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INTERVENTION EFFECT OF FOLIC ACID AND VITAMIN B_{12} ON VASCULAR COGNITIVE IMPAIRMENT COMPLICATED WITH HYPERHOMOCYSTEINEMIA

EFEKAT INTERVENCIJE FOLNOM KISELINOM I VITAMINOM B_{12} NA VASKULARNI KOGNITIVNI POREMEĆAJ KOMPLIKOVAN HIPERHOMOCISTEINEMIJOM

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Summary

Background: Hyperhomocysteinemia (HHcy) may be correlated with cognitive function. Although intervention with folic acid and $VitB_{12}$ can decrease the homocysteine (Hcy) level, its effect on cognitive function remains uncertain. This prospective study aimed to explore the effects of folic acid and $VitB_{12}$ on the Hcy level and cognitive function in patients with vascular cognitive impairment–no dementia (VCIND) complicated with HHcy.

Methods: A total of 120 patients with VCIND complicated by HHcy were randomly selected. They were divided into intervention and control groups. The intervention group was given 5 mg of folic acid per day and 500 μg of $VitB_{12}$ thrice per day apart from conventional therapy. Folic acid, $VitB_{12}$, and Hcy were determined and Montreal cognitive assessment (MoCA) and event-related potential P300 determination were performed before and after treatment.

Results: Before treatment, no significant differences in the folic acid, $VitB_{12}$, Hcy, MoCA, and P300 parameters were observed between the groups. After treatment, the folic acid and $VitB_{12}$ levels increased and the Hcy level decreased in the intervention group compared with that before treatment and in the control group. At 24 weeks, the MoCA score and P300 outcomes in the intervention group improved compared with those before treatment and in the control group.

Conclusions: Folic acid and $VitB_{12}$ effectively decrease the Hcy level in VCIND patients and improve their cognitive functions.

Keywords: cognitive impairment, cerebrovascular disorder, hyperhomocysteinemia, folic acid, vitamin B_{12}

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Kratak sadržaj

Uvod: Hiperhomocisteinemija (HHcy) nekada koreliše sa kognitivnom funkcijom. Iako se nivo homocisteina (Hcy) snižava posle intervencije folnom kiselinom i vitaminom B_{12}, njihov efekat na kognitivnu funkciju ostaje nejasan. Cilj ove prospektivne studije bio je da se istraži uticaj folne kiseline i vitamina B_{12} na nivo homocisteina i kognitivnu funkciju kod pacijenata sa vaskularnim kognitivnim poremećajem bez demencije (eng. vascular cognitive impairment – no demetia, VCIND) komplikovanim hiperhomocisteinemijom.

Metode: Nasumično je izabrano ukupno 120 pacijenata sa vaskularnim kognitivnim poremećajem bez demencije komplikovanim hiperhomocisteinemijom. Oni su podeljeni u intervencionu i kontrolnu grupu. Intervenciona grupa je primala 5 mg folne kiseline jednom dnevno i 500 μg vitamina B_{12} tri puta dnevno, uz uobičajenu terapiju. Određeni su nivoi folne kiseline, vitamina B_{12} i homocisteina a pre i posle tretmana su urađeni kognitivni testovi Montreal Cognitive Assessment (MoCA) i P300 kognitivni potencijal.

Rezultati: Pre tretmana, između grupa nisu uočene značajne razlike u nivoima folne kiseline, vitamina B_{12}, homocisteina, niti u parametrima MoCA i P300. Posle tretmana, nivoi folne kiseline i vitamina B_{12} su porasli, dok se nivo homocisteina u intervencionoj grupi snizio u poređenju sa nivoom pre tretmana i u kontrolnoj grupi. Posle 24 nedelje, u intervencionoj grupi su se popravili rezultati MoCA i P300, u poređenju sa onima pre tretmana i u kontrolnoj grupi.

Zaključak: Folna kiselina i vitamin B_{12} efikasno snižavaju nivo homocisteina kod pacijenata sa vaskularnim kognitivnim poremećajem bez demencije i poboljšavaju njihove kognitivne funkcije.

