Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model

Yin-Qiu Fan¹, Jun Yang²*, Jing-Cheng Cui¹, Pin Wang³, Yue Li¹ and Li Gui¹

¹Department of Postgraduate Education, Anhui University of Traditional Chinese Medicine, Hefei, Anhui, China
²1st Affiliated Hospital, Anhui University of Traditional Chinese Medicine, Hefei, Anhui, China
³Clinical Department of Acu-moxibustion and Orthopaedic, Anhui University of Traditional Chinese Medicine, Hefei, Anhui, China

*Corresponding Author: Jun Yang, 1st Affiliated Hospital, Anhui University of Traditional Chinese Medicine, Hefei, Anhui, China.

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Abstract

Neuron apoptosis due to ischemia has been considered to play one of the major roles in the pathogenesis of vascular dementia (VaD) and dysfunction of delay memory appears in the early phase of VaD. Previous study suggested that Huayutongluo Moxibustion could improve ischemia-induced cognitive impairments by stimulating angiogenesis in injured brain. However, whether delayed memory improvement is associated with neurogenesis and the effect of Huayutongluo Moxibustion on neurogenesis remains unclear. In this study, an animal model of VaD was established via bilateral common carotid arteries occlusion (BCCAo) to investigate the alteration of delayed memory, neurogenesis and angiogenesis. VaD rats suggested impairments in delayed memory as shown in Morris Water Maze. By NSCs+EPCs combinative implants or NSCs transplanted three days after ischemia injury and three courses of moxibustion treatment, VaD-induced delayed memory deficits were ameliorated. Expression of Nestin, DCX and CD34 significantly increased after treatment, which might contribute to the neurogenesis and angiogenesis in hippocampus. In addition, moxibustion significantly increased expression of Nestin, DCX and CD34 in NSCs+EPCs transplanted group of rats compared with those rats transplanted with NSCs. These findings suggested that Huayutongluo Moxibustion reversed VaD-induced delayed memory deficits, which might attribute to the promotion of neurogenesis and angiogenesis.

Keywords: Moxibustion; Vascular Dementia; Delayed Memory; Neurogenesis; Angiogenesis; Hippocampus

Abbreviations

VaD: Vascular Dementia; BCCAo: Bilateral Common Carotid Arteries Occlusion; AD: Alzheimer's Disease; NSCs: Neural Stem Cells; CNS: Central Nervous System; SGZ: Subgranular Zone; SVZ: Subventricular Zone; EPCs: Endothelial Progenitor Cells; EGFP: Enhanced Green Fluorescent Protein; ECM: Extracellular Matrix; AUCTM: Anhui University of Chinese Traditional Medicine; SPF: Special Pathogen Free; MWM: Morris Water Maze; PFA: Phosphate Buffer; MVD: Microvessel Density; CBF: Cerebral Blood Flow; WM: White Matter; NPCs: Neural Precursor Cells; VEGF: Vascular Endothelial Growth Factor

Introduction

Vascular Dementia (VaD) is a progressive disease that caused by reduced blood flow to the brain, which can affect cognitive abilities. VaD patients may suffer from slowed thinking, forgetfulness, depression and anxiety, disorientation, and loss of executive functions such

as problem solving, working memory, thinking, reasoning, judgment, planning and execution of tasks, with performance declining accompanied by increasing task complexity. VaD accounts for about 17 - 20% of all dementia patients making it the second leading form of dementia after Alzheimer’s disease (AD), and is prevalent among the older population [1]. Memory dysfunction especially immediate and delayed memory occurs in the early phase of VaD.

Recently, some pieces of research have demonstrated that neural stem cells (NSCs), which have a proliferation capacity of self-renewal and generation of both neurons and glia [2], play a key role in endogenous restoration in the mammalian central nervous system (CNS) [3]. Although neural regeneration in the brain declines with age [4], NSCs survive throughout life in a few distinct neurogenic zones, such as the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles [5]. Endogenous NSCs in these regions are activated after injury [6] and subsequently develop to mature neurons through a complex sequence of developmental steps, including self-renewal, differentiation, migration, targeting, and synaptic integration [5,7]. However, the quantity of activated endogenous NSCs is not sufficient to completely repair nerve injury [3].

