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The effects of an herbal medicine Bu-Wang-San on learning and memory of ovariectomized female rat

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ABSTRACT

Ethnopharmacological significance: Bu-Wang-San (BWS) is a traditional Chinese herbal medicine for the treatment of learning and memory impairment. The effect of BWS on neuroprotection and how BWS increases CA1 dendritic spine synapse density in menopaused women was investigated in the model of ovariectomized (OVX) rats.

Materials and methods: Sixteen OVX rats were divided into two groups, the OVX group and OVX + BWS group. After 3 months, Morris water maze was used to assess spatial acquisition and spatial retention. Swim time, swim distance, swim speed, quadrant time and platform crossing were recorded. The ultrastructure of the pyramidal cell and spine synapse density were examined by transmission electron microscopy (TEM).

Results: In the spatial acquisition and spatial retention phase of testing, BWS group functioned significantly better than control group. Ultrastructural observation of the hippocampal CA1 region of OVX group showed swelling of mitochondria, the broken and reduced cristas and even crista dissolution; however, the mitochondria were protected well in BWS group. In addition, BWS significantly increased spine synapse density.

Conclusions: These results suggested that BWS could improve cognitive ability of menopause-induced learning and memory impairment. The positive effect of BWS on rat learning and memory was associated with increase of spinal synapse density and protection of mitochondrial function of the pyramidal cell in hippocampal CA1 region from menopause-induced injury.

1. Introduction

Menopause marks the end of reproductive capacity of women and results from the permanent cessation of ovarian function. Natural or surgical menopause is confirmed by absence of menstrual periods for 12 consecutive months, excluding other obvious pathologic or physiologic causes (Notelovitz, 1989). Some symptoms such as hot flashes, tiredness, irritability, insomnia, palpitations, memory or concentration difficulties, and mood swings or depression begin in the peri-menopause and increase as women progress through the menopause (Hardy and Kuh, 2002). Many studies have shown that learning and memory may be negatively affected altered by the loss of estrogen after menopause (Sherwin, 1988). These changes can be ameliorated by estrogen replacement therapy (ERT) (Ditkoff et al., 1991; Sherwin, 1994; Kimura, 1995). As a neuroprotective and neurotrophic factor, estrogen (E_2) helps maintain memory and cognition (Sughrue and Merchenthaler, 2000; Wise et al., 2001), decreases the risk and delays the onset of neurological disorders, e.g. Alzheimer's disease (AD). Indeed, estrogen has been shown to increase cerebral blood flow, to act as an anti-inflammatory agent and enhance neural synapse activity (Toran-Allerand et al., 1999; Roof and Hall, 2000). Numerous studies indicate that estrogen is essential for optimal brain function (Toran-Allerand et al., 1999; Roof and Hall, 2000; Sughrue and Merchenthaler, 2000; Wise and Dubal, 2000; Wise et al., 2001). However, the above-mentioned health benefits of ERT were often overshadowed by the serious side-effects of estrogen use in menopaused women. Specifically, long-term use of estrogen in postmenopausal women may lead to the increased risk of endometrial and breast cancer (Hammond, 1994; Grady et al., 1995; Anonymous, 1997). Accordingly, there has been a growing interest in alternative therapies. One alternative employs phytoestrogens, the other alternative employs Chinese herbal medicine.

Phytoestrogens as nonsteroidal plant compounds that are structurally or functionally similar to estrogens and may have similar beneficial effects. These substances have a 2-phenylnaphthalene-type chemical structure and bind to estrogen receptors in vitro and in vivo (Welshons et al., 1987; Lephart et al., 1996; Pan et al.,

Abbreviations: OVX, ovariectomized; E_2, estrogen; TEM, transmission electron microscopy; ERT, estrogen replacement therapy; BWS, Bu-Wang-San.

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1999a,b; Patisaul et al., 1999; Jacob et al., 2001; Kim et al., 2001). Specifically, dietary supplementation with isoflavones or lignans had an estrogenic effect, as shown by the maturation of vaginal epithelium on cytology (Wilcox et al., 1990).

Chinese herbal medicine has been used for thousands of years in China and other Asian countries. In clinical practice of traditional Chinese medicine (TCM), it is common to modify original formula by adding or substituting herbs in accordance with a patient's condition to enhance the efficacy of the original formula. Most of the regular use of traditional Chinese medicine is not associated with serious side effects. Specific formula of traditional Chinese herbal medicines has been reported to be effective against cognitive disorder (Junping et al., 2005; Hai-Fa et al., 2006; Young-Ju et al., 2007). However, there was little information available in literature about whether herbal medicines with neuroprotective effect could affect postmenopausal cognitive disorder. Bu-Wang-San (不忘散, BWS), a classical Chinese herbal formula, has been used to treat the postmenopausal cognitive disorder for years in clinic. The mechanism as to how BWS protects learning and memory has not been studied. Pyramidal neurons in the hippocampal CA1 are particularly vulnerable from impairment. We have used ovariectomized rat as the estrogen-depleted, postmenopausal model (Kalu, 1991) to examine the effect of BWS on postmenopausal cognitive disorder. In addition, we have studied the effect of BWS, for the first time, on the change of ultrastructure of CA1 region and spinal synapse density with electron microscope.

