

Effect of acupoint with moxa cone on ^1H -MRS of hippocampus and posterior cingulate gyrus in patients with mild cognitive impairment

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Research

Keywords: Moxibustion, Magnetic resonance spectroscopy (MRS), Mild cognitive impairment (MCI)

Posted Date: May 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-424514/v1>

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Abstract

Background: Mild cognitive impairment (MCI) is often the prodromal stage to Alzheimer's disease (AD). Most patients with MCI harbor the pathologic changes of AD and experience progressive brain metabolites change. We investigated the changes of metabolites in bilateral hippocampus (HIP) and posterior cingulate gyrus (PCG) in MCI patients, and observed the effects of moxa cone moxibustion on the metabolites of bilateral hippocampus and posterior cingulate gyrus in patients with MCI.

Methods: Sixty-nine patients with MCI and sixty-seven cases of age-matched normal controls (NC) were enrolled in this study. MCI patients were randomly divided into drug group (Drug, $n=24$), sham acupoint group (Sham, $n=20$) and acupoint group (Acupoint, $n=25$). All subjects received proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) for measurement of NAA/tCr and Cho/tCr ratios in bilateral hippocampus and posterior cingulate gyrus before and after treatment. Mini-Mental Status Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to evaluate cognitive function.

Results: MCI patients were characterized by lower NAA/tCr in bilateral hippocampus and posterior cingulate gyrus and Cho/tCr in right hippocampus compared to those in NC group ($P < 0.05$). After two months treatment, NAA/tCr in bilateral hippocampus and posterior cingulate gyrus and Cho/tCr in right hippocampus were increased in the three treatment groups, and there was no significant difference compared to NC group ($P > 0.05$). NAA/tCr in right hippocampus and Cho/tCr of right posterior cingulate gyrus of acupoint group was significantly higher than those in sham acupoint group ($P < 0.05$) and NC group ($P < 0.05$). The scores of MMSE and MOCA in the acupoint group and sham acupoint group were better than those in the drug group, especially in the long-term efficacy.

Conclusions: Moxa cone moxibustion may improve the cognitive function of MCI patients by regulating the abnormal metabolites of bilateral hippocampus and posterior cingulate gyrus. MRS can provide objective imaging basis for moxibustion prevention and treatment of MCI.

Trial Registration: ChiCTR-IPR-16009144 (Retroactively registered on 26 AUG 2016)

Background

The size of the elderly population has been dramatically increasing worldwide. About 962 million people aged 60 or older accounted for 13% of the global population in 2017, it is predicted to rise to 1.4 billion, 2.1 billion, and eventually 3.1 billion people by 2030, 2050, and 2100, respectively [1]. Cognitive frailty is defined as the cooccurrence of physical frailty and cognitive decline in older people without dementia. Mild cognitive impairment (MCI) is generally referred as a transition state between normal cognition and Alzheimer's disease (AD) [2]. The prevalence of MCI in adults older than 60 is approximately 6.7–25.2%. It increases with age and lower level of education and is more prevalent in men [3–6]. The annual rate of progression to dementia is approximately 5–17% [2, 7–9]. For example, in 2010, approximately 4.7 million Americans were living with AD, the most common neurodegenerative disease; by 2050 it is projected to be around 16 million [10]. Medical expenses to treat these patients, most over the age of 65

[11], exceed those to treat individuals with cancer and cardiovascular disease combined, placing an immense burden on Medicare and Medicaid [12]. AD is the most common cause of dementia and a growing health problem. It affects more than 35.6 million people living with dementia worldwide, with approximately 6.5 million AD patients in China [13], while the global rates are expected to more than triple by 2050 [14]. At present, the pathogenesis of AD is not clear, and there is no effective way to prevent and treat it [15]. Therefore, early detection and prevention of AD is particularly important.

Neuroimaging is becoming increasingly important in the study and clinical diagnosis of AD [16–17]. Hydrogen 1 (^1H) magnetic resonance (MR) spectroscopy is a quantitative biochemical imaging technique and follows structural MR imaging as one of the most extensively investigated MR modalities used to assess AD [18]. ^1H -MRS is a non-invasive technique that allows detection and quantification of certain biochemical compounds in brain tissue, such as *N*-acetylaspartate (NAA), creatine (Cr), choline (Cho), and lipids [19, 20]. NAA is found at relatively high concentrations in the human central nervous system and is particularly localized within neurons and related to neuronal processes [19, 21]. A decrease in NAA concentration is routinely considered as an indicator of neuronal loss or dysfunction [22, 23] and has been observed in different brain regions in various neurodegenerative disorders [20, 24–28]. NAA decrease is mostly observed at the moment when the disease is in progression. Cr, which is known to play an important role in energy metabolism, has been reported to be constant throughout the brain and resistant to change in several degenerative brain diseases [19, 29, 30]. Cr is often used as an internal reference to measure the content of other metabolites in many spectral studies [31, 32]. Occasionally Cr can't be used as a reference value [33], because of abnormal energy metabolism in some diseases, such as high malignant tumor. Cho is considered a marker for cell turnover [19], which can reflect the damaged cholinergic neurons in AD. ^1H -MRS technique show tissue damage that may precede the evidence of atrophy on morphological imaging and the accuracy of this technique was demonstrated by the correlation of metabolic MR biomarkers with clinical and neuropathological severity in neurodegenerative disorders such as AD [34]. In addition, the MRS Consensus Group have indicated the ^1H -MRS as a complementary tool to conventional MRI for diagnosing and monitoring disease progression and treatment response in neurodegenerative disorders [35]. ^1H -MRS has been used for early diagnosis and differential diagnosis of AD [36]. Prior studies have demonstrated that atrophy of the medial temporal lobe, including the hippocampal area, is a common structural feature of AD [37]. The hippocampus is involved in learning and memory and implicated early in the pathophysiology of AD [38]. Ishiwata et al reported that the hippocampal atrophy was correlated with the severity of cognitive impairment in AD [39]. Since the hippocampus is involved early in the AD process, it is particularly useful to understand the metabolic change of the hippocampus for early diagnosis and evaluation of intervention [40]. Some structural MRI studies have demonstrated that AD-associated brain atrophy may involve not only the medial temporal lobe but also the posterior cingulate gyrus (PCP), and parietotemporal area particularly in early onset AD [41, 42]. Patients with AD and MCI were reported to exhibit tissue degeneration in the posterior cingulate gyrus [43].