Endothelial progenitor cells (EPCs) are the precursor cells of vascular endothelial cells, which contribute to new vessel formation in postnatal angiogenesis. They are mobilized by physiological and pathological stresses, such as exercise, trauma, tumor, and inflammation [8-10]. Increases in circulating EPCs are reported to improve clinical outcomes of stroke and myocardial infarction [11,12]. We have previously demonstrated that increasing EPCs could promote the functional recovery of brain trauma in a rat model but the effect was not good enough [13,14].

Using retroviral vector carrying enhanced green fluorescent protein (EGFP) to label rat fatal NSCs that cultivated in vitro. Then to construct the combinative implants which consist of NSCs, EPCs and appropriate extracellular matrix (ECM), and to transplant the combinative implants or NSCs into the lateral ventricle of the VaD rats respectively in order to simulate the micro-environment of neurogenesis and angiogenesis. Finally to explore the mechanism of Huayutongluo Moxibustion on VaD rats post-plantation by observing the variation of delayed memory, Nestin, DCX and CD34.

**Materials and Methods**

**Animals**

The study was carried out at the Animal Care Facility of the Anhui University of Chinese Traditional Medicine (AUCTM) and the experimental procedure followed the Guidelines for the Care and Use of Laboratory Animals which published in 2006 by the Ministry of Science and Technology of the People’s Republic of China. One hundred and forty male Special Pathogen Free (SPF) Wistar rats weighing 250 - 300g (at the start of the experiment) were purchased from the Animal Experimental Corporation of the Beijing Weitonglihua (Certification No. SCXK (Jing) 2012-0001). All animals were housed in a controlled environment (12h light/dark cycles, 25 ± 1°C).

One hundred and thirty-five rats were chosen for the experiment by Morris Water Maze (MWM) and 12 rats were separated into the sham group. The other rats were operated on the improved 2-VO method to make the VaD models. VaD models were identified three days after operation by MWM. The qualified models were randomly divided into three groups: VaD model group (n = 36); NSCs+EPCs group (n = 36); NSCs group (n = 36). In sham group and VaD model group, rats did not undergo any procedure unless the fixation. Moxibustion was administered on rats in NSCs+EPCs group and NSCs group once per day for seven consecutive days and a one-day rest after that. Seven days comprised a course of treatment. Each group of rats was subdivided into three subgroups considering of the course of treatment. Every subgroup had 12 rats. Rats of every subgroup were executed 24 hours after the first and second course. Rats of the third course would be tested by MWM 24 hours after treatment and then be sacrificed. The brains of rats were made into frozen sections and used to do immunofluorescence experiment.
Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model

Vascular dementia model

Bilateral common carotid arteries occlusion (BCCAo) was performed to make the VaD model. Rats were forbidden from eating 12 hours before the surgery. Briefly, rats were anesthetized with the mixture of 10% chloride hydrate (Guangfu, Tianjin, China) and 25% urethane (Solarbio, Beijing, China) (5.0 mL/kg, intraperitoneally) and then fixed. After being disinfected regularly, the rats were incised in the middle of the neck and the bilateral common carotid arteries were separated and occluded. The wounds were sutured and injected with gentamycin to prevent from infection. The rats in sham group were performed the same as the VaD group except for BCCAo. All rats were kept in different cages respectively after the surgery.

Model identification

Rats in every group were identified with MWM three days after making models. To compare the latency of every rat been operated with the mean latency of rats in sham group on the fourth day of visible platform experiment. The rats which showed statistically different were chosen for the VaD models.

