Effect of Cistanche deserticola Ma extract on memory of aged mice

Gui-fang Zhang¹, Li-shi Yin², Chao Zhang² and Hai yan Luo¹`

¹Department of Neurology Medicine, The Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, ²Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China

*For correspondence: Email: luohaiyan494@126.com; Tel: +86 15310692461

Sent for review: 15 December 2016   Revised accepted: 8 July 2017

Abstract

Purpose: To assess the memory-enhancing effect of Cistanche deserticola Ma (Orobanchaceae) extract (CDME) on normal aged mice.

Methods: An open-field test was used to study the effects of various doses of CDME on mouse locomotive activity. The mice were sacrificed following the locomotor test and the brain dissected to investigate the effects of CDME on catalase (CAT) and acetyl cholinesterase (AChE) activities in mouse brain tissue.

Results: Compared with the control aged group, CDME (400 mg/kg) significantly reduced the total distance traveled (3312.5 ± 119.3 cm, p < 0.05), increased brain CAT activity (107.6 ± 11.8U/mg pro, p < 0.05), and inhibited brain AChE activity (0.89 ± 0.13 U/mg pro, p < 0.05) in CDME-exposed mice.

Conclusion: The results show that CDME improves memory function in mice, probably by increasing the activity of CAT and decreasing AChE activity.

Keywords: Cistanche deserticola Ma, Memory function, Catalase, Acetyl cholinesterase, Open-field test

INTRODUCTION

Age-related learning and memory disorders have become more prevalent as human life expectancy has increased and constitute a major global healthcare issue. To combat these issues, it is vital to develop effective prophylactic and therapeutic agents for enhancing and maintaining memory functions before the onset of memory impairment in the aging population [1-3]. Traditional Chinese medicine has been used for improving memory and treating cognitive deficits for thousands of years. Cistanche deserticola Ma (Orobanchaceae) is a well-known medicinal shrub-like herb that has been commonly used as memory enhancer in China. Cistanche deserticola Ma (Orobanchaceae) extract (CDME) is also included in some traditional prescriptions for treating central nervous system disturbances [4-6]. This study was designed to investigate the effects of CDME on learning and memory impairment in normal aged mice. Learning and memory parameters were evaluated using an open-field test, and brain tissue catalase (CAT) and acetyl cholinesterase (AChE) activities were analyzed to determine potential mechanisms for the observed changes.

EXPERIMENTAL

Plant material and extraction

Cistanche deserticola Ma (Orobanchaceae) whole plant was collected in Kashi City, Xinjiang
Province, China, in October 2015. Taxonomic identification of the plant was made by Professor Heping Hu of Chongqing Medical University (Chongqing, China). A voucher specimen of *Cistanche deserticola* Ma (no. RRL 20151004) was deposited in the herbarium of the College of Pharmacy, Chongqing Medical University for future reference.

The *Cistanche deserticola* Ma plant material was dried in an oven at 100 °C for 12 h. The plant extract (CDME) was obtained by steeping the dried *Cistanche deserticola* Ma in 60 °C water for 1 h. This extraction process was repeated three times. The extract was freeze-dried to obtain a powder. One gram of powder was obtained from about 2.0 g of dried sample, giving a yield of approximately 50.0 %.

**Animals and study design**

Female C57BL/6J mice (60 mice aged 15 months and 12 mice aged 3 months) were purchased from the Experimental Animal Center of Chongqing (certificate no. SYXK 2005-0004). The mice were housed in groups of five animals per cage and kept under conditions of constant temperature (23 ± 2 °C) and humidity (50 ± 10 %) and a 12 h:12 h light-dark cycle. The animals had free access to standard chow diet and sterilized drinking water in the SPF Animal House. The 3-month-old mice were used as the 3-month-old normal control group. The 15-month-old mice were randomly assigned to six groups of 10 each, including the 15-month-old normal control group, the aged control group, the positive control galantamine (3 mg/kg) group, and the CDME (100, 200, and 400 mg/kg) groups. The mouse experiments were approved by the Animal Care and Use Committee of The Second Affiliated Hospital of Chongqing Medical University (approval ref. no. 20111006) and were carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [7].

Drug administration and behavioral assays were carried out using double-blind methodology. After 2 days of feeding, the 15-month-old mice received water alone orally (normal control group and the aged control group), galantamine at 3 mg/kg (positive control drug galantamine group), or CDME at 100, 200, and 400 mg/kg (L-CDME, M-CDME, and H-CDME groups, respectively) for a period of 4 weeks.

Locomotive activity of mice was evaluated using an open-field test on the first day of behavioral testing. Then, the mice were sacrificed by dislocation of cervical vertebrae, and the brain tissues were used for assessment of the CAT and AChE contents.