Currently, no drug has proven effective in treatment of MCI. Cholinesterase inhibitors have not been shown to decrease risk of progression from MCI to dementia at 1 and 3 years. In addition, cholinesterase inhibitors have limited or no significant effects on cognitive function over the short term and may substantially increase adverse effects, based on a meta-analysis of 4 trials (1960 participants) and another meta-analysis of 9 trials (5149 participants). Consequently, cholinesterase inhibitors and memantine are not recommended for MCI treatment and there are currently no FDA-approved medications for MCI [44, 45]. We have known a series of studies in men and animals that acupuncture and moxibustion can improve memory on AD or MCI [46–48]. Moxibustion could help prevent cognitive impairment, and prevention is currently the only suitable management technique for this disorder [49]. Moxibustion could improve clinical symptoms, regulated blood lipid levels and neuropeptides associated with learning and memory in dementia patients [50, 51]. Although studies have evaluated the cognitive effect of moxibustion, no one has tried to prevent mild cognitive impairment by moxibustion at acupoints. The effect of moxibustion on neuroimaging in patients with MCI is still unclear. In this study, the effect of moxibustion on cognitive impairment was evaluated from the perspective of prevention for the first time, and the effect of moxibustion on brain metabolites in patients with MCI was evaluated by $^1\text{H-MRS}$.

Methods

Subjects

Sixty-nine patients with MCI and sixty-seven cases of age-matched normal controls (NC group) were recruited from January 2014 to December 2017 at The First Affiliated Hospital of Guangxi University of Chinese Medicine. MCI patients were randomly divided into acupoint group with twenty-five cases, sham point group with twenty cases, drug group with twenty-four cases.

The inclusion criteria were: (1) Presenium and elderly people aged 55–82, educational objects, (2) MCI group should meet the MCI diagnostic criteria of 2006 edition of Chinese guidelines for the diagnosis and treatment of dementia [52], with the main complaint of memory impairment and abnormal MoCA score [53–55], All MCI patients included in this study belong to aMCI. (3) In the NC group, the main complaint of noncognitive impairment and the total score of MMSE (Chinese version) > 27 [56] were required. The overall cognitive function was normal, and no cerebral infarction and other brain lesions were found on CT or MRI. The exclusion criteria were: (1) non age group, (2) cardiovascular risk factors were hypertension or diabetes mellitus, history of acute cardiovascular events, clinical stroke, (3) ataxia and subjects with history of ataxia, (4) patients with a history of malignancy, (5) current or previous smoking history, alcohol intake or drug abuse, and known patients who had used other drugs that may cause cognitive function change.

All the participants (or their legal representative) gave their formal written consent. The research protocol was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi University of Chinese Medicine (Lot number: [2016]009).

Treatment

Acupoint group: Baihui acupoint (DU 20) (located in the middle of the posterior hairline, 7 individual cuns straight up), Guanyuan acupoint (RN 4) (located in the front midline, 3 individual cuns below the umbilicus.), Zusanli acupoint (ST 36, bilateral) (located on the anterior aspect of the lower leg, about one finger-breadth lateral to the tibia, just inferior to the tibial tuberosity.), Xuanzhong acupoint (GB 39, bilateral) (located 3 individual cuns above the high point of lateral malleolus and at the front edge of fibula.) (Fig. 1). Sham point group: The left side of the head was 1 individual cun above Shuaigu acupoint (GB 8, about 2.5 cm), 3 individual cuns (about 7.5 cm) to the right side of Guanyuan (RN 4), the lower edge of patella (bilateral), and 1 individual cun (about 2.5 cm) above the tip of lateral malleolus (bilateral). Manipulation: the patient was in supine position. The above acupoints were selected according to "acupuncture and acupoint" edited by Shi Xuemin [57]. Bilateral acupoints were taken each time. Thin vaseline was applied to the acupoints to protect the patient's skin and facilitate fixation of moxa cone (produced by Nanyang Iyong moxa grass *Biological Products Co.Ltd.*, 12 mm×15 mm) and ignited with alcohol lamp, 3–5 minutes each moxa cone, when patients felt burning hot, changed the moxa cone, after acupoint, gently pressed the acupoints with cotton swab, 3 moxa cones/per acupoint, 6 acupoints per day, a total of 18 moxa cones for 30 minutes one time treatment in a day. Once every other day, 15 times as a course of treatment, 2 days after rest to continue the next course of treatment, a total of 2 courses. Drug group: oral donepezil hydrochloride [Produced by Weicai (China) Pharmaceutical Co.Ltd.: H20050978, batch number: 131224a, 130820a, 15060111610044, 5mg×7 tablets] 5 mg, once every night, 30 days as a course of treatment, a total of 2 courses.