Cell culture

The NSCs (Weikai Biotechnology Corporation, Tianjin, China) were cultivated in special medium for rats' NSCs (Weikai Biotechnology Corporation, Tianjin, China). The EPCs (MRC Biotechnology Corporation, Jiangsu, China) were cultivated in special medium for rats' EPCs (MRC Biotechnology Corporation, Jiangsu, China). Cells were cultured at 37°C with 5% CO₂ in an incubator (Thermo Scientific, Barrington, IL, USA). The NSCs medium was changed every two or three days and cells were passaged (1:2 or 1:3) in about 5 days. The EPCs medium was changed every two or three days and cells were passaged (1:3 or 1:4) in about 5 days. NSCs and EPCs that had been passaged three to five times were used for the experiment, which strongly maintained their proliferation and differentiation ability.

EGFP labeling of NSCs

To use retroviral vector carrying EGFP (Hongshan Biotechnology Corporation, Hefei, China) to infect rat fatal NSCs (MOI = 50) that cultivated in vitro for labeling. NSCs were cultivated in suspension and cultured at 37°C with 5% CO₂ in an incubator (Thermo Scientific, Barrington, IL, USA). To observe NSCs under fluorescence microscopy (OLYMPUS, Japan) with 450 - 490 nm laser and the green fluorescence could be seen even in the middle of NSCs.

Combinative implants construction

To digest the passaged EPCs with parenzyme (Solarbio, Beijing, China) until almost cells began to change in form and to use EPCs special medium to stop cells digesting. Digested EPCs were mixed with special medium together and cultivated in 6-well plates (Corning, America) then they grew to monolayer after 24 hours.

Primarily got rid of the medium and cultured suspended NSCs in the 6-well plates added with NSCs special medium after coating laminin (Sigma, America). NSCs grew to monolayer after 24 hours. Then got rid of the medium and cultured EPCs in the 6-well plates added with the mixture of NSCs and EPCs special medium after coating laminin. Two kinds of cells grew to multilayers after 24 - 48 hours.

Transplantation of combinative implants and NSCs

Three days after VaD model identification, the NSCs+EPCs group and NSCs group of rats were anesthetized by using the mixture of 10% chloride hydrate (Guangfu, Tianjin, China) and 25% urethane (Solarbio, Beijing, China) (5.0 mL/kg, intraperitoneally, respectively) and received stereotaxic (RWD-68505, Shenzhen, China) transplantation. Combinative implants or NSCs suspension with 1 × 10⁶ cells in 10 μL PBS was injected into the lateral ventricle of the right hemisphere in rats by micro- syringe pump respectively, with the following coordinates: Bregma, − 2.4 mm; M–L, −3.6 mm; D–V, −3.5 mm. Suspension was delivered at 1 μL/min and the needle was kept in situ for 5 minutes post-injection before being withdrawn slowly. The wound was then sutured and the animals were returned to the cages separately for follow-up experiments.

**Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model**

**Therapeutic methods**

Three days after the cells injection, rats in the NSCs+EPCs and NSCs group received moxibustion stimulation at DU14, DU20 and DU24 with homemade moxa sticks (L: 120 mm, D: 5 mm). Every acupoint was cured about 20 min every day. This treatment continued at 24-hour intervals for seven consecutive days as one course and involved three courses. Rats in the sham and VaD model group were also kept on the shelf for the same period without moxibustion and then all groups of rats were returned to their cages respectively.

**Morris Water Maze task**

Delayed memory was assessed by using MWM, n = 36 per group. Briefly, a rat-used tank measuring 180 cm in diameter and 40 cm in height was separated into four quarters and filled with water 25 cm in depth at 26 ± 2°C. A target platform (10 cm in diameter) was hidden 2 cm below the water surface in the first quarter halfway between the center and the wall of the maze. Rats were allowed to adapt the maze on the platform for 30 seconds before the visible platform experiment per day for three days. Afterwards, the rats were forced to swim in water and try to locate the submerged escape platform. Rats were ordered to swim to find the platform directly without adaption on the fourth day of the task. A computerized tracking system (Etho-vision 3.0; Noldus Information Technology, Wageningen, Netherlands) was used to record latency (time to reach the platform). Four trials from four random start positions in four different quarters were tested daily (each trial lasted for two minutes with 5 minutes intervals) for 4 consecutive days (from 1 day through 4 days post-treatment). Rats which failed to find the platform within 2 minutes were recorded for a maximum latency score of 120 seconds.