**Open-field test**

The effect of CDME on mice locomotor activity was evaluated automatically using an open-field computer-aided controlling system [8, 9]. The apparatus consisted of four metal tanks (30 cm in diameter and 40 cm in height) with a video camera fixed at the top. The apparatus was illuminated by a light source (120 Lux) on the ceiling.

Experiments were performed in a quiet room, and four mice were tested simultaneously. Thirty minutes after drug administration, the mice were placed at the center of the metal tank and allowed to explore freely for 5 min. The distance traveled by each mouse was measured to evaluate the locomotive activity of the mouse.

**Preparation of brain tissue samples and biochemical evaluation**

After behavioral measurements, all the mice were sacrificed by dislocation of cervical vertebrae. The brain tissues were quickly removed, washed with cold saline solution followed by 50 mM Tris-HCl buffer (pH 7.4), and weighed. Then, the tissues were placed in a glass bottle, labeled, and stored in a deep freezer (-25 °C) for a maximum of 10 h. The tissues were cut into small pieces and then homogenized in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Elektrocrafts, Mumbai, India) for 2 min at 5000 rpm.

The homogenate was then centrifuged (Remi, India) at 1000 rpm for 10 min to remove the debris. The upper supernatant fluid was extracted with an equal volume of ethanol and centrifuged at 17000 rpm for 30 min. The clear upper ethanol layer was removed and used for biochemical assay. All the preparations were performed at 4 °C. The CAT and AChE contents were estimated using commercial kits according to the manufacturer's protocols.

**Determination of CAT activity in mouse brain tissue**

CAT activity was assessed by measuring hydrogen peroxide at 405 nm [10, 11]. One unit (U) of CAT corresponds to the amount of the enzyme that hydrolyzes a specified amount (in mmol) of hydrogen peroxide per minute at 25 °C. Catalase activity was expressed as n moles of H$_2$O$_2$ metabolized/mg protein/h.
Determination of AChE activity in mouse brain tissue

AChE activity was determined as described by Ellman et al. [12] with some modifications. In brief, diluted homogenate (30 µL) was added to 2 mL of the reaction mixture, which contained 100 mM phosphate buffer (pH 8.0) and 1.0 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB), and incubated at 37 °C for 6 min.

Hydrolysis was monitored by determining the formation of the thiolate dianion of DTNB at 412 nm for 3 min in a spectrophotometer. AChE activity was calculated from the quotient between lymphocyte AChE activity and protein content.

One unit (U) of enzyme activity was defined as 1 µmol of AChE hydrolyzed/hour/milligram of brain homogenate or 1 µmol of AChE hydrolyzed/hour/milliliter of blood (pH 8.0, 25 °C).

Data analysis

All data were processed using Statistical Package SPSS 16.0 (SPSS Inc, Chicago, IL, USA), expressed as mean ± standard error of mean (SEM), and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s t-test. A p value of < 0.05 was considered statistically significant.

RESULTS

Effect of CDME on mouse locomotive activity in the open field test

Medium and high doses of CDME (200 and 400 mg/kg) significantly decreased locomotive activities of mice in the open-field test (p < 0.05; Table 1). Furthermore, galantamine (3 mg/kg) significantly reduced the total distance traveled compared with the aged control group (p < 0.05; Table 1).

Table 1: Effect of CDME on mouse locomotive activity

<table>
<thead>
<tr>
<th>Group</th>
<th>CDME (mg/kg)</th>
<th>Total distance (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>3429±127</td>
</tr>
<tr>
<td>Agedb</td>
<td>0</td>
<td>3842±115</td>
</tr>
<tr>
<td>Gal</td>
<td>3</td>
<td>3228±119</td>
</tr>
<tr>
<td>L-CDME</td>
<td>100</td>
<td>3689±122</td>
</tr>
<tr>
<td>M-CDME</td>
<td>200</td>
<td>3515±112</td>
</tr>
<tr>
<td>H-CDME</td>
<td>400</td>
<td>3295±116</td>
</tr>
</tbody>
</table>

Effect of CDME on CAT activity in mouse brain tissue

The CAT activity in brain tissue in aged control mice was significantly lower than that in 15-month-old normal control mice (p < 0.05; Table 2). However, mice treated with medium and high doses of CDME (200 and 400 mg/kg, respectively) and galantamine (3 mg/kg) had significantly higher CAT activities than aged control mice (p < 0.05).