Ključne reči: kognitivni poremećaj, cerebrovaskularni poremećaj, hiperhomocisteinemija, folna kiselina, vitamin B_{12}

Introduction

Vascular cognitive impairment–no dementia (VCIND) refers to early or mild cognitive impairment caused by cerebral vascular injury, whose severity has not yet met the diagnostic criteria of dementia; it has an insidious onset (1, 2). VCIND has an incidence as high as 39.5% within one year after apoplexia (3). Early diagnosis and timely intervention can improve the prognosis of VCIND; otherwise, it may develop into dementia (1). Because VCIND has excellent reversibility, it has become a research hotspot. Hyperhomocysteinemia (HHcy) has been assumed to be an independent risk factor of cerebrovascular diseases (4). HHcy may also be closely related to cognitive impairment (5, 6). Folic acid and vitamin B_{12} can decrease the level of homocysteine (Hcy) in patients with dementia, but whether they can improve cognitive function remains undefined (7). In addition, VCIND patients in whom folic acid and vitamin B_{12} can achieve the best intervention effect have only been rarely studied.

To provide a useful clue for the prevention and cure of VCIND, we explored the intervention effects of folic acid and vitamin B_{12} on the Hcy level in VCIND patients, as well as their cognitive function improvement.

Subjects and Methods

Inclusion criteria

VCIND was diagnosed based on the recommendations by Rockwood et al. (8). The inclusion criteria included: 1) cerebrovascular disease; 2) evidence of cognitive impairment according to psychological evaluation; 3) cognitive impairment within 3 months after cerebral apoplexy; 4) causality between cerebrovascular disease and cognitive impairment, other than other diseases; 5) Hanchinski ischemia index ≥7; and 6) severity without meeting the diagnostic criteria of dementia.

Exclusion criteria

Patients meeting any of the following criteria were excluded from this study: 1) Alzheimer disease; 2) other cognitive disorders, mental diseases, or aphasia that affects Montreal cognitive assessment (MoCA) and P300 determination; 3) administration of drugs that influence Hcy level within one month (such as contraceptives, antiepileptics, dopaminergics, and folic acid and/or vitamin B_{12}; and 4) systemic diseases that influence the function of the central nervous system, such as thyroid disease, severe ischemia, deficiency of vitamin B_{12} and folic acid, severe malnutrition, and severe cardiac, hepatic and renal diseases.

General data

A total of 120 patients with VCIND complicated by HHcy were randomly selected. They were all patients with cerebral apoplexy that received treatment at the First Hospital Affiliated to the Chinese PLA General Hospital. Among them, 78 were males and 42 were females with an average age of 63± 1.87 years. Thirty-nine patients had concurrent high blood pressure, 43 had diabetes, and 35 had hyperlipidemia. Fifty-six smoked; 18 patients had college education degrees, 89 had senior middle school education degrees, and 13 had junior middle school education degrees. Patients with especially bad habits such as alcoholism were excluded. This study was conducted in accordance with the Declaration of Helsinki. The study was conducted with approval from the Ethics Committee of the First Hospital Affiliated to the Chinese PLA General Hospital. Written informed consent was obtained from all participants.

Grouping and treatment

The patients were equalized randomly into intervention and control groups. The intervention group was given 5 mg of extra folic acid per day (Changzhou Pharmaceutical Factory, China; 5 mg/tablet; state medical permitment No.: H32023302) and 500 μg of mecobalamin thrice per day (Eisai China Inc., China; brand name: methycobal; 500 μg/tablet; state medical permitment No.: H20030812) for 24 weeks, apart from conventional treatment. The control group only received conventional treatment. Both groups were prohibited from taking any other nootropic drug during treatment. No significant differences were observed in the ages, sex, education degrees, inpatient proportions, and incidences of basic diseases such as hypertension and diabetes between the groups (t-test was performed to compare the ages and body mass indices and χ^2 test was used for other indices; all $P>0.05$). Also, no significant differences were observed in the levels of serum folic acid, vitamin B_{12}, Hcy, MoCA scores, and P300 determination outcomes between the two groups (*t*-test: $P>0.05$).