**Immunofluorescent staining**

Rats in the VaD, NSCs+EPCs and NSCs group were deeply anesthetized and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde dissolved in 0.1M phosphate buffer (PFA). Next, the brains were quickly harvested, postfixed in 4% PFA for 24 - 48 hours, and immersed in 30% sucrose solution for storage at 4°C prior to sectioning and frozen serial coronal brain sections (10 μm) were prepared on a cryostat (Leica-CM1900, Germany). The sections located in SGZ of the hippocampal dentate gyrus and SVZ of the lateral ventricle were blocked with 0.1% Triton X-100 and 10% normal blocking serum which had original species the same as the secondary antibody in PBS at room temperature for 2 hours to avoid unspecific staining. All sections were incubated with mouse primary antibody for anti-EGFP (1:100, MRC Biotechnology Corporation, Jiangsu, China) firstly. Then rabbit primary antibody for anti-Nestin (1:100, Abcam, UK), anti-DCX (1:100, Abcam, UK) or anti-CD34 (1:100, Abcam, UK) was added respectively for different target detection. Briefly, sections were incubated with both primary antibodies overnight at 4°C, followed by anti-mouse FITC (1:200, Santa cruz, USA) and anti-rabbit TRITC (1:500, SIGMA, USA) for 2h at room temperature without light. Then sections were counterstained with DAPI for visualization of nuclei for 10 minutes, followed by three washes of 15 minutes each in PBST (0.1% Triton in PBS) and coverslipped for microscopic observation.

**Quantification and imaging**

All the quantification was done using the Image J program. Nestin-positive, DCX-positive and CD34-positive cells were mainly observed in SGZ of the hippocampal dentate gyrus and SVZ of the lateral ventricle. At least three sections per rat were used for Nestin and DCX quantification by calculating MOD of the target protein. Quantification of CD34 needed at least three sections every rat. Six fields of view were chosen per section and new-born microvessel density (MVD) was quantified by counting CD34-positive cells. Microscopic imaging was done using OLYMPUS LSM confocal microscope. Images were acquired as tile scans with Objectives Lens: 40 X, Sampling Speed: 8.0 us/Pixel, Image Size: 2048 x 1536, Integration Type: Frame Kalman and analyzed using the OLYMPUS FV 1000 image-analysis software. Images for different experimental interventions were acquired under the same laser and microscopic parameters for the purpose of consistency.


Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model

Statistical analysis

Statistical analysis was conducted using Statistical Package of Social Science SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were expressed as Mean ± SEM and were subjected to test the normality. The data according with normality distribution were aimed to statistical analysis using one-way analysis of variance (ANOVA) followed by LSD test or Dunnett T3 test for multiple group comparison according to the homogeneity of variance. Values of p < 0.05 were considered statistically significant. The data according with skewed distribution were aimed to statistical analysis using Kruskal-Wallis H rank-sum test and the value of p < 0.05 were considered statistically significant. Mann-Whitney U test was used for the following multiple group comparison and the value of p < α’ were considered statistically significant (α’ = 2α/k (k-1), k was represented for the groups compared following).

Results

Model identification

Kruskal-Wallis H rank-sum test should be used to analyze since the data presented skewed distribution because of much censored data. Values of p < 0.05 were considered statistically significant. Mann-Whitney U test was used for the following multiple group comparison and the value of p < α’ were considered statistically significant. In this experiment k equaled four and α’ was 0.008.

It has been shown that the latency of VaD, NSCs+EPCs moxibustion and NSCs moxibustion group was longer than that of sham group on the fourth day of visible platform experiment (p < 0.008) whereas no statistically significant has be shown between VaD, NSCs+EPCs moxibustion and NSCs moxibustion group (p > 0.008) (Figure 1). It could be considered successful VaD model.