Proton MRI and spectroscopy

The 3.0T Siemens superconducting MRI system (Siemens, Verio 3.0T) was used to collect the multi voxel spectrum of MCI and NC groups. PRESS-CSI sequence were used. The TR/TE was 1700/135ms and the frequency was 1200Hz. FOV is 160mm×160mm, and matrix size is 16×16. The volume of interest (VOI) is 80mm×66mm×15mm for HIP and 50mm×50mm×15mm for PCG. The voxel size is 10.0mm×10.0mm×15.0mm for two of them. The VOI size of HIP was designed to matching bilateral HIP, which is located in the skull base, to avoid the interference from other factors, such as skull, cerebrospinal fluid. Automatic pre scanning program was used to adjust the gain of voxel, receive/transmit, semi-automatic shimming, weak water suppression, FWHM < 25Hz, Water suppression level > 95%. After the scanning, A series of post-processing steps are used to obtain the MRS data include water reference processing, Filter, Zero-filling, Fourier transformation, Frequency shift correction, Baseline correction, Phase correction, and Curve fitting. The chemical shifts of metabolites were 2.02ppm for NAA, 3.03ppm for tCr and 3.22ppm for Cho. tCr as internal parameter, NAA/tCr, Cho/tCr ratio was calculated. MMSE (Chinese version) and MoCA (Beijing version) were evaluated by the same clinician before MRS examination in MCI group and NC group.

Neuropsychological assessment

Cognitive assessments include MMSE (Chinese version) [56], and MoCA (Beijing version). The MMSE consists of several tasks, testing direction, memory, attention, calculation, language (naming, repetition, auditory comprehension, reading and writing) and visual special ability, the highest total score is 30. MoCA tests 8 cognitive areas, visual spatial ability, attention, executive function, immediate memory, delayed memory, language, abstraction, calculation and orientation, the highest total score is 30. If the subjects' education years are less than 12 years, the measured total score is the test score plus 1 point. All tests were administered and graded by professionals trained in neuropsychological testing.

Statistical analysis

Statistical analyses were performed using SPSS 22.0. Demographic clinical characteristics and metabolite ratios relative to tCr across the MCI and NC groups were assessed by one way ANOVA tests and repeated measurement analysis, followed by post hoc multiple comparisons of least-significant difference (*LSD*). Statistical significance was set at $P < 0.05$.

Results

Demographic and neuropsychological assessment

The demographic and neuropsychological assessment data of the four groups are listed in Table 1. ANOVA revealed group differences of MMSE scores ($F = 78.428$, $P < 0.0001$) and MoCA scores ($F = 78.428$, $P < 0.0001$), with a significant decrease in MMSE scores and MoCA scores in the three treatment groups compared to the NC ($P < 0.0001$).

Table 1
Demographic and neuropsychological assessment data

	NC	Drug	Sham	Acupoint	F/ χ^2	P
N (M/F)	67(25/42)	24(7/17)	20(4/16)	25(9/16)	2.350	0.503
Age (years)	64.76 \pm 5.73	66.00 \pm 6.42	64.25 \pm 7.50	63.52 \pm 6.20	0.686	0.562
Course of disease	21.75 \pm 27.58	34.67 \pm 18.35	33.8 \pm 25.41	29.48 \pm 15.55	0.479	0.622
Educations (years)	11.85 \pm 3.04	10.13 \pm 2.82	10.85 \pm 2.94	11.36 \pm 2.00	2.410	0.070
MMSE	29.16 \pm 0.75	25.46 \pm 1.38*	26.25 \pm 1.07*	25.80 \pm 1.41*	113.399	< 0.0001
MoCA	26.06 \pm 2.01	21.46 \pm 3.26*	21.95 \pm 2.72*	21.44 \pm 2.66*	36.438	< 0.0001

Data represent number or mean \pm SD. Data were analyzed by one-way ANOVA. NC = normal control; M = Male; F = Female; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; Compared with NC: * $P < 0.001$

Changes of metabolites in hippocampus and posterior cingulate gyrus of four groups before treatment

The changes of metabolites in bilateral hippocampus and posterior cingulate gyrus were shown in Table 2. NAA/tCr in bilateral hippocampus, posterior cingulate gyrus and Cho/tCr in right hippocampus of the three treatment groups were lower than those in NC group ($P < 0.05$). There was no significant difference in Cho/tCr in left hippocampus and bilateral posterior cingulate gyrus between the three treatment groups and NC group ($P > 0.05$). There was no significant difference in NAA/tCr and Cho/tCr in bilateral hippocampus and posterior cingulate gyrus among the three treatment group ($P > 0.05$).

