide could reduce the cerebral ischemia injury in the VaD rat model, as well as improve learning and memory ability [29]. At the same time, it was found that butylphthalide could enhance the expression of BDNF in hippocampus. BDNF level in peripheral blood is positively correlated with the level of BDNF in brain. We found that BDNF level was significantly increased after treatment, especially in the observation group, indicating that Butylphthalide combined with Naofukang can significantly increase the expression of BDNF, which may play a protective role in neurons and improve cognitive function.

Conclusion

In conclusion, Butylphthalide combined with Naofukang can improve the cognitive function and self-care ability of patients, which may be related to the changes of BDNF level.

Disclosure of conflict of interest

None.

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