![Figure 1: VaD model identification with MWM. * p < 0.008 vs sham group.](image)

EGFP labeling of NSCs

Neurospheres began to form 48 hours after retroviral vector labeling EGFP infecting NSCs. Cells grew in suspension and green fluorescence could be seen in the neurospheres under fluorescence microscope (Figure 2).

Figure 2: EGFP labeling of NSCs. NSCs was shot by fluorescent microscope under fluorescent or optical light source with 100 or 200 times enlargement respectively (A1-B2).
- A1 shot under fluorescent light source×100
- A2 shot under optical light source×100
- B1 shot under fluorescent light source×200
- B2 shot under optical light source×200.

Combinative implants construction

The combinative implants were irregular under microscope. NSCs had characteristic of reflective rays and formed into big neurospheres. EPCs grew in the shape of rhombus or fusiform and had bigger nucleuses than that of NSCs. NSCs were encircled by EPCs or cohered each other (Figure 3).

Figure 3: NSCs+EPCs combinative implants. Combinative implants were shot by fluorescent microscope under fluorescent, optical or double light source with 200 times enlargement respectively (A1-A3).
- A1 shot under fluorescent and optical light source×200
- A2 shot under fluorescent light source×200
- A3 shot under optical light source×200.

Improved recovery of delayed memory after NSCs+EPCs combinative implant transplanted into rats’ brains treated with Huayutongluo moxibustion

As expected, latency was significantly shortened on the fourth day of visible platform experiment compared with before treatment (p < 0.05) except VaD model group, suggesting that delayed memory had developed in all rats except VaD model group of rats (Figure 4). However, the escape latency of all rats was influenced by grouping. The rats subjected to NSCs+EPCs combinative implant transplantation and moxibustion had shorter latency than those in VaD model group (p < 0.008), which indicated that Huayutongluo Moxibustion improved the recovery of delayed memory after NSCs+EPCs combinative implants transplanted into VaD rats brains.

![Figure 4: Delayed memory of different groups of rats after different treatments.](image)

*"p< 0.05 vs before treatment in the within-group comparison; # p < 0.008 vs VaD model group in the between-group comparison.

Increased neurogenesis and angiogenesis in the right lateral SVZ after Huayutongluo Moxibustion coupled with NSCs+EPCs combinative implant transplantation

VaD group should be excepted to do comparison since no positive cells could be found under microscope. The level of neurogenesis was assessed by quantifying Nestin and DCX positive cells, a specific marker of NSCs proliferation and differentiation respectively. The level of angiogenesis was assessed by counting CD34 positive cells, a specific marker of new-born micro vessel. Our results proved that neurogenesis and angiogenesis increased in the lateral SVZ after Huayutongluo moxibustion but the changes was different according to groups and treatment courses.

Quantification of Nestin positive cells showed significant decrease after the 2nd and 3rd treatment courses compared with the 1st treatment course in NSCs+EPCs moxibustion group (p < 0.05). Similar variation trend was found in NSCs moxibustion group that quantification decreased after 3rd treatment course compared with the 1st treatment course (p < 0.05). And quantification in NSCs moxibustion group was significantly less than that in NSCs+EPCs moxibustion group after the 1st and 3rd treatment courses (p < 0.05) (Figure 5).

Figure 5: Expression of Nestin in the right hippocampus of rats.
DAPI: Blue; Nestin/EGFP: Yellow; Yellow Arrow: Target
C1-C3: Expression in NSCs+EPCs moxibustion group. C1: course 1, C2 course 2, C3: course 3.
D1-D3: Expression in NSCs moxibustion group. D1: course 1, D2 course 2, D3: course 3.
E: MOD of Nestin in different groups on different courses(*: p < 0.05 vs course 1 in the within-group comparison; #: p < 0.05 vs NSCs+EPCs moxibustion group in the between-group comparison).

