Basic Investigations

Study on Perfume Stimulating Olfaction with Volatile Oil of Acorus Gramineus for Treatment of the Alzheimer's Disease Rat

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Objective: To probe into the therapeutic efect of perfume stimulating olfaction with volatile oil of Acorus Gramineus on the Alzheimer's disease (AD) rat.
Methods: Totally 50 adult SD rats, male,weighing 300±10 g, were randomly divided into 5 groups, normal group (group A), olfactory nerve severing model group (group B), AD model group (group C), AD model plus perfume stimulation group (group D), AD model olfactory nerve severing plus perfume stimulation group (group E), 10 rats in each group. After perfume stimulation, Morris maze test was conducted for valuating the learning and memory ability; Malondaldehyde (MDA) content, and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the brain, and the brain weight were detected.
Results: Compared with the AD model group,the average escape latency and swimming distance in 6 days were significantly shorter than those in the group A, B, D (P<0.01), with no significant differences between the group C and the group E (P>0.05); Compared with the group A, B and D, MDA content in the group C significantly increased (P<0.01), and SOD and GSH-Px activities significantly decreased (P<0.01), and brain weight/body weight decreased significantly in the group C (P<0.01), with no significant differences between the group C and the group E (P>0.05).
Conclusion: Perfume stimultating olfaction with volatile oil of Acorus Gramineus can significantly increase the learning-memory ability, decrease MDA content and increase SOD and GSH-Px activities and weight of brain in AD rats.

Keywords: *Alzheimer's disease; olfaction; learning and memory brain weight; MDA; SOD; GSH-Px; volatile oil of Acorus Gramineus*

Alzheimer's diease (AD) is a senile nerve system progressive retrograde disease, characterized mainly by cognition dysfunction, including memory, comprehension and orientation, etc. At present, cholinergic replacement, calcium channel blocker and neurotrophic factor often are used for its treatment, with indefinite therapeutic effects and larger adverse effects. In the study, according to the achievements of studies on olfaction-related etiology of senile demantia at home and abroad, the AD rat was treated with perfume stimulating nasal mucosa to induce resuscitation by means of aromatics, and Morris maze test was conducted for valuating the learning and memory ability, and malondaldehyde (MDA) content and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in the brain, and brain weight were detected, so as to assess the therapeutic effects and probe into the mechanism.[1-4]

MATERIALS AND METHODS

Animals and Grouping

Fifty adult Sprague Dawley rats, weighing 300±10 g, were supplied by the Laboratory Animal Center, the Forth Military Medical University. The rats were

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Preparation of AD Rat Model[5]

Amyloid protein Aβ1-40 peptide segment was dissolved in sterilized distilled water (5 g/L, Aβ1-40 reagent was purchased from Beijing Zhongshan Jinqiao Bio-tech Co.). Before use, it was incubated at 37℃ for 48 h, making it become agglutinative state. The rat was anesthtized by interperitoneal injection of 10% chloral hydrate (0.4 mL/ 100 g body weight) and fixed on a stereotaxic apparatus. With routine ascept operation, the skin was incised, and in reference with " *the Rat Brain Stereotasic Orientation Atlas"* drawed by Bao XM, et al., left hippocampus was selected as injection target area (3.5 mm behind anterior fontanelle, 2.0 mm lateral to left median suture, 2.8 mm below cerebral dura mater) and a small hole of 1 mm in diameter on the skull corresponding to the position was made with a dental drill and the cerebral dura mater was explored, the Aβ1-40 peptide segment 2 μl (10 μg) was vertically and slowly (0.5 μl/min) injected with a micro-injector from the brain surface into 2.8 mm in depth, and the injector was retained for 5 min to ensure full diffusion of the solution, and then the injector was slowly withdrawn (3 min), and the incision was sutured. The operative area was applied with an appropriate amount of penicillin for anti-infection, and the rats were routinely raised.

Preparation of the Olfactory Nerve Severing Rat Model

In reference to the method of Wei YX, the rat after AD modeling was anesthetized with chloral hydrate and fixed on a stereotaxic apparatus.[6] The skin on the skull top medial line was incised, and the anterior fontanelle was separated in a small range and explored. Two small holes with 1 mm in diameter at 2 mm lateral to the anterior fontanelle, 5 mm front the anterior fontanelle on the skull top median line was respectively drilled with such a depth to explore olfactory bulb and not to injure it; The cerebral dura mater of the anterior end of the oflatory bulb was lifted with a micro-anatomic tweezers, and the olfactory nerve was sharply cut off, avoiding injury of the olfactory bulb as far as possibly, and then gelatin sponge was used for pressing hemostasis. The wound was dropped with penlicillin and streptomycin and then was sutured. For the rats in the control group, only a small hole on the skull top was drilled and the skin was immediately sutured, not injuring the olfactory bulb and olfactory nerves.

Perfume Stimulation Method with Volatile Oil of Acorus Gramineus

After sussessiful modeling, the rats in the group D and the group E were treated by perfume stimulating olfaction with volatile oil of Acorus Gramineus, which was purchased from Jiangxi Provincial Zhangsu City Herbal Natural Medicinal Oil Factor with purification of 99%. In a self-made perfume-smoking airtight wuden box of 1 m × 1 m × 0.3 m with a square opening of 10 cm adge length on the bottem with a wire meshes nailed, and with 5 ventilatie holes of 1 cm radiometer on the top, and a small glass bottle containing 5 ml volatile oil of Acorus Gramineus was placed straight below the wire meshes of the opening on the box bottom, which was slowly heated with a alcohol burner via an asbestos wire, making volatile oil gradually volate. Each session of perfume smoking lasted for 60 min, once each day, for 42 consecutive days.

Morris Maze Place Navigation Test[7]

After the end of treatment, Morris maze test was conducted in a Morris maze with a water depth of 41 cm, water temperature of 22℃–26℃. Four water-inlet points on the pool walls were marked, thus the pool was equally divided into 4 quations by the 4 points, and a platform was placed on the middle of a randomly selected quation and soaked into 1cm below waler level with a foamed plastic covered. The rat was placed into water and faced to the pool wall in order of the 4 water-inlet points of E,S, W, N clockwise. The escape latency (the time for finding out the platform within 2 min) was recorded. If the rat found out the platform within 2 min, the real escape latency and swimming distance would be recorded; If the rat did not find out the platform within 2 min, it wowld be guided to the platform by the experimenter and it was stayed on the platform for 10 s. The escape latency was recorded 2 min. The eaperiment was conducted for 6 days, once each day. The mean escape latency and the mean swimming distance in the 6 days were used for assessing learning and memory ability of the rat.

Determination of Brain Weight/Body Weight and Free Radials of Brain Tissue

After end of the Morris maze test, the rat was weighted and the blood was taked rapidly by decapition, and the brain was quickly taken on an ice plate and weighted, and brain weight/body weight was calculated. The brain was homogenazed with cold saline, and 10% homogenate was prepared and centrifuged at 3000 rpm for 20 min. The supernatant was kept at -80℃. MDA content, and GSH-Px and SOD activities were detected with sodium thiobarbiturate (TBA) colorimetry, dithio-dinitrobenzoic acid method (DTNB) and xanthine oxidase method, respectively, following the instruction of the kits perchased from Nanjing Jiancheng Biological Engineering Academy.

Statistical Analysis

SPSS12.0 software was adopted and the data were expressed as $\bar{x} \pm s$, One-way ANOVA was used for comparisions among the means of groups and *t* test was used for comparison between two groups.

RESULTS

Comparisons of Escape Latency and Swimming Distance among the Groups

The mean escape latency and the swimming distance in the 6 days in the group A, B and D were significantly shorter than those in the group C (all $P<0.01$), with no significant differences between the group C and the group E ($P>0.05$), as showed in Table 1.

Table 1. Comparisons of escape latency and sweaming distance among the groups ($\bar{x} \pm s$, n=10)

Group	Escape latency (s)	Swimming distance (cm)
A	15.88±7.18**	102.56±17.12**
B	18.68±5.31**	118.66±16.52**
C	112.18±15.11	182.18±15.13
D	65.16±8.12**	136.16±12.26**
E	101.12±9.15*	176.18±14.18*

Notes: Compared with the group C, $^{*}P>0.05$, $^{**}P<0.01$.

Comparisons of MDA Content, and GSH-Px and SOD Activities in the Rat Brain Tissue among the Groups

Compared with the group A, B and D, MDA content significantly increased and GSH-Px and SOD activities significantly decreased (all $P<0.01$) in the group C, with no significant difference between the group C and the group E ($P>0.05$, Table 2).

Table 2. Comparisons of MDA content, and GSH-Px and SOD activities in the rat brain tissue among the groups ($\bar{x} \pm s$, n=10)

Group	MDA(nmol/mg)	GSH-Px(U/mg)	SOD(U/mg)
A	2.11±0.12**	4.45±1.28**	64.66±3.22**
B	2.46±0.11**	3.81±1.58**	61.16±2.11**
C	4.51±1.15	2.56±0.62	39.88±2.24
D	2.87±0.26**	3.38±1.12**	57.06±2.25**
E	4.22±0.61*	2.98±0.36*	41.21±2.12*

Notes: Compared with the group C, $^{*}P>0.05$, $^{**}P<0.01$.

Comparison of Brain Weight/Body Weight in Rats among the Groups

Compared with the group A, B, D, the brain weight/body weight in rats significantly decreased in the group C ($P<0.01$), with no significant difference between the group C and E in the brain weight/body weight ($P>0.05$, Table 3).

Table 3. Comparison of brain weight/body weight in rats among the groups ($\bar{x} \pm s$, n=10)

Group	Brain weight/body weight (%)
A	0.4468±0.0123**
B	0.4386±0.0116**
C	0.3569±0.0152
D	0.4198±0.0268**
E	0.3657±0.0533*

Notes: Compared with the group C, $^{*}P>0.05$, $^{**}P<0.01$.

DISCUSSION

Modern medicine in studies about olfactory function has attained great achievements and got Nobel Prize in 2004. The available scientific data indicate that the olfactory receptor on olfactory mucosa of nasal cavity can receive and distinguishing different odor molecules, which produce nerve impulse and transmit into olfactory bulb via olfactory nerves. The nerve cells in the olfactory bulb transmit the signals into olfactory tubercle of rhinencephalon, amygdaloid nucleus, pyriform anterior cortex, medial olfactory cortex, hippocampus, etc. in the center via the olfactory tract. Because hippocampus is closely related with learning and memory functions, olfaction–related memory produced by the olfactory impulse of transmitting into hippocampus can induce remodeling of nerve synapse, so as to directly participate in distinguishing, coding and storing processes of learning and memory information.[1-3] It is found that the information of volatile oil of Chinese medical herbs

stimulating the olfactory system transmitting into hippocampus can improve learning and memory , which is possibly due to change of a certain neurotransmitter expression in hippocampus, or the violatile oil of Chinese medical herbs possibly intervenes neurotransmitters of the olfactory system, influencing change of olfactory conductive signals, so as to strengthen learning and memory functions.[8]

Shi Chang Pu (石菖蒲 Rhizoma Acori Graminei) has functions of removing dampness by means of aromatics, inducing resuscitation and relieving mental stress, and *Shen Long Ben Cao Jing* (神农本草经) definitely indicated that it had anti-aging function. It is used as main medicine for antiaging by physiciants of later ages. In the study, perfume smoking with volatile oil of Acori Graminei did not strengthen learning and memory ability in the AD model rats with severing of olfactory nerve; However, it could significantly strengthen learning and memory ability in the AD model rat, indicating that the effect of volatile oil of Acori Graminei strengthening learning and memory ability is produced by means of olfactory conduction pathway entering the central nerve system, which is the same as previous study results of the authors.[9-12]

Studies on AD at home and abroad find that AD is a chronic progressive mental decline disease, and cerebral atrophy is a main change of morphological structures, and peroxidation injury of free radials is one of main pathogeneses. At present, it is generally recognized that ageing has a certain tendency towards decline or decrease of anti-oxidation ability in the organism, manifested as abnormal metabolism of free radicals.[13] Pathological injury of free radicals on AD mainly manifestes as destruction and peroxidation of unsaturated fatty acid and production of MDA on the cell membrance. MDA is a kind of macromolecular cross-linking agent and it can crossly link with protein and nuclear acids, directly influencing transmition, transcription and duplication of biologic information, manifecting as decrease of memory and intelligence. SOD and GSH-Px are important anti-oxidant metal enzymes in the organism and can directly clear away free radicals, avoiding peroxidatic injury.[14,15] In the present study, it is indicated that perfume stimulating olfactory system of the AD rat with volatile oil of Acori Graminei can significantly increase SOD and GSH-Px activities and decreae MDA content, as so to block the pathologic process of peroxidatic injury induced by free radicals. In addition, AD model rats show obvious learning and memory dysfunction, with brain tissue weight significantly reduced. After perfume stimulating olfactory system of the AD rat with volatile oil of Acori Graminei, the learning and memory function in the AD model rat significantly was improved and the brain tissue weight significantly restored. Thus, it is proved that volatile oil of Acori Graminei stimulating olfraction is effective for treatment of the AD model rat, which provides a new thinking and unique administration way for clinical prevention and treatment of AD.

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