Cognitive function assessment

Cognitive function levels were assessed using the MoCA scale (9). The scale covers eight cognitive fields and consists of 11 assessment items, including visual and spatial implementation, naming, memory, concentration, language fluency, abstract thinking, delayed memory and calculation, with a full mark of 30 points. One point is added to those with less than 12 years of education. A higher score indicates better cognitive function. The evaluation criterion for cognitive disorders is MoCA <26 points.

Potential P300 determination

All the patients were subjected to potential P300 determination within 48 h after grouping. The equipment used was an OXFORD multimedia myoelectricity evoked potential system (UK). The patient was positioned supinely, conscious and concentrating, in a quiet, screened room. According to the international standard electrode placement method for 10/20 electroencephalogram systems, the recording electrode was placed at the medial line, with the reference electrode at the right ear lobe and the forehead grounded. The resistance between the electrodes was <5KΩ and the analysis time was 600 ms. Short sound stimuli were utilized. Non-target stimuli (1000 Hz) were given regularly with a probability of 80% and intensity of 80 dB. Target stimuli (4000 Hz) went randomly between non-target stimuli with a probability of 20% and intensity of 90 dB. The subject pressed the button on a target stimulus. The reaction time was recorded automatically by the equipment. The experiment was repeated twice, and a mean was obtained.

Determination of serum Hcy as well as of folic acid and vitamin B_{12}

Venous blood at 2–3 mL was extracted from the patient on an empty stomach in the morning. The sample was sent for determination within half an hour. The concentration of serum Hcy was determined by a Hitachi 7180 automatic biochemistry analyzer (Japan) using the enzymatic conversion method. The kit was supplied by Beijing Strong Biotechnologies, Inc. (China). The normal Hcy concentration range is between 5 μmol/L and 14 μmol/L. When a value is above the upper limit, HHcy will be accounted for. Meanwhile, 3–4 mL of blood was extracted from the ulnar vein for the determination of the concentrations of folic acid and vitamin B_{12} on the same day using the Access automated chemiluminescent immunoassay system and its supporting kit (Beckman, USA).

Evaluation indices

The concentrations of folic acid, vitamin B_{12}, and Hcy were determined 4, 12, and 24 weeks after treatment. Meanwhile, MoCA scoring and event-related potential P300 determination were performed to make comparisons before and after treatment as well as between groups.

Statistical analysis

All data were presented as $\bar{x} \pm s$ and analyzed using SPSS16.0 software. ANOVA and t-test were performed. Numeration data were presented as percentages and tested using χ^2 test. $P<0.05$ was considered statistically significant.

Results

The levels of serum folic acid, vitamin B_{12}, and Hcy

Before treatment, the intervention group and the control group did not show significant differences in the levels of folic acid, vitamin B_{12}, and Hcy ($P>0.05$). The control group did not exhibit variations in the levels of folic acid, vitamin B_{12}, and Hcy throughout the experiment ($P>0.05$). In the intervention group, the levels of folic acid and vitamin B_{12} at 4 weeks increased compared with those before treatment, whereas the level of Hcy decreased ($P<0.05$). At 12 weeks, the levels of folic acid and vitamin B_{12} increased compared with those at 4 weeks, whereas the level of Hcy decreased ($P<0.01$). At 24 weeks,

Table I The levels of serum folic acid, vitamin B_{12}, and Hcy at 0, 4, 12, 24 weeks in the intervention group and the control group ($\bar{x} \pm s$).

