

Clinical Observation

Influence of acupuncture on cognitive function and markers of oxidative DNA damage in patients with vascular dementia

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assess independence in activities of daily living (ADL). Life quality was evaluated using the DEMQOL (Dementia quality of life questionnaire) questionnaire, and syndromes and expression of vascular dementia were evaluated with the Scale for the Differentiation of Syndromes of Vascular Dementia (SDSVD). In addition, the urine concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG)—a marker of oxidative damage—was quantified with enzyme-linked immunosorbent assay.

RESULTS: The MMSE-R and DEMQOL scores were higher after acupuncture than before ($P < 0.05$), while there were no obvious differences in the ADL-R or SDSVD scores ($P > 0.05$). The 8-OHdG content in urine significantly decreased after acupuncture ($P < 0.05$).

CONCLUSION: Acupuncture reduces the levels of 8-OHdG and improves cognitive function and quality of life in VD patients, suggesting that acupuncture is beneficial at least in part by preventing oxidative damage.

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Key words: Acupuncture; Vascular dementia; Cognitive function; Life quality; 8-OHdG.

Abstract

OBJECTIVE: To test the influence of acupuncture on cognitive function and a marker of oxidative DNA damage in patients with vascular dementia (VD).

METHODS: Sixteen VD patients were evaluated before and after acupuncture, using the Folstein Mini-Mental State Examination-Revised (MMSE-R) to assess cognitive function, and the ADL-R scale to

INTRODUCTION

Vascular dementia (VD) is a clinical syndrome of high-level cognitive dysfunction caused by damage to cerebral tissue by various cerebrovascular factors^[1]. The quality of life of VD patients is seriously influenced by declined cognitive function and impaired social behav-

ior, as well as by the long duration of the illness (several months to more than half a year)^[2]. VD is the second most common senile dementia after Alzheimer's disease (AD), accounting for 10%-50% of patients^[3]. With the aging population and the resulting increase in cerebrocardiovascular diseases, VD incidence may well end up as the most common cause of dementia^[4]. Oxides produced by reactions of free radicals with DNA can be used as markers of the levels of free radicals or oxidative stress^[5]. One accepted biomarker of oxidative DNA damage is 8-hydroxy-2'-deoxyguanosine (8-OHdG)—a product of oxidative damage to DNA by free radicals. In this study, we investigated the effects of acupuncture on cognitive function and 8-OHdG levels in VD patients, to explore the mechanisms of anti-oxidant effects of acupuncture. We used internationally accepted scales to assess VD patients for cognitive function, function in daily life, quality of life, TCM syndromes, expression of dementia, and urine 8-OHdG levels.

This study was approved by the appropriate ethics committees and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All study participants provided written, informed consent prior to their inclusion.

METHODS

Subjects

Sixteen VD patients from the First Hospital Affiliated to Tianjin TCM University and the Tiantuo Hospital in Tianjin took part in the study, which was conducted from June 2008 to July 2009.

Diagnostic standard

Standards for clinically diagnosing VD have been stipulated by the US National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS/AIREN)^[6].

Inclusion criteria

The inclusion criteria were as follows: 1) meeting the diagnostic criteria for VD; 2) a duration of illness of >2 weeks; 3) an age of 50-80 years; 4) Hachinski ischemic index ≥ 7 ; 5) mild or moderate dementia (MMSE-R score from 10 to 26); 6) recognition of the significance of acupuncture research and good compliance with the instructions; 7) having signed a consent form.

Exclusion criteria

The exclusion criteria were: 1) symptoms of dementia symptoms before the onset of cerebrovascular disease; 2) diagnosis of AD or other types of dementia; 3) severe dysfunction of heart, liver, or kidney, or severe primary diseases in hematopoietic or endocrine systems; 4) severe aphasia, depression, or mental illness.

Needles

Disposable needles for acupuncture (0.25 mm×40mm; and 0.25 mm×25mm) were produced by the Beijing Hanyi Medical Apparatus Center.

Acupuncture treatment

Main points: Baihui (GV 20), Sishencong (EX-HN1), Shenting (GV 24), Neiguan (PC 6), Tanzhong (CV 17), Zhongwan (CV 12), Qihai (CV 6), Xuehai (SP 10), and Zusanli (ST 36).

Adjunct points: Xuanzhong (GB 39) for deficiency of kidney essence, Fenglong (ST 30) for stagnation of phlegm in the interior, Shexia for obstruction of collaterals by blood stasis (blood-letting puncture), Taichong (LR 3) for hyperactivity of the liver-*yang*, Neiting (ST 44) for exuberant heat in the interior, Tianshu (ST 25) for stagnation of turbid *Qi* in fu-organs, and Guanyuan (CV 4) for deficiency of both *qi* and blood.

Manipulation: Baihui and Sishencong were obliquely inserted to a depth of 0.2 *cun* using the twirling reinforcing method with small amplitude and high frequency. Shenting was horizontally inserted (0.5-0.8 *cun* deep) using the twirling reinforcing method with small amplitude and high frequency. Neiguan was vertically inserted (0.5-1.0 *cun*) using the uniform twirling reinforcing-reducing method. Tanzhong was inserted (0.5 *cun*) with the needle tip upward using the twirling reinforcing method with small amplitude and high frequency. Zhongwan and Qihai were vertically inserted (1.0-1.5 *cun*) using the twirling reinforcing method with small amplitude and high frequency. Xuehai was obliquely inserted (0.5-1.0 *cun*) with the needle tip toward the inner thigh using the twirling reducing method with large amplitude and low frequency. Zusanli was vertically inserted (0.5-1.0 *cun*) using the twirling reinforcing method with small amplitude and high frequency. Xuanzhong and Guanyuan were inserted using the twirling reinforcing method. Taichong, Fenglong, Neiting, and Tianshu were inserted with the twirling reducing method. The needles were retained for 30 min. Acupuncture was given once every other day for 6 w.