Quantification of DCX positive cells indicated significant increase after the 2nd treatment course compared with the 1st and 3rd treatment courses in NSCs+EPCs moxibustion group (p < 0.05) and the quantification in NSCs+EPCs moxibustion group was significantly more than that in NSCs moxibustion group after the 2nd treatment course (p < 0.05) (Figure 6).

**Figure 6:** Expression of DCX in the right hippocampus of rats.

DAPI: Blue; DCX/EGFP: Yellow; Yellow Arrow: Target

C1-C3: Expression in NSCs+EPCs moxibustion group. C1: course 1, C2: course 2, C3: course 3.
D1-D3: Expression in NSCs moxibustion group. D1: course 1, D2: course 2, D3: course 3.

E: MOD of DCX in different groups on different courses(*: p < 0.05 vs course 2 in the within-group comparison; #: p < 0.05 vs NSCs+EPCs moxibustion group in the between-group comparison).
It was revealed that counting of CD34 positive cells expressed significantly most after the 2\textsuperscript{nd} treatment course compared with the 1\textsuperscript{st} and 3\textsuperscript{rd} treatment courses in both NSCs+EPCs moxibustion group and NSCs moxibustion group (p < 0.05). The expression after the 3\textsuperscript{rd} treatment course was the least (p < 0.05). The counting in NSCs moxibustion group was always less compared with the NSCs+EPCs moxibustion group after the parallel treatment course (p < 0.05) (Figure 7).

**Figure 7**: Expression of CD34 in the right hippocampus of rats.

**DAPI**: Blue; **CD34/EGFP**: Yellow; **Yellow Arrow**: Target


**C1-C3**: Expression in NSCs+EPCs moxibustion group. C1: course 1, C2: course 2, C3: course 3.

**D1-D3**: Expression in NSCs moxibustion group. D1: course 1, D2: course 2, D3: course 3.

**E**: Number of CD34 in different groups on different courses (*: p<0.05; ※: p < 0.05 vs course 1 and course 2 respectively in the within-group comparison; #: p < 0.05 vs NSCs+EPCs moxibustion group in the between-group comparison).
Discussion

Vascular dementia (VaD) belongs to dementia according to theory of Traditional Chinese Medicine and always has some abnormal behaviors in psychology. Mental activities are regulated by brain and marrow in brain is the foundation of brain activity. Consequently, sea of marrow insufficient is the main pathology of VaD and block of blood stasis in collaterals as the main cause of sea of marrow insufficient also has close relations with VaD. Huayutongluo Moxibustion can be operated with compressive moxibustion on DU20 and suspended moxibustion on DU14 and DU24. The treatment can clear the meridian, remove the stasis, replenish the sea of marrow and refresh the consciousness by connecting the role of moxibustion and acupoints.

In VaD, chronic hypoperfusion and thromboembolic events can lead to a decrease in cerebral blood flow (CBF), hypoxia, oxidative stress and trigger inflammatory responses [15]. These changes can cause damage due to vessel endothelia, glial and neural cells that will further result in neurovascular uncoupling [16-18], neuro-degeneration, cell death [19], the impairment of neurogenesis, neural progenitor cell proliferation, synaptic plasticity, dendritic spine density, CBF reduction and so on [20]. The periventricular white matter (WM), basal ganglia, and hippocampus are highly susceptible to hypoperfusion. When the hypoperfusion occurs, hippocampus based learning and memory deficits ensue [15].

Current studies have demonstrated that acupuncture can improve VaD syndrome by reducing oxidative stress [21,22], attenuating neuron apoptosis [23,24], increasing the number of pyramidal neuron in the hippocampus CA1 region (Cornu Ammonis 1) [25], relieving neuroinflammation, regulating glucose metabolism [26], modulating neurotransmitters [27] and improving synaptic plasticity [28] and blood vessel function [29-31], etc.

