Effects of Chongmyung-tang on Learning and Memory Performances in Mice

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Chongmyung-tang(CMT, 聰明湯), oriental herbal medicine which consists of Polygaglae Radix(遠志), Acori Graminei Rhizoma(石菖蒲) and Hoelen(白茯神) has effect on amnesia, dementia. In order to evaluate effect of CMT on memory and learning in mice, CMT extract was used for studies. This paper describes the effects of CMT extract on memory and learning processes by using the passive and active avoidance performance tests, novel object recognition task and water maze task. The CMT extract ameliorated the memory retrieval deficit induced by ethanol in the passive avoidance responses but did not affect ambulatory activity of normal mice. These results suggest that CMT has an ameliorating effect on memory retrieval impairment. CMT extract decreased spontaneous motor activity(SMA) in the latter sessions of memory registration in active avoidance responses. These results suggest that CMT has partly transquilizing or antianxiety effects. In novel object recognition task to measure visual recognition memory, CMT-administered mice enhanced in long term memory for 1-3 days. In water maze task to measure spatial learning, which requires the activation of NMDA receptors in the hippocampus, spatial learning in CMT-administered mice was faster than in wild-type mice. These results suggest that CMT enhances memory and activates NMDA receptors.

Key words : Chongmyung-tang(CMT, 聰明湯), Learning and Memory Performances, NMDA

Introduction

In oriental medical theory, conception of memory and learning is included in function of the heart(Xin, 心), the mind(Shen, 神), the essence(Jing, 精), the energy(Qi, 氣), and the brain(Nao, 腦). The heart(Xin, 心) controls function of spirit or vital activity. The mind(Shen, 神) is considered as conception of vital penomenon or thinking consciousness, the essence as conception of vital original matter, the energy as conception of the vital activity. This all is expressed by the mind externally[1,2]. The brain is place where the essence changes into the mind and the converted mind from the brain emerges in the whole body, the sensory organs and carries vital function[3].

Amnesia which is representative disease of memory disorder, has been studied with dementia. In korean oriental medical study, there is bibliographic study on the type of differential diagnosis of amnesia written by Choi[4], bibliographic study on the cause, treatment method and medicine of amnesia by Kim[5], study on effect of Chongmyung-tang on memory and learning in memory impaired mice by Kim[6], study on effect of Jowisungcheongtang on the learning and memory of radial arm maze in mice by Woo[7], study on correlation of memory and function of kidneys by Lee[8].

In Dong-Eu-Bo-Gam(東醫寶鑑) compiled by Hu Jun(許浚), CMT has been known to tranguilizing the mind by nourshing the heart, dissipating phlegm retention, inducing resuscitation and has effect on amnesia caused by deficiency of the heart and phlegm retention[9]. CMT consists of 3 herbs of Polygaglae Radix(遠志) 12 g, Acori Graminei Rhizoma(石菖蒲) 12 g and Hoelen(白茯神) 12 g.

This paper describes the effects of CMT extract on memory and learning processes by using the passive and active avoidance performance tests, novel object recognition task and water maze task, which may provide clues for the clinical application of CMT to amnesia or dementia.

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· Received : 2005/07/14 · Revised : 2006/02/20 · Accepted : 2006/04/04

Materials and Methods

1. Animals

The C57B/6 mice were used as experimental animals. Also, male mice of ddY-strain of 5-6 weeks old(KRIBB, Tawjon,

Japan) were used. Ten mice were kept in one cage in a room where the temperature and humidity were controlled for a week before the experiment.

2. Isolation of hot water extract from CMT

CMT consists of 3 herbs, which are Polygaglae Radix(遠志), Acori Graminei Rhizoma(石菖蒲) and Hoelen(白茯神)[1]. The aqueous extracts of CMT and its 3 composed Korean herbs, which were massproduced for clinical use, were kindly supplied by the Oriental Medical Hospital of Dongguk University. Amounts of the CMT which were studied in this work were as follows: Polygaglae Radix(遠志) 12 g, Acori Graminei Rhizoma(石菖蒲) 12 g, Hoelen(白茯神) 12 g were added to 500 ml of water and boiled for 2 hr, filtered and then concentrated to 10 ml. The extracts were lyophilized and separately stored at -20 ℃ for next experiments