Table 2
Changes of metabolites in hippocampus and posterior cingulate gyrus before treatment

	NC	Drug	Sham	Acupoint	F	P
L.HIP NAA/tCr	1.38 ± 0.23	1.10 ± 0.21*	1.17 ± 0.22*	1.05 ± 0.19*	18.893	<0.0001
L.HIP Cho/tCr	1.00 ± 0.18	0.94 ± 0.24	0.98 ± 0.28	0.96 ± 0.17	0.533	0.661
R. HIP NAA/tCr	1.36 ± 0.25	1.07 ± 0.17*	1.16 ± 0.14*	1.05 ± 0.14*	20.365	<0.0001
R.HIP Cho/tCr	1.04 ± 0.19	0.90 ± 0.18*	0.91 ± 0.20*	0.89 ± 0.19*	7.042	<0.0001
L. PCG NAA/tCr	2.07 ± 0.21	1.85 ± 0.27*	1.85 ± 0.20*	1.81 ± 0.17*	12.954	<0.0001
L.PCG Cho/tCr	1.07 ± 0.18	1.03 ± 0.13	1.06 ± 0.24	1.06 ± 0.32	0.175	0.913
R.PCG NAA/tCr	2.00 ± 0.24	1.82 ± 0.30*	1.86 ± 0.18*	1.77 ± 0.20*	7.870	<0.0001
R.PCG Cho/tCr	1.07 ± 0.22	1.02 ± 0.19	1.09 ± 0.24	1.01 ± 0.14	0.934	0.426

Left = L.; Right = R. HIP = hippocampus; PCG = posterior cingulate gyrus; NAA = N-acetylaspartate; Cho = choline; tCr = total creatine; Compared with NC group: * $P < 0.05$.

Changes of metabolites in hippocampus and posterior cingulate gyrus of four groups after treatment

After two months of treatment, NAA/tCr in bilateral hippocampus, posterior cingulate gyrus and Cho/tCr in right hippocampus were increased in the three treatment groups, and there was no significant difference compared to NC group ($P > 0.05$, Table 3). NAA/tCr in right hippocampus and Cho/tCr of right posterior cingulate gyrus of acupoint group was significantly higher than those in sham point group ($P < 0.05$) and NC group ($P < 0.05$, Table 3). There was no significant difference in NAA/tCr and Cho/tCr in bilateral hippocampus and posterior cingulate gyrus between drug group and sham point group and acupoint group (Fig. 2).

Table 3
Changes of metabolites in hippocampus and posterior cingulate gyrus after treatment

	NC	Drug	Sham	Acupoint	F	P
L.HIP NAA/tCr	1.38 ± 0.23	1.38 ± 0.21	1.29 ± 0.21	1.40 ± 0.21	1.145	0.333
L.HIP Cho/tCr	1.00 ± 0.18	1.06 ± 0.18	1.03 ± 0.23	1.03 ± 0.19	0.750	0.524
R.HIP NAA/tCr	1.36 ± 0.25	1.37 ± 0.23	1.26 ± 0.17	1.41 ± 0.22 ^Δ	1.589	0.195
R.HIP Cho/tCr	1.04 ± 0.19	1.09 ± 0.24	0.99 ± 0.18	1.10 ± 0.28	1.169	0.324
L.PCG NAA/tCr	2.07 ± 0.21	2.16 ± 0.32	1.98 ± 0.20	2.08 ± 0.25	2.063	0.108
L.PCG Cho/tCr	1.07 ± 0.18	1.13 ± 0.21	1.11 ± 0.19	1.14 ± 0.22	1.232	0.301
R.PCG NAA/tCr	2.00 ± 0.24	2.09 ± 0.27	1.96 ± 0.22	2.09 ± 0.25	1.742	0.162
R.PCG Cho/tCr	1.07 ± 0.22	1.11 ± 0.20	1.12 ± 0.23	1.18 ± 0.23*	1.708	0.169
Left = L.;Right = R.;HIP = hippocampus;PCG = posterior cingulate gyrus; NAA = N-acetylaspartate; Cho = choline;tCr = total creatine; Compared with NC: * $P < 0.05$;Compared with sham point group: ^Δ $P < 0.05$.						

Comparison of MMSE scores in four groups at different time points

There were significant differences in MMSE scores between the three treatment groups and the normal control ($F = 22.460$, $P < 0.0001$). The results showed that acupoint group and sham point group had better effect, and there was interaction between group and time ($F = 21.910$, $P < 0.0001$). The MMSE scores at different time points after treatment also had statistical significance ($F = 107.689$, $P < 0.0001$), showing that the effect was the best after 2 months of treatment, MMSE showed a downward trend in the fifth month compared with that after 2 months of treatment (Table 4 and Fig. 3).