Neuroscience has found that the cognitive function and memory of VaD rats can be improved by inducing neurogenesis in the hippocampus [32]. Neurogenesis is a process that NSCs reside and continuously proliferate and differentiate into new neurons [33]. Nestin is a kind of intermediate filament protein which is recognized as a marker of NSCs located in the brain neurogenic niches [2,34]. Nestin+ neural precursor cells (NPCs) are reported to have a potential source of newborn brain neurons [35,36]. DCX is a microtubule-associated protein expressed by NPCs, and is associated with neural regeneration [37]. It is associated with the normal brain development processes of neural cell birth and migration [38-40]. It has been demonstrated that ischemia and nerve injury can cause increased Nestin and DCX expression but the effect is not enough for functional recovery [33,41]. Acupuncture has been proved to improve ischemia induced neuronal damage, mainly by promoting neural regeneration, which is shown by increased Nestin and DCX expression [42,43]. EPCs are a population of cells which participate in vessel formation in both physiological and pathological processes, and demonstrate characteristics of both endothelial and progenitor cells [44]. They have great potential as a source of cells for the repair of vasculogenic injuries [45] and been used to repair ischemic or damaged cardiac tissue in animal models [46] by the creating of living blood vessels in postnatal angiogenesis [47,48]. VEGF is one of the important factors of angiogenesis. There are some reports that moxibustion could increase VEGF expression in VaD rat brain [49]. CD34 has been regarded to express on EPCs [50]. There is a micro-environment called neurovascular niche which is crucial for the neurogenesis and angiogenesis following stroke in adult brains [51,52]. In this neurovascular niche, angiogenesis can upregulate NSCs expansion for neurogenesis and in turn NSCs can regulate development of functional integrity in the neurovascular niche by secreting the factors that induce angiogenesis [53,54]. Accordingly, we proposed the hypothesis that Huayutongluo Moxibustion can promote neurogenesis and angiogenesis by inducing the proliferation, differentiation, maturation of NSCs and EPCs respectively.

Using a well-characterized VaD model, we examined dysfunction of delayed memory in rats subjected to BCCAo and correlated such changes with no Nestin+, DCX+ and CD34+ cells in SGZ of the hippocampal dentate gyrus and SVZ of the lateral ventricle.

We found that rats developed delayed memorial defects after making VaD models and those treated with doing moxibustion after NSCs+EPCs or NSCs transplantation improved delayed memory after treatment. The delayed memory of rats treated with NSCs+EPCs transplantation and moxibustion had higher development compared with rats in VaD group. In our study, doing moxibustion after NSCs+EPCs or NSCs transplantation to VaD rats significantly showed the trend of increasing level of Nestin, DCX and CD34 compared
Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model

with rats in VaD group. An obvious advantage could also be exhibited on NSCs+EPCs transplantation and moxibustion group than NSCs transplantation and moxibustion group. Therefore, apparent reversal of the BCCAo-induced delayed memorial dysfunction and other effects observed in SGZ of the hippocampal dentate gyrus and SVZ of the lateral ventricle could be considered as a consequence of the moxibustion and cells transplantation manipulation. Additionally, the different effects between two moxibustion groups may be caused by the interaction of NSCs and EPCs. Regarding the previous researches, it has been proved that neurogenesis and angiogenesis could be mutually facilitated. Based on these considerations, we conformed experimentally that the moxibustion could promote neurogenesis in brains of VaD rats and angiogenesis can regulate neurogenesis actively likewise.

Conclusion

In conclusion, rats treated with Huayutongluo Moxibustion alleviated VaD-associated dysfunction of delayed memory. This benefit from Huayutongluo Moxibustion treating is associated with improving neurogenesis and angiogenesis in the injured brain by regulating neurovascular niche. In addition, a high proliferating and differentiating level of NSCs and EPCs is critical for neurogenesis and angiogenesis respectively.

Conflicts of Interests

The authors state that there are no competing interests regarding the publication of this paper.

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Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model


Huayutongluo Moxibustion Ameliorate Delayed Memory Dysfunction Via Promoting Neurogenesis and Angiogenesis of Hippocampus in a Vascular Dementia Rat Model


642