3. Passive avoidance performances(Step through test)

The chamber apparatus had a partition wall with a hole, which divided the chamber into two compartments, one bright and the other dark. As soon as a mouse entered the dark compartment from the bright one, a punishing electric shock was given through the foot grids. The time needed for the mouse to enter the dark compartment was recorded. On the first day, each mouse received a learning trial, by which it was taught that if it entered the dark compartment, it was to be punished. 24 hr later, 12 mice were placed again in the bright compartment, and were left there for 300 sec. The latency and the number of mice, which did not enter the dark compartment, were recorded[10].

1) Experimental procedures

(1) Effects of CMT extract on memory processes in normal mice

CMT extract was orally administered 30 min prior to the learning trial, immediately after the learning trial and 30 min before the testing trial to demonstrate the effects of CMT extract on memory registration, consolidation, and retrieval processes respectively. 12 mice were randomly used in each group.

(2) Effects of CMT extract on memory impaired mice

Mice were randomly divided into wild-type group, and experimental group. Experimental group was administered with 50 mg/100 g of CMT extract. 12 mice were used in each group.

① Memory registration impairment

10 min after the administration of CMT extract, 30 % ethanol(50 ml/kg, p.o.) were given to the mice to interfere with memory registration process. 20 min later, the learning trial was given to them.

② Memory consolidation impairment

CMT extract was give 30 min before the learning trial. Electric convulsive shock (0.4 ms in width, 100 Hz, 94 mA for 0.2 sec) was given to the mice through the ears immediately after the learning trial in order to impair the memory consolidation process.

③ Memory retrieval impairment

On the first day, the learning trial was given to mice. On the second day, 10 min after the treatment with CMT extract, 40 % ethanol(10 ml/kg, p.o.) or electric convulsive shock(0.4 ms in width, 100 Hz, 80 mA for 0.2 sec) was given to the mice to impair the memory retrieval process. 20 min later, the testing trial was given.

4. Active avoidance performances(Shuttle box test)

Two sets of infra-red beams were arranged apart at the long side of the shuttle box apparatus. A buzzer and a lamp were set on the ceiling and the grid floor of the box connected with an electric stimulator. The programme of one trial was as follows: (1) 40 sec interval; (2) 10 sec for conditional stimulation(CS), (the warning buzzer and the lamp were operated on during this CS period); (3) 10 sec for unconditioned stimulation(US), (electric stimulation, an intensity of 36 V AC, as well as buzzer and lamp were operated on during the US period).

If a mouse interrupted both of the two infra-red-beams during the CS or US period, the electric shock was canceled immediately. The photobeam interruption during the CS period was considered as a conditioned avoidance response(CAR) and during the US period as an unconditioned avoidance response(UAR). The trial without interruption during CS and US period was considered as a failure. The movement of mouse which was irrelevant to CAR or UAR was counted as spontaneous motor activity(SMA). One session was composed of 60 trials[11].

1) Experimental procedures

Mice were randomly divided into wild-type group and experimental group. Experimental group was administered with 50 mg/100 g of CMT extract. CMT extract was orally administrated 15 min prior to the test. Shuttle box performances were tested for 7 days, once daily, at the same time of the day. The number of animals in each group was 8.

5. Novel object recognition task

Mice were individually habituated to an open-field box(20 × 20 × 10 high inches) for 3 days. Mice were randomly divided into Wild-type group and experimental group. Experimental group was administered with 50 mg/100 g of CMT extract. During training sessions, two novel objects were placed into the open field and the animals were allowed to explore for 5 min. The time spent exploring each object was

recorded. During retention tests, the animals were placed back into the same box, in which one of the familiar objects used during training was replaced with a novel object, and allowed to explore freely for 5 min. A preference index, a ratio of the amount of time spent exploring any one of the two objects(training session) or the novel one(retention session) over the total time spent exploring both objects, was used to measure recognition memory.

6. Water maze task

The water-maze apparatus is a circular pool(1 m in diameter). The training protocol consisted of six sessions(4 trials per session per day). The navigation of the mice was tracked by a videocamera and the escape latency to the platform was recorded. The time spent in each quadrant was recorded[12].

7. Statistics

The results of the rate of the successful mice were analyzed by the chi-square test, novel object recognition task by Dunnett's test, water maze task by Student's t-test. All the other data were analyzed by the Mann-Whitney's U-test.

Results

1. Effects of CMT extract on passive avoidance performances (step through test)

1) In normal mice

In normal mice, CMT extract has no effect on the latency in the step through test, which therefore suggested that CMT extract has no influence on passive avoidance performances in normal mice.

2) In memory impaired mice induced by 40% Ethanol

CMT extract neither ameliorated the memory registration impairment caused by 30% ethanol, nor improved the memory consolidation deficit induced by electric convulsive shock when tested by the step through test.

In the case of mice having a memory retrieval impairment induced by 40% ethanol, CMT extract increased the latency to enter the dark shock compartment($P<0.05$)(Table 1). CMT extract significantly increased the percentage of successful mice in the step through test ($P<0.05$)(Table 2).

Table 1. Effects(time elapse) of CMT extract on memory retrieval impairment induced by 40% ethanol in the step through test.

	Wild-type	Ethanol	CMT 50
Latency (S)	213.7±7.0	191.7±9.1*	207±10.4#

Time(sec) elapsed before the mice entered the dark compartment (latency) is shown. Wild-type(n=12), Ethanol : mice orally treated with 40% ethanol 20 min before the testing trial(n=12), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=12). Data is expressed as mean ± S.E. * : P<0.05 vs wild-type, # : P<0.05 vs ethanol group, Mann-Whitney's U-test.

Table 2. Effects(percentage of mice not entering the dark compartment in the testing trial) of CMT extract on memory retrieval impairment induced by 40% ethanol in the step through test

	Wild-type	Ethanol	CMT 50
No of successed mice(%)	70	45*	64#

Wild-type(n=12), Ethanol : mice orally treated with 40% ethanol 20 min before the testing trial(n=12), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=12). Data is expressed as mean ± S.E. * : P<0.05 vs wild-type, # : P<0.05 vs ethanol group, chi-square test

2. Effects of CMT extract on active avoidance performances in normal mice (Shuttle box test)

In the memory registration experiment, wild-type mice and CMT- administered mice gradually obtained higher CAR level (Fig. 1), however the CMT-administered mice gave a significant decrease of SMA on the final session day(Fig. 2). No significant influence was observed in their CAR, UAR and errors.

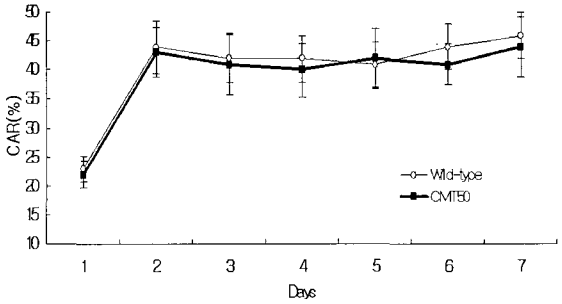

Fig. 1. Effects conditioned avoidance response(CAR) of CMT extract on memory registration in the shuttle box test. Wild-type(n=8), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=8). Data is expressed as mean ± S.E.

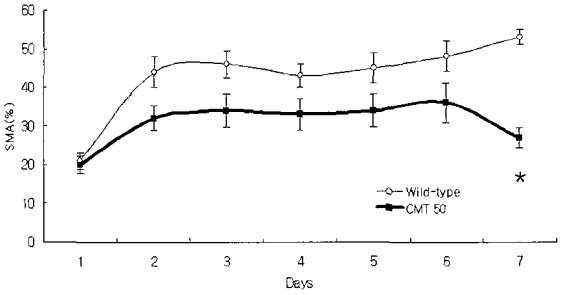

Fig. 2. Effects spontaneous motor activity(SMA) of CMT extract on memory registration in the shuttle box tests Wild-type(n=8), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=8. Data is expressed as mean ± S.E. * : P<0.05 vs wild-type, # : P<0.05 vs ethanol group, Mann-Whitney's U-test.

3. Effects of CMT extract on novel object recognition memory

All behavioural experiments were performed with the experimenter blind to the CMT-administered of each mouse.

We used the novel object recognition task to measure visual recognition memory, which is evolutionarily conserved in species including humans and rodents and requires the hippocampus[13-15]. To increase the difficulty of this task, we used 5 min training protocol. During the training, there was no significant difference in the amount of the time which the mice spent exploring the two novel objects(Table 3). The result

indicates that both types of mouse have the same curiosity and motivation to explore the objects. During the retention test, one of the familiar objects used in the training session was replaced with a third novel object and mice were allowed to explore for 5 min. Both CMT-administered mice and wild type mice exhibited similar preference towards the novel object at the 1-hr retention test(Fig. 3).

Table 3. Exploratory preference in the training session.

	Wild-type	CMT 50
Exploratory preference(%)	49.3±1.2	50.7±0.6

The base line is 50% corresponding to preference at chance. Wild-type(n=8), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=12). Data is expressed as mean ± S.E.

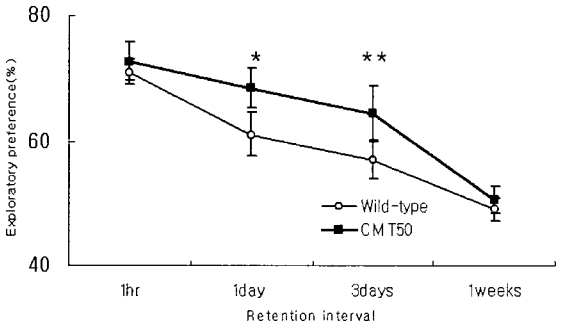

Fig. 3. Exploratory preference in retention test. Wild-type(n=8), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=12). Data is expressed as mean ± S.E. * : P<0.05, ** : P<0.01 between CMT-administered and wild-type mice, Dunnett's test.

However, when retention tests were conducted either 1 day or 3 day later(Fig. 3), CMT-administered mice exhibited much stronger preference for the novel object than did the wild-type mice. The result indicates that CMT-administered mice have better long term memory. A statistical analysis using Dunnett's test reveals a significant difference between wild-type and CMT-administered mice at the 1-day($P<0.05$) or 3-day retention tests($P<0.01$). However, 1 week after training, the preference shown by CMT-administered mice also returned to the basal level.

4. Effects of CMT extract on escape latency in water maze task

Spatial learning was tested in CMT-administered mice using the hidden-platform water maze, which requires the activation of NMDA receptors in the hippocampus[16,17]. As shown in Fig. 4 the latency to escape to the platform in both wild-type and CMT-administered mice decreased following the training sessions. However, there was a significant group difference throughout sessions($P<0.05$). The result indicates that spatial learning in CMT-administered mice was faster than in wild-type mice. Moreover, a statistic analysis revealed a significant difference at the fourth session($P<0.01$), confirming better spatial learning in CMT-administered mice.

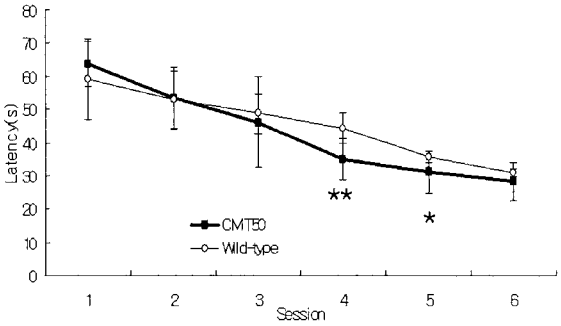

Fig. 4. Effects of CMT extract on escape latency in water maze task Wild-type(n=8), CMT 50: 50 mg/100 g CMT was orally given 30 min before the testing trial(n=12). Data is expressed as mean ± S.E. * : P<0.05, ** : P<0.01 between CMT-administered and wild-type mice, student's t-test.

Discussion

The correlation of learning and memory is inside and outside. Memory is descrived as change of vital activity, which is induced by training, whereas learning is described as change of behavior which is reflected by memory[18].

Construction of memory is divided sensory store, short term memory, long term memory[19]. Because sensory store is associated with sensory route of the sight, hearing, touch and etc, it retains the sensory information from the exterior for 1 sec or 2 sec. However, if previous information is not converted by new information, it may obscure. Short term memory is restrictive lasting maximum 30 sec. In order to convert short term memory to long term memory, repetive training and practice are required. Though long term memory is stored in somewhere not in present consciousness, it can be recalled whenever it is necessary and lasts for a long time[7].

In oriental medical theory, course of memory is considered as The Xin(心), Yi(意), Zhi(志), Si(思), Lu(慮), Zhi (智). If this conception is compared with psychologic course of memory, The Xin(心) is considered as sensory store, The Yi(意) as short term memory, The Zhi(志) as long term memory[7].

The reason of amnesia was phlegm retention, deficiency both the heart and spleen, breakdown of the coordination between the heart and kidney, deficiency of the kidney, blood stasis. The method of medical treatment is invigorating both the heart and spleen, invigorating the kidney, invigorating the heart and nourishing blood, removing blood stasis, dissipating phlegm, and relieving mental stress. The prescription is commonly used as Guibitang, Insuksan, Chongmyung-tang, Chunwangbosimdan and etc[5].

In Dong-Eu-Bo-Gam(東醫寶鑑) compiled by Hu Jun(許浚), CMT has been known to relieving mental stress, reducing phlegm damp, inducing resuscitating and has effect on amnesia caused by deficiency of the heart and phlegm retention as well[9].

CMT, oriental herbal medicine consists of Polygaglae Radix(遠志), Acori Graminei Rhizoma(石菖蒲) and Hoelen(白茯神). Polygaglae Radix(遠志) has effected on inducing resuscitation, relieving mental stress. Acori Graminei Rhizoma (石菖蒲) has effected on inducing resuscitation, reducing phlegm damp. Hoelen(白茯神) has effected on relieving mental stress, diuresis.

In order to evaluate the anti-amnesic and learning effects of CMT, CMT extract was tested for its effects on the novel object recognition task, water maze task, passive and active avoidance responses in normal and memory impaired model mice.

CMT extract has no effect on the latency in the step through test, which means no influence on passive avoidance performances in normal mice. According to this, it is suggested that CMT extract has no direct memory improving effects in normal animals. CMT extract improves only the memory retrieval deficit induced by 40% ethanol, which suggested that CMT extract might therefore interfere with the action of ethanol, that CMT extract might mainly act on the memory retrieval process.

The neurotoxicity of ethanol has been well studied[20-22]. Ethanol induces short and long term memory impairment and the results of administration of ethanol before and after the tests suggested that the ethanol-induced memory deficit was due to its effect on the neurotransmitters, especially chollinergic and adrenergic, and that the neocortex might be the major taget of ethanol in the brain[17,20]. In the present experiment, CMT extract improved the memory impairment induced by ethanol. CMT extract decreased SMA in the latter sessions of memory registration in active avoidance responses. These results suggest that CMT has partly transquilizing or antianxiety effects.

In novel object recognition task to measure visual recognition memory, which is evolutionarily conserved in species including humans and rodents and requires the hippocampus[13-15], there was no significant difference in the amount of the time which the mice spent exploring the two objects in training session. CMT-administered mice and wild type mice exhibited similar preference towards the novel object at the 1-hr retention test. However, when retention tests were conducted either 1 day or 3 day later, both CMT-administered mice exhibited much stronger preference for the novel object than did the wild-type mice. The result indicates that CMT-administered mice have better long term memory. But after 1 week training, the preference shown by CMT-administered mice also returned to the basal level.

In water maze task to measure spatial learning, which requires the activation of NMDA receptors in the hippocampus[16,17], spatial learning in CMT-administered mice was faster than in wild-type mice.

This study reveals a strategy for the creation of other oriental medically administered mammals with enhanced intelligence and memory. Further experiments on MNDA recepters should be progressed[12].

Though further research is required to elucidate the effects of CMT on memory and learning processes, the results presented in this paper may provide fundamental data for the study about the effects of CMT on the central nervous system.

Acknowledgement

This work is supported by the Dongguk University Research Fund

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