Table 4
Comparison of MMSE scores in four groups at different time points

group	n	Time			
		Before	One month	Two months	Fifth month
NC	67	29.16 ± 0.75	29.13 ± 0.76	29.16 ± 0.75	29.13 ± 0.76
Drug	24	25.46 ± 1.38	27.58 ± 2.45**	28.04 ± 2.35**	27.71 ± 2.81**
Sham	20	26.25 ± 1.07	28.40 ± 1.14**	28.50 ± 1.76**	28.00 ± 0.80**
Acupoint	25	25.80 ± 1.41	28.8 ± 1.29**	29.00 ± 1.32**	28.24 ± 2.19**#

Before = Before treatment; One month = After one month of treatment; Two months = After two months of treatment; Fifth month = Return visit in the fifth month. Compared with the same group before treatment, ** $P < 0.01$; Compared with the same group after 2 months of treatment, # $P < 0.05$.

Comparison of MoCA scores in four groups at different time points

There was significant difference in MoCA scores between the three treatment groups and the normal group ($F = 8.621$, $P < 0.0001$). The MoCA scores of acupoint group and sham point group were higher than those of drug group. There was interaction between group and time ($F = 24.415$, $P < 0.0001$), and MoCA scores at different time points after treatment were also significantly different ($F = 111.378$, $P < 0.0001$). After 2 months of treatment, MoCA scores of acupoint group and sham point group were increased, and still maintained good effect in the fifth month of follow-up. The MoCA score in the drug group was better after 2 months of treatment, but with the extension of time, the MoCA score at the fifth month of follow-up was lower than that after 1 month and 2 months of treatment (Table 5 and Fig. 3).

Table 5
Comparison of MoCA scores in four groups at different time points

group	n	Time			
		Before	One month	Two months	Fifth month
NC	67	26.06 ± 2.01	26.03 ± 2.01	26.06 ± 2.01	26.03 ± 2.01
Drug	24	21.46 ± 3.26	23.88 ± 3.08**#	24.88 ± 3.28**	23.33 ± 3.95**#
Sham	20	21.95 ± 2.72	26.15 ± 2.28**	26.40 ± 2.60**	26.90 ± 1.83**
Acupoint	25	21.44 ± 2.66	25.36 ± 3.24**	26.12 ± 3.15**	26.24 ± 3.40**

Before = Before treatment; One month = After one month of treatment; Two months = After two months of treatment; Fifth month = Return visit in the fifth month. Compared with the same group before treatment, ** $P < 0.01$, * $P < 0.05$; Compared with the same group after 2 months of treatment, # $P < 0.05$.

Discussion

As far as we know, this is the first study using MRS to observe moxibustion in the prevention and treatment of mild cognitive impairment. Moxibustion is a Chinese medical therapy widely used in East Asian countries [58]. As part of traditional acupuncture practice, moxibustion applies the heat of burning moxa floss, primarily from *Artemisia vulgaris*, to stimulate specific spots, known as acupuncture points on the surface of skin [59]. Heat transfer and radiation play an important role in the therapeutic mechanism of moxibustion [60]. The temperature on surface during the burning moxa was controlled to be lower than 60°C, which is the highest temperature that human skin can endure and without scarring, it was found Tissue up to 3 cm deep can be heated during the treatment by an examination magnetic resonance imaging (MRI) of the three-dimensional temperature elevation [61]. Increasing of the temperature in skin via burning moxa can lead to activation of thermosensitive receptors (TRPV channels), which can induce different biological and chemical reactions in the body [62]. It has been suggested recently TRPV1 activation as a novel therapeutic target that activation of TRPV1 in rodents protects neurons from cytotoxic effects of Amyloid- β peptide (A β), and reverses hippocampal damage and memory impairment [63]. This study observed that after moxibustion treatment for 2 months, the long-term effect of MMSE and MoCA scores was superior between acupoint and non acupoint groups than that of drug group. We speculate that heat transfer and radiation of moxa cone on the acupoint are the critical mechanisms for improving brain metabolites in MCI patients.

A systematic review showed that moxibustion might help prevent AD, and its mechanism of action might include increasing neurotrophin and heat shock protein activity, regulating cell cycle factors, and/or suppressing apoptosis and inflammation [47]. Studies have examined the mechanism of moxibustion as related to anti-aging [64]. Preventive and therapeutic moxibustion on Baihui (GV 20) and Shenshu (BL 23) acupoints could reduce cell apoptosis in the hippocampus and improve the learning and memory abilities in AD, which has a beneficial effect for the prevention of AD development [65]. Moxibustion on Guanyuan (RN 4) could improve the learning and memory function of AD animals by regulating amyloid production pathway, which would be an effective regulation point for the efficacy of moxibustion [66]. Moxibustion and moxa smoke could improve the learning and memory function of AD animals by regulating the metabolism of tricarboxylic acid and unsaturated fatty acid [67]. Our previous study observed that Moxibustion Zusanli (ST 36) and Xuanzhong (GB 39) could be used to complement free radical scavenging, increase endogenous SOD in cerebral tissues and delay the process of aging in the brain of D-galactose-induced senescent mice [68] and animal experiments have confirmed that moxibustion on Baihui (DU 20), Zusanli (ST 36), Xuanzhong (GB 39) and Guanyuan (RN 4) acupoints could improve the learning and memory ability of brain aging rats, and delay brain aging [69].