Table 2: Effect of CDME on CAT activity in mouse brain tissue

<table>
<thead>
<tr>
<th>Group</th>
<th>CDME (mg/kg)</th>
<th>CAT activity (U/mg pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>122.7±14.8</td>
</tr>
<tr>
<td>Agedb</td>
<td>0</td>
<td>72.8±12.4</td>
</tr>
<tr>
<td>Gal</td>
<td>3</td>
<td>96.3±15.3</td>
</tr>
<tr>
<td>L-CDME</td>
<td>100</td>
<td>78.7±12.6</td>
</tr>
<tr>
<td>M-CDME</td>
<td>200</td>
<td>86.4±11.5**</td>
</tr>
<tr>
<td>H-CDME</td>
<td>400</td>
<td>112.2±10.7</td>
</tr>
</tbody>
</table>

**15-month-old control; *aged control; **galantamine; a low dose of CDME; b middle dose of CDME; c high dose of CDME; significantly different (p < 0.05) versus 15-month-old control group (n = 10); **significantly different (p < 0.05) versus aged control group (n = 10)

Effect of CDME on AChE activity in mouse brain tissue

As shown in Table 3, the aged mice showed increased AChE activity in the brain tissue of aged control mice compared with the 15-month-old normal control mice (p < 0.05). On the contrary, the activities of AChE in the brain tissue of mice markedly decrease (p < 0.05) when treated with CDME (200 and 400 mg/kg) and galantamine (3 mg/kg).

Table 3: Effect of CDME on AChE activity in mouse brain tissue

<table>
<thead>
<tr>
<th>Group</th>
<th>CDME (mg/kg)</th>
<th>AChE activity (U/mg pro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>1.27±0.21</td>
</tr>
<tr>
<td>Agedb</td>
<td>0</td>
<td>1.76±0.14</td>
</tr>
<tr>
<td>Gal</td>
<td>3</td>
<td>0.92±0.15</td>
</tr>
<tr>
<td>L-CDME</td>
<td>100</td>
<td>1.65±0.12</td>
</tr>
<tr>
<td>M-CDME</td>
<td>200</td>
<td>1.51±0.16</td>
</tr>
<tr>
<td>H-CDME</td>
<td>400</td>
<td>0.87±0.12</td>
</tr>
</tbody>
</table>

**15-month-old control; *aged control; **galantamine; a low dose of CDME; b middle dose of CDME; c high dose of CDME; significantly different (p < 0.05) versus aged control group (n = 10)

DISCUSSION

Learning and memory abilities are important functions of the brain in humans and rodents. Aging in humans is associated with deterioration of cognitive performance, particularly learning...
and memory abilities [13]. Aging animals have traditionally been used as a model of memory impairment [14]. Behavioral tests are one of the most reliable methods of investigating learning and memory abilities of animals. *Cistanche deserticola* Ma has been used as memory enhancer in Asia for thousands of years. Various animal models have demonstrated that *Cistanche deserticola* Ma can improve brain functions [15]. In the present study, the memory-enhancing effects of CDME on normal aged mice were investigated by using an open-field test.

The exact mechanisms responsible for the memory impairments with aging are still unclear, but evidence has accumulated that oxidative stress plays an important role [16]. Oxidative stress occurs when pro-oxidant and antioxidant levels become imbalanced. With aging, there is an increased production of reactive oxygen species and diminished endogenous antioxidant enzyme levels, leading to an increased oxidizing cellular environment. CAT, the main endogenous antioxidant enzyme, plays an important role in the intracellular antioxidant defense in the brain. Our study results showed that decreasing activities of CAT in the aged control mice could be partly reversed by CDME. These findings demonstrate that the memory-enhancing effects of CDME on the aged mice may be via the antioxidant system.

Aging is often accompanied by some alterations in neurotransmitter systems, such as acetylcholine and monoamine transmitters [17,18]. The transmission of these neurotransmitters in the brain has been long considered an important modulator of synaptic plasticity, memory consolidation, and other cognitive processes [19]. Under normal conditions, metabolic controls that are responsible for maintaining the levels of ACh and monoamine transmitters are catalyzed by AChE [20]. Our experimental data suggest that the CDME-mediated enhancement in spatial and non-spatial learning and memory abilities could be, at least partially, due to the decreasing activity of AChE in aged control mice. This suggestion is consistent with multiple behavioral tendencies.

**CONCLUSION**

The findings of this study indicate that CDME ameliorates learning and memory deficits in aged mice. Thus, CDME is a promising treatment for enhancing memory but further studies are required in this regard.

**DECLARATIONS**

**Acknowledgement**

This study was supported by Fund of Chongqing Medical University (no. XB200518).

**Conflict of Interest**

No conflict of interest associated with this work.

**Contribution of Authors**

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

**Open Access**

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/ 4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

**REFERENCES**


from: