Experimental Study on Multi-Infarct Dementia Treated with Principle of Yijing Tishen (益精提神法)*

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ABSTRACT
Objective: To explore the mechanism of multi-infarct dementia (MID) treated with the principle of Yijing Tishen (YJTS, reinforcing Kidney-essence and refreshing mental activities). Methods: MID rat models were established successfully with injecting sterile and naturally dried blood clots of the homologous rat into common carotid artery and screened by the first jumping-off latency of diving-platform reflex, based on which, the effect of YJTS in learning and memorizing, monoamine neurotransmitters content in brain tissue, malondialdehyde (MDA) content and superoxide dismutase (SOD) activity in serum and brain tissue, and brain morphosis of multi-infarct rats were observed. Results: Obvious malfunction of learning and memorizing was found in MID rat models, and there were also significant decreasing of monoamine neurotransmitters content in partial brain zones, decreasing of SOD activity in brain and increasing of MDA content in serum and brain. YJTS could obviously improve learning and memorizing, raise SOD activity and monoamine neurotransmitters content in brain tissue, lower MDA content in serum and brain of MID rat models, protect brain morphosis of multi-infarct rats. Conclusion: YJTS might treat MID by restraining lipid peroxidation, improving monoamine neurotransmitters content in partial brain zones and decreasing ischemic damage of brain tissue.

KEY WORDS principle of Yijing Tishen, vascular dementia, malondialdehyde, superoxide dismutase, monoamine neurotransmitters

Multi-infarct dementia (MID) is the most common senile dementia in China, which does great harm to senile physical and psychological health. According to the pathologic features of Kidney-essence deficiency and mental activity dysfunction in MID patients, we put forward the principle of Yijing Tishen (YJTS, 益精提神法, reinforcing Kidney-essence and refreshing mental activities) as the chief therapeutic principle based on theory of "essence-Qi-mentality" in traditional Chinese medicine (TCM). Yizhi Capsule (YZC, 益智胶囊) was formed according to this principle and has shown good effect in preventing and treating MID. (1,2) To further explore the mechanism of MID treated with YJTS, we observed the effect of YJTS in learning and memorizing, monoamine neurotransmitters content in brain tissue, malondialdehyde (MDA) content and superoxide dismutase (SOD) activity in serum and brain tissue, and brain morphosis of multi-infarct rats based on MID rat models established successfully, so as to provide treating MID by Chinese herbal medicine with valuable clues.

METHODS

Animals
Male Wistar rats, aging 12 – 15 months, weighing 350 – 450 g, provided by Animal Center of Shandong Epidemic Prevention Station.

Drugs
YZC consists of Panax ginseng 6 g, Liguisticum chuanxiong 12 g, Epimedium grandiflorum 12 g, Acorus gramineus 9 g, Polygnum multiflorum 15 g, Anemarrhena asphodeloides 9 g, Leech 6 g, Polygala tenuifolia 6 g. It was produced by Shandong Analyzing and Testing Center. Each capsule contains 0.228 g medicinal powder which corresponds to 2.28 g crude herbs. It was prepared by making up 0.2 g/ml suspension. Piracetam was the product of North-East Pharmaceutical Factory and prepared by making up 0.032 g/ml suspension.

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Reagents and Instruments

MDA and SOD testing kit were purchased from Nanjing Jiancheng Bioengineering Institute. Diving-platform reflex box and water labyrinth were made according to Methodology of Pharmacological Experiment in Chinese Herbal Medicine. (3) The 723 spectrophotometer was manufactured by Shanghai Factory of Analyzing Instrument. RF-540 fluorospectrophotometer was the product of Japan Shimatzu Company.

Establishing of MID Models

The total of 100 Wistar rats aging from 12 to 15 months were tested with diving-platform experiment and the first jumping-off latency of diving-platform reflex was regarded as the screening index of dementia rats. First, the rats were put in the diving-platform reflex box to be adapted for 3 minutes, then tested preliminarily by electrifying for 3 minutes and tested formally after 24 hours. The first jumping-off latency of each rat was recorded and the mean ($\bar{x}$) and standard deviation ($s$) were calculated. The minimum of the normal range was 72.50 s, calculated by formula $\bar{x} \pm 1.96s$ according to statistical method. Besides, multiple cerebral infarction models were established with similar rats according to slightly modified method introduced by CHEN Junpao. (4) At the 10th day after surgery, the first jumping-off latency of each survived rat was tested. The rats with the first jumping-off latency less than 72.50 s were regarded as MID models.

Grouping and Medication

The total of 36 survived rats after surgery were randomly divided into 4 groups with 9 rats in each group: sham-surgery group, model group, piracetam group and YZC group. The surgical operation of sham-surgery group was similar to model group except injecting equivalent normal saline (NS) instead of embolus-saline suspension into the common carotid artery. Based on establishing models, all groups were medicated from the 11th day after surgery for consecutive 15 days with NS, NS, piracetam and YZC respectively, once a day, 1 ml/100g body weight.

Examination of Learning and Memorizing

Water labyrinth were used to test learning and memorizing of rats. The time needed to swim from starting point of water labyrinth to safe platform as destination and the frequency of going into wrong way were regarded as indexes of learning-acquiring and memory-consolidating. The rats were examined at the 21st day after surgery according to the method introduced by WU Jiang. (3)

Measurement of MDA and SOD

The rats were sacrificed at the 26th day after surgery. Blood was gained from eyeball and brain tissue and drawn quickly after cutting off head. The measurement of MDA and SOD were based on manual instruction of kits.

Measurement of Noradrenalin, Dopamine, and Serotonin

The rats were sacrificed at the 26th day after surgery. Brain tissue was drawn quickly after cutting off head. Hippocampus, thalamus, brain stem and cortex (frontal lobe) were separated on ice-cold glass plate. The measurement of noradrenaline (NE), dopamine (DA), serotonin (5-HT) were based on related literature. (3)

Observation of Brain Morphosis of Multi-Infarct Rats

The animal, grouping and dosage of medication were the same as above experiment. Establishing of models was still based on slightly modified method introduced by CHEN Junpao. (4) Medication began 3 days before surgery and ceased at the 15th day after surgery. Brain tissue was drawn quickly after cutting off head, fixed with formalin, embedded with paraffin, cut section routinely, stained with hematoxylin and eosin. Brain morphosis was observed by optical microscope.

Statistical Analysis

Double student $t$ test was used to compare the significance of the difference among various groups.