The functional significance of the NAA variance is underlined by studies showing that NAA levels covary with cognitive performance [70]; ¹H-MRS of the hippocampal region in AD and MCI patients demonstrated a decrease in NAA and NAA/Cr ratios were predictive of future conversion from MCI to AD [36, 71]. Overall, NAA decrease in AD has been shown in at least 18 reports, including in vitro studies showing a correlation with AD pathology [72, 73]. Cognitive change has also been related to MI and NAA

alteration in the hippocampus individuals with AD [74, 75]. There have been conflicting reports regarding Cho levels and Cho/Cr ratios in patients with AD. Some studies [76, 77] investigators have reported elevated Cho levels and/or Cho/Cr ratios with AD, while others have not [78–80]. It is known that Cho levels in the hippocampus of normal individuals are higher than when compared with other brain regions [81], reports have so far failed to show a difference in hippocampal Cho levels between patients with AD and control subjects [74, 75]. Of the 15 in vivo MRS studies reviewed, however, only two reported a significant choline elevation in AD [82, 83]. The decrease in the level of Cho/Cr ratio observed in study following treatment is presumably considered as a quantitative biomarker for monitoring of the AD progression [84]. The current study observed that NAA/tCr in bilateral hippocampus, posterior cingulate gyrus and Cho/tCr in right hippocampus of the MCI patients were decreased. Chung-Man found that the hippocampal volume was positively correlated with the K-MMSE score and the ratio of NAA/Cr [84]). Lower levels of neuronal integrity marker NAA to Cr ratio in the posterior cingulate gyrus on MRS is associated with a higher risk of progression to AD dementia in patients with MCI [85]. This study showed that after treatment NAA/tCr in bilateral hippocampus, posterior cingulate gyrus and Cho/tCr in right hippocampus were increased, which showed that moxibustion and donepezil could improve brain metabolites in MCI patients. After moxibustion and drug treatment, MMSE and MoCA scores of MCI patients were improved. It is implied that moxibustion and drug therapy can improve the cognitive function of MCI patients, which may be related to regulating the metabolites of NAA/tCr and Cho/tCr in hippocampus and posterior cingulate gyrus. The scores of MMSE and MoCA in the acupoint group and sham point group were better than those in the drug group, especially in the long-term efficacy. NAA/tCr in right hippocampus and Cho/tCr in right posterior cingulate gyrus of acupoint group was significantly higher than sham point group, it may be related to the therapeutic effect of acupoints. In this study, we observed that sham point group can also improve cognitive ability and brain NAA/tCr, Cho/tCr metabolites in MCI patients. We considered that the first is that the sham point is opened near the left temporal lobe and the right abdominal Guanyuan(RN 4), which also has a local treatment effect; the other is the combined effect of local heat effect and light spectrum radiation produced by moxibustion, thus causing physiological and biochemical changes of the body [86]. The therapeutic effect of sham point group needs further observation and analysis. A fMRI study using a memory face-name recognition paradigm demonstrated greater hippocampal activation, especially right hippocampal and right frontal activation, in lowperforming normal older adults [87]. We found that NAA/tCr and Cho/tCr in the right hippocampus of MCI patients were lower than those in the normal control group, suggesting that NAA/tCr and Cho/tCr in the right hippocampus may be sensitive markers for early diagnosis of MCI.

Our results suggest that right hippocampus NAA/tCr and Cho/tCr ratios measured at MRS may be sensitive markers of future progression to dementia in a clinically defined elderly population with isolated, mild memory complaints. Moxa cone moxibustion can effectively regulate the metabolism of NAA/tCr and Cho/tCr in the brain of MCI patients, so as to improve the cognitive function. The application of ¹H-MRS for the metabolic evaluation of consequences of hippocampus and posterior cingulate gyrus in the brain may help understand disease symptoms and progression as well as the mechanisms of brain plasticity in general.

Limitations

There were some limitations in our research. First of all, we used long TE in this study, which may lead to biased results, because the T2 relaxation time between queues is unknown, which is a deficiency of this study. Secondly, due to the influence of time, manpower and material resources, this research did not study the stimulation amount of moxibustion. Is the influence of stimulation amount of moxibustion on clinical efficacy related to the size of moxa cone, temperature, time and times of moxibustion? These are the problems that need to be optimized and further studied in the future.

Conclusions

The NAA/tCr and Cho/tCr ratios in right hippocampus may be sensitive markers of future progression to dementia. Moxa cone moxibustion can effectively regulate the metabolites of NAA/tCr and Cho/tCr in the brain of MCI patients, so as to improve the cognitive function. The application of ¹H-MRS for the metabolic evaluation of consequences of hippocampus and posterior cingulate gyrus in the brain may help understand disease symptoms and progression as well as the mechanisms of brain plasticity in general.

Declarations

Acknowledgements

Not applicable.

Author Contributions

LZ, WM designed the study, WM, LT, LZ, ZZ, JT, JS, XN, BY, LS performed the experiments. CL, YW, GD, DD, SC analyzed and interpreted the data. WM, LZ wrote the manuscript. All authors read the manuscript, approved the final manuscript and have substantial contribution in the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81360561, 81760886, 81973922); The Science and Technology plan of Guangxi (Gui, Grant No. 14124004-1-27), the Science and Technology plan of Guangdong (Grant No. 2016A020215202), Plan of Shenzhen Science and Technology Innovation Committee (Grant No. JCYJ20190809151013581).