Items	Groups	Cases (n)	Treatment time			
			Before treatment	4 weeks	12 weeks	24 weeks
Hcy	Intervention group	60	24.95±5.64	18.69±5.82[1,2]	13.36±2.84[2,3]	12.13±3.58[2,3,4]
	Control group	60	25.05±4.36	24.28±3.11	24.57±7.29	23.28±3.12
Folic acid	Intervention group	60	2.74±0.65	3.74±0.70[1,2]	5.14±1.10[1,2]	6.10±1.18[1,2]
	Control group	60	2.83±0.80	2.51±0.77	2.94±0.63	2.93±0.50
VitB_{12}	Intervention group	60	241.78±40.26	277.71±24.84[1,2]	316.46±49.55[1,2]	362.16±35.80[1,2]
	Control group	60	253.25±24.31	260.20±21.82	260.51±22.32	266.18±31.76

Note: Compared with the other time point in the same group, [1]$P<0.05$; Compared with the control group at the same period, [2]$P<0.05$; Compared with before treatment, [3]$P<0.05$; Compared with the 12 weeks, [4]$P>0.05$.

Table II Changes of MoCA scores at 0, 4, 12, 24 weeks in the intervention group and the control group ($\bar{x} \pm s$).

Items	Groups	Cases (n)	Before treatment	4 weeks	12 weeks	24 weeks
MoCA	Intervention group	60	22.39±2.01	22.79±2.26	23.05±2.39	24.90±1.79[1,2]
	Control group	60	22.50±2.12	22.61±2.18	23.06±2.36	23.20±1.58

Note: Compared with the other time point in the same group, $^1P<0.05$; Compared with the control group at the same period, $^2P<0.05$.

Table III Changes of P300 latency period and amplitude at 0, 4, 12, 24 weeks in the intervention group and the control group ($\bar{x} \pm s$).

P300	Groups	Cases (n)	Before treatment	4 weeks	12 weeks	24 weeks
Latency period	Intervention group	60	380.77±25.97	373.78±27.20	378.35±32.56	347.06±20.03[1,2]
	Control group	60	378.26±28.41	361.88±16.20	371.35±37.56	368.16±15.53
Amplitude	Intervention group	60	4.37±0.64	4.23±0.96	3.76±0.87	4.05±0.54
	Control group	60	4.16±0.85	3.77±0.68	3.59±0.73	3.91±0.67

Note: Compared with the other groups, $^1P<0.05$; Compared with before treatment, $^2P<0.05$.

the levels of folic acid and vitamin B_{12} further increased compared with those at 12 weeks, whereas the level of Hcy did not show a significant difference (P>0.05), but was still lower than that in the control group at the same time point (P < 0.01) (*Table I*).

MoCA scores

The control group did not exhibit significant differences in the MoCA scores throughout the experiment (ANOVA: P>0.05). In the intervention group, the scores at 4 weeks and 12 weeks did not show a marked difference (ANOVA: P>0.05). At 24 weeks, the score improved compared with those at the previous time points (ANOVA: P<0.01). Compared with that in the control group at the same time point, the score was also significantly higher (*t*-test: P<0.01) (*Table II*).

P300 latency periods and amplitude changes

The control group did not show changes in the latency period and amplitude throughout the experiment (ANOVA: P>0.05). In the intervention group, the latency periods and amplitudes at 4 and 12 weeks did not exhibit significant differences (ANOVA: P>0.05). At 24 weeks, the P300 latency period shortened compared with those at the previous time points, and was also significantly shorter than that in the control group at the same time point (ANOVA: P<0.05). However, such changes were not observed in the P300 amplitude (*Table III*).

Discussion

Numerous studies have demonstrated that HHcy may be correlated with cognitive impairment or dementia, for which it has been attracting more and more attention from scholars (10, 11). VCIND has excellent reversibility. Therefore, determining the correlation between VCIND and Hcy and then exerting intervention is of great clinical significance.