2. Materials and methods

2.1. Composition and preparation of Bu-Wang-San

BWS consists of four medicinal compositions as shown in Table 1. All of these herbs were purchased from Jian-lian Company of Traditional Crude Drugs (Jinan, China), and carefully authenticated by Dr. Li-Hong Zhong, Pharmaceutical Preparation Section, Qilu Hospital Affiliated to Shandong University, Jinan, China. Voucher specimens (numbers were listed in Table 1) were deposited at the Herbarium of Shandong University (Jinan, China). After drying, these herbs were mixed in proportion. Forty-eight grams of the mixed material was mixed with 300 ml of distilled water and boiled for 1 h at 100 °C. The extract was filtered, and the residual medicine was boiled in water following the same procedure once more. Finally, the pool of the extracts from two boiling and filtering was lyophilized to form a dried powder. The yield of BWS extract was 25% (w/w) of the original herbs. The resulting lyophilized powder, stored at 4 °C, was diluted to the appropriate concentrations with distilled water and filtered before use.

2.2. Animals and treatment

2.2.1. Animals

Ten-week-old virgin female Wistar rats weighing 260–300 g were purchased from Shandong University Laboratory Animal Shelter (Shandong, China). Rats were housed in individual cages under controlled environmental conditions (22 ± 2 °C relative humidity 40–60%, 12-h dark/light cycles, food and water *ad libitum*). All rats were treated parallelly in terms of daily manipulation.

Table 1
Recipe of Bu-Wang-San (BWS) formulation

Components	Voucher specimens	Part used	Amount used (g)
Panax ginseng C.A. Mey	SDU0810	Root	6
Acorus gramineus Soland	SDU0156	Stem and root	12
Poria cocos (Schw.) Wolf	SDU0123	Dried sclerote	20
Polygala tenuifolia Willd	SDU0241	Root	10

2.2.2. Ovariectomy and medical treatment

The rats were divided randomly into three groups; the first group was given a sham operation (Sham group), the others were ovariectomized (OVX) (El-Bakri et al., 2004). They were either bilaterally ovariectomized or sham-operated through dorsal incision under anaesthesia with 3% sodium pentobarbital (30 mg/kg, i.p.). The next day, ALL post-operative rats were injected with penicillin (22,000 u.i./kg) for 3 days, and the vaginal smear was taken from each rats for 4 days. Rats of the Sham group with a classic estrous cycle were selected ($n = 8$). Success of the ovariectomization was confirmed by demonstration of predominantly leukocytes with few epithelial cells in vaginal smears over at least 4 days. The successful OVX rats were further randomly divided into two groups ($n = 8$ equally): OVX group, OVX + BWS group. The next day, the Sham group and the OVX group received distilled water; and the OVX + BWS group received Bu-Wang-San (2.4 g/(kg day)) by the oral gavage daily for 3 months. The dose of BWS was determined by conversion of regular dose for human to that for rat and also by a pilot experiment with different doses of BWS for rat. All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals, which was approved by the Shandong University Animal Care and Use Committee.

2.3. Morris water maze

All rats were put into the Morris water maze to assess learning and memory performance on a spatial orientation task (Morris, 1981). A circular 180-cm diameter swimming pool made of black polyethylene was filled 32-cm deep with 25 ± 2 °C water. The water was made opaque by the addition of pure milk powder (Inner Mongolia Yili Industrial Group Co., Ltd., China). A platform, which consisted of a round transparent lucite platform (10 cm diameter and 30 cm high) invisible to the rat, was hidden below the surface of water in one of the four quadrants of the pool. Conspicuous visual cues outside the pool were provided for orientation. A video camera suspended above the pool was connected to a video tracking system (MI-200, Chengdu Taimeng Technology & Market Co., Ltd., China) that recorded that recorded the swimming pattern including the length of the swim path on each trial.

2.4. Behavioral test

2.4.1. Spatial acquisition

Testing was conducted between 8:30 to 11:30 a.m. and 1:30 to 4:30 p.m.; rats were trained throughout 4 days, with eight trials per day, and there was a 2-min break between each trial. The first day, rats were initially placed on the platform and allowed to stay there for 30 s. They were then placed in the pool at the edge of the platform with their front paws touching it and were allowed to climb out of the water onto the platform and stand for 30 s. This was repeated three additional times. Finally, they were placed at the edge of the pool and allowed to swim to the cued platform and climb onto it. Animals that failed to locate the platform within 120 s were manually guided to it. From the second day to the fourth day, the test was carried out in following way: rats were placed at the edge of the pool. The point of placing rats was each alternative midpoint of the four quadrants. Rats were allowed to swim to the submerged platform and climb onto it and stayed there for 30 s. Rats that failed to locate the platform within 120 s were manually