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by the Medicine Ethics Committee of the First Affiliated Hospital, Guangxi University of Chinese Medicine. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this manuscript.

Consent for publication

All authors have approved of the manuscript and agree with its submission.

Competing interests

The authors declare that they have no competing interests.

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Figures

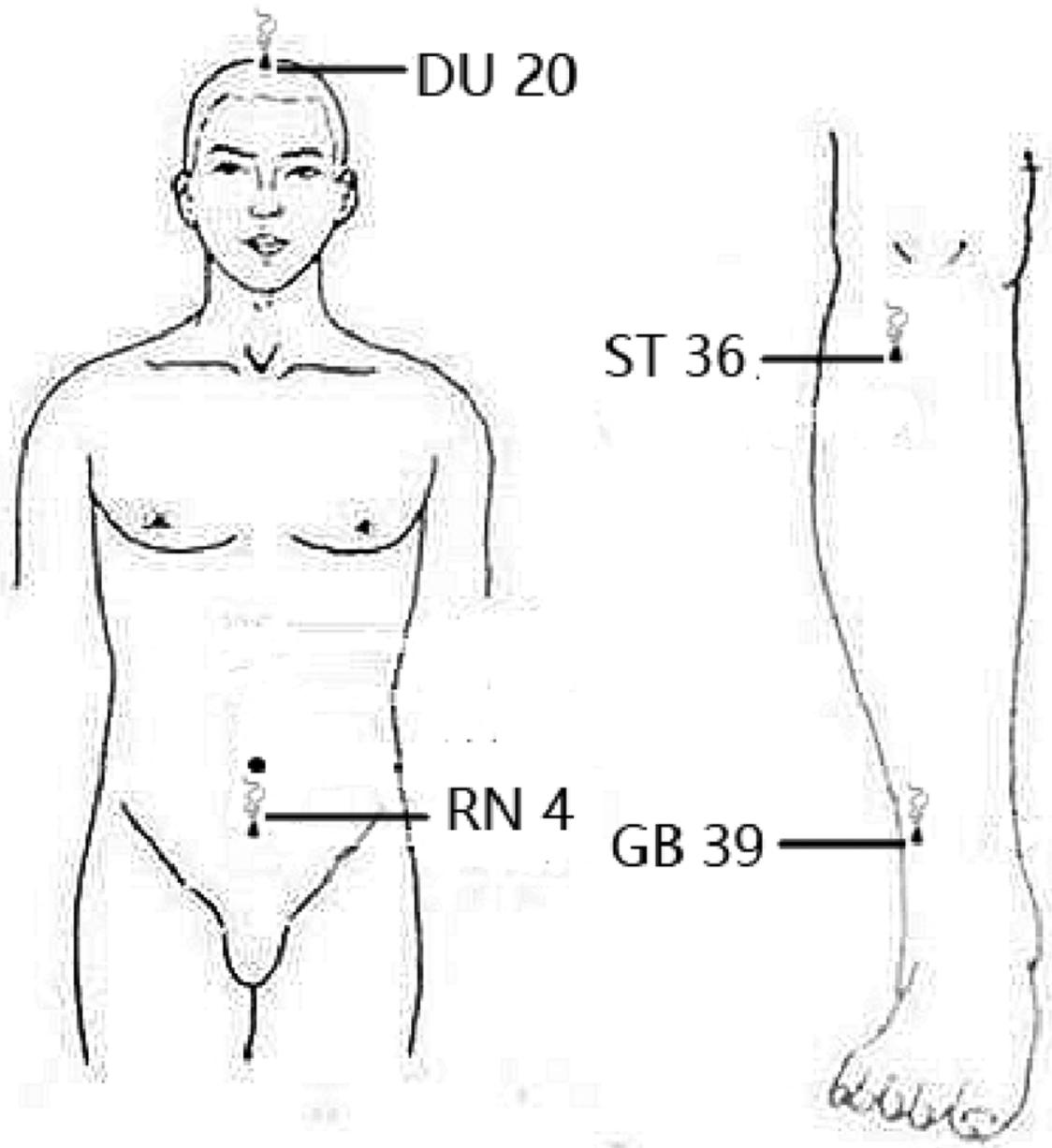


Figure 1

Location of acupoints used in the experiment. DU 20 located in the middle of the posterior hairline, seven inches straight up. RN 4 located in the front midline, three inches below the umbilicus. ST 36 located on the anterior aspect of the lower leg about one finger-breadth lateral to the tibia, just inferior to the tibial tuberosity. GB 39 located three inches above the high point of lateral malleolus and at the front edge of fibula.