Hcy is a mercaptoamino acid produced by the metabolism of methionine *in vivo*. The metabolism of Hcy is primarily manifested by its transformation into methionine and tetrahydrofolic acid under the catalyses of methionine synthetase and methylenetetrahydrofolate reductase; this re-methylation process requires the participation of folic acid and vitamin B_{12} (12, 13). Deficiency of these cofactors is very likely to affect enzymatic activity, as well as the metabolism and transformation of Hcy, ultimately leading to HHcy. Replenishment of folic acid and vitamins can reduce a high level of Hcy (14, 15). In this study, the results showed that the Hcy levels in the intervention group at 4, 12, and 24 weeks significantly decreased compared with those before treatment as well as those in the control group at the corresponding time points (P<0.01). The concentrations of folic acid and vitamin B_{12} in this group at 4, 12, and 24 weeks significantly increased compared with those before treatment as well as those in the control group at the corresponding time points (P<0.01). In contrast, the control group did not exhibit changes in the levels of Hcy, folic acid, and vitamin B_{12} throughout the experiment. These findings indicate that the replenishment

of folic acid and vitamin B_{12} can effectively decrease the Hcy level in patients with VCIND complicated by HHcy. Furthermore, in the intervention group, the Hcy levels at 12 and 24 weeks did not show a noticeable difference. This phenomenon may be explained by the assumption that even after the replenishment of folic acid and vitamin B_{12}, the Hcy level will not further decrease when a certain level is reached.

To date, an agreement on the correlation between HHcy and cognitive function has not been reached. Some scholars believe that HHcy serves as an independent risk factor of cognitive disorder or a decrease in cognitive function (16, 17), whereas some hold that HHcy has nothing to do with cognitive function (19). To the formers two channels through which HHcy leads to cognitive disorder are believed to exist. The first is that HHcy indirectly leads to cognitive impairment by causing cerebrovascular disease through the channel of vascular endothelial cell dysfunction and lipid metabolism disorder, and the other implies that it leads to cognitive impairment by increasing glutamate excitotoxicity, decreasing neuronal DNA repair capacity, accelerating oxidative stress and the formation of Aβ, and causing damage to hippocampal neurons (20). In this study, the results showed that in the intervention group, the Hcy level at 24 weeks significantly decreased compared with those at the previous time points in the same group, the MoCA score at 24 weeks noticeably improved compared with those at the previous time points (as well as that in the control group at the same time point), and the event-related potential P300 latency period greatly shortened compared with those at the previous time points. In contrast, in the control group, the Hcy, MoCA, and P300 latency period at 24 weeks did not show marked changes compared with those at the previous time points. These findings indicate that the cognitive function of the VCIND patients improved with the decrease in Hcy. In addition, the intervention and control group did not exhibit significant differences in the P300 amplitudes. This phenomenon may be correlated with the facts that the severity of the cognitive impairment in the patients enrolled in this study was mild and that amplitudes cannot serve as a valid index, as they vary greatly according to different individuals. To date, the direct correlation of folic acid and Vit B_{12} with cognitive function remains uncertain. Considering that this study was not designed for this purpose, future studies may be needed.

Furthermore, this study revealed that the improvement of cognitive function occurred rather late compared with the increases in folic acid and vitamin B_{12} and a decrease in Hcy, showing a relative delay. Nowadays, the results of the correlation between Hcy and cognitive function from different studies still differ from one another. This phenomenon is presumably attributable to different ethnic groups as well as different types of patients included in different studies. Different nationalities differ in apolipoprotein e (Apo E) genes which may be associated with cognitive function and partially associated with memory function (21). Therefore, the results from one country may not be applicable for another. Further, different subjects may also lead to different findings. In this study, all the patients suffered from a mild vascular cognitive disorder. Treatment and intervention achieved relatively satisfactory effects. Therefore, a positive result was obtained. By contrast, negative results have been reported in some studies. These results might be associated with the fact that the enrolled patients in those studies had severe dementia. Due to relatively severe pathological conditions, satisfactory treatment and intervention effects were not achieved.

This study has some limitations. First, the subjects were primarily old patients, and their number was rather small. Therefore, these subjects cannot be representative of all VCIND patients. Second, the outcomes of this study might be affected by the sensitivity of the cognitive function assessment scale used. Third, the observation time was short. Last, the individual effects of folic acid and vitamin B_{12} on cognitive function were not taken into consideration. Therefore, more subjects, prolonged observation time, and modified statistical measures are needed in further studies.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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