Behavioral evaluations

The Folstein MMSE-R scale was used to evaluate changes in cognitive function, such as orientation, calculation, attention, immediate memory, delayed memory, and language. The ADL-R scale was used to evaluate the ability of patients to take care of themselves in daily life. The DEMQOL scale was used to evaluate quality of life. The SDSVD scale was used to evaluate the syndromes and severity of dementia. These scales were used for evaluation before treatment, at the end of the 6 w treatment, and 4 w after treatment.

OHdG detection

Morning urine was collected before and after acupuncture treatment and immediately stored in a refrigerator

at -80°C . ELISA was used to quantify the 8-OHdG levels (Reagent kit from Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analysis

Data are presented as mean \pm standard deviation. Paired *t*-tests in SPSS10.0 were used to compare the data before and after acupuncture. $P < 0.05$ was considered a statistically significant difference.

RESULTS

Baseline characteristics of VD patients

The 16 patients comprised 9 males and 7 females aging 50-80 years (average age 63.83 ± 9.30 years). Their medical history revealed 7 cases (43.75%) of coronary

heart disease, 2 cases (12.50%) of diabetes, 12 cases (75.00%) of hypertension, 1 case (6.25%) of kidney disease, and 3 patients (18.75%) with a family history of apoplexy.

Influence of acupuncture on cognitive function of VD patients

The MMSE-R and DEMQOL scores were significantly higher after acupuncture than before ($P < 0.05$), whereas there were no obvious differences in ADL-R or SDSVD scores ($P > 0.05$) (Table 1).

Influence of acupuncture on 8-OHdG content in urine

The 8-OHdG content in the urine of VD patients after acupuncture (14.70 ± 1.69 ng/mg creatinine) was significantly lower than before acupuncture (15.95 ± 2.67 ng/mg creatinine) ($P < 0.05$).

Table 1 Change in scores of cognitive function of VD patients before and after acupuncture ($\bar{x} \pm s$)

	MMSE-R	ADL-R	DEMQOL	SDSVD
Before acupuncture	18.24 \pm 0.91	41.63 \pm 3.59	73.56 \pm 1.57	15.56 \pm 1.24
After acupuncture	20.62 \pm 0.99	39.44 \pm 3.38	80.06 \pm 2.51	14.18 \pm 1.13
<i>P</i>	0.000	0.309	0.005	0.071

DISCUSSION

At present, the effects of treatments in VD are mainly evaluated based on changes in the following three aspects: 1) objective evaluation of cognition or memory; 2) ability to function independently in daily life; 3) general clinical impression^[7]. In this study, we used the MMSE-R, ADL-R, DEMQOL, and SDSVD scales to comprehensively evaluate the influence of acupuncture on cognitive function in VD patients. We discovered that a 6-week acupuncture treatment improved cognitive function and enhanced quality of life, whereas there were no obvious influences on the patients' ability to take care of themselves in daily life or on the expression of TCM syndromes.

Free radicals with highly active chemical properties can destroy molecular structures and damage cells. Such oxidation is an important pathological basis of cerebral ischemia-reperfusion, cerebral atherosclerosis, neural retrograde affection, and other diseases of the nervous system^[8,9]. Compared with other tissues, brain tissue has lower levels of antioxidants, lower membrane levels of unsaturated fatty acids, and a higher rate of oxidative metabolism. Because the normal function of the brain fundamentally relies on synaptic transmission, cerebral tissue is particularly sensitive to damage by reactive oxygen species^[10]. The close relationship of oxidative stress with pathophysiological mechanisms of dementia is generally considered a key factor for neural damage^[11,12]. Previous research has shown that cerebral ischemia and super oxide radicals are associated with

impairments in spatial cognitive function^[13,14]. Acupuncture, a safe and effective therapy, is widely used to prevent and treat VD^[15]. Our previous studies have shown that acupuncture can reduce free radical generation in rats with VD by enhancing antioxidant ability through actions on superoxide dismutase and glutathione peroxidase^[16,17].

Wide-spread oxidative damage is mainly manifested as damage to the structure and function of biomacromolecules, such as DNA, proteins, and lipids. This causes genetic mutations, cellular canceration, senility, and other phenomena. 8-OHdG is formed when free radicals attack guanine bases of the DNA chain, causing hydroxidation at the C-8 position. Therefore, 8-OHdG is one of the main products of oxidative DNA damage^[18]. At present, 8-OHdG is widely accepted as a marker of oxidative damage to DNA. Quantification of 8-OHdG levels can be used to evaluate the extent of oxidative damage and repair, and to investigate the relationships between oxidative stress and DNA damage and between oxidative stress and disease^[19]. In this study, we have shown that acupuncture can reduce the 8-OHdG content in urine of VD patients, suggesting that acupuncture can prevent oxidative DNA damage.

By comparing the cognitive function and 8-OHdG levels of VD patients before and after acupuncture, we have discovered that acupuncture can reduce 8-OHdG levels in the urine of VD patients and improve cognitive function and life quality of VD patients. However, due to the complexity of processes involved in oxida-

tive damage, the above-mentioned indexes can be influenced by factors both inside and outside the body. In this study, we investigated the antioxidant effect of acupuncture only based on the marker molecule 8-OHdG. Future studies will further investigate the dynamic balance between oxidation and anti-oxidation, to comprehensively analyze the mechanisms by which acupuncture improves the cognitive function of VD patients.

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