A Acupoint group: before moxibustion treatment

B Acupoint group: after moxibustion treatment

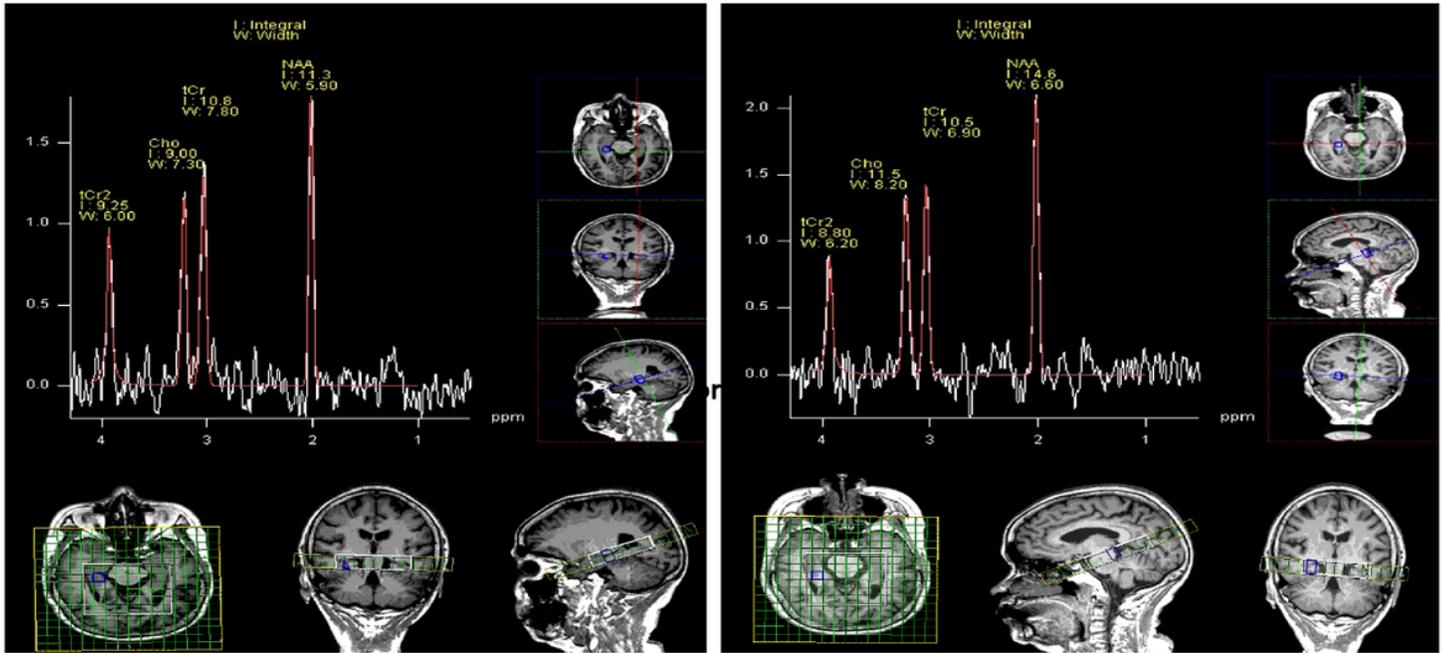


Figure 2

An example of 1H-MRS data obtained in a seventy-four year old MCI female volunteer from acupoint group. NAA=N-acetylaspartate; Cho=choline; tCr=total creatine. Part A demonstrates that before moxibustion treatment the metabolites ratios are (NAA/Cr=1.05, Cho/Cr=0.84) in the Right HIP. Part B demonstrates that after moxibustion treatment the metabolites ratios are (NAA/Cr=1.40, Cho/Cr=1.10) in the Right HIP.

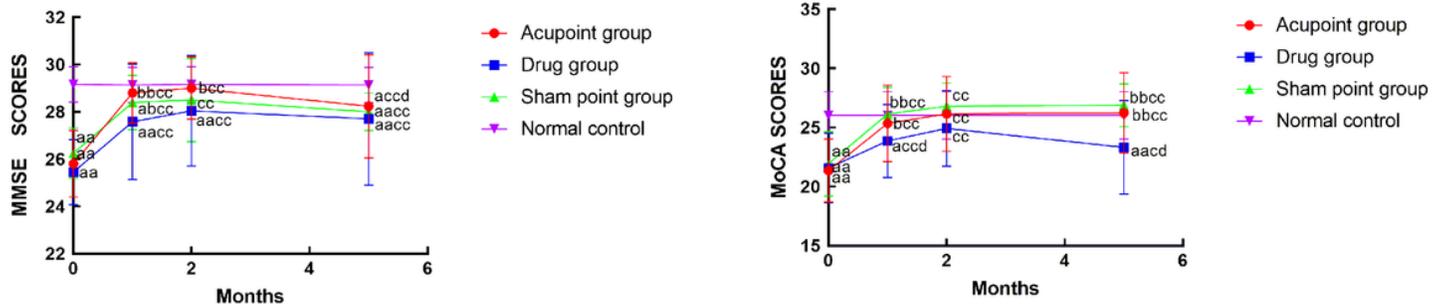


Figure 3

Scores of MMSE and MoCA in the normal control, drug, sham point and acupoint groups. aaP<0.01, aP<0.05, vs normal control group; bbP<0.01, bP<0.05, vs drug group; ccp<0.01, cP<0.05, vs the same group before treatment; dP<0.05, vs the same group after 2 months of treatment.