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Research Paper

Effect of traditional Chinese herbal Bu-Wang-San on synaptic plasticity in ovariectomised rats

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Abstract

Objectives The neuroprotective effects of Bu-Wang-San (BWS) and its effects on spine synapse plasticity were investigated in ovariectomised rats.

Methods Thirty-six ovariectomised rats were divided into three groups: untreated controls, treatment with 17β-estradiol or with BWS. After 3 months, spatial acquisition and spatial retention were measured using the Morris water maze. Swim time, swim distance, swim speed, quadrant time and platform crossing were recorded. Spine synapse density in the hippocampus was examined by transmission electron microscopy. The expression of synaptophysin P38 (P38) mRNA was examined by real-time PCR and the protein expression of P38 was examined by Western blot.

Key findings In spatial acquisition and spatial retention, the BWS group functioned significantly better than the control group. Ultrastructural observation of the hippocampus showed that BWS significantly increased spine synapse density compared with the ovariectomised group. In addition, BWS significantly increased P38 mRNA and protein expression in the hippocampus. Thus, the positive effect of BWS on learning and memory in rats was associated with increased spinal synapse density and increased P38 mRNA and protein expression in the hippocampus following menopause-induced injury.

Conclusions These results suggest that BWS could improve cognitive ability following menopause-induced impairment of learning and memory.

Keywords Bu-Wang-San; Chinese herbal medicine; learning; memory; menopause

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 menstruation for 12 consecutive months, excluding other obvious pathological or physiological causes.[1] Many studies have shown that learning and memory may be impaired by the loss of estrogen after the menopause.[2] These changes can be ameliorated by estrogen replacement therapy.[3–5] As a neuroprotective and neurotrophic factor, estrogen helps to maintain memory and cognition,[6,7] and decreases the risk and delays the onset of neurological disorders such as Alzheimer's disease. Indeed, estrogen has been shown to increase cerebral blood flow and enhance neural synapse activity.[8,9] Numerous studies indicate that estrogen is essential for optimal brain function.[6–10] However, the health benefits of estrogen replacement therapy in menopausal women are often overshadowed by the side-effects. Specifically, long-term use of estrogen in postmenopausal women may increase the risk of endometrial and breast cancer.[11–13] Accordingly, there has been a growing interest in alternative therapies.

Chinese herbal medicine has been used for thousands of years in China and other Asian countries. Most of the regular use of traditional Chinese medicine is not associated with serious side-effects. Specific formulas of traditional Chinese herbal medicines have been reported to be effective against cognitive disorder.[14–16] Bu-Wang-San (BWS), literally meaning the decoction for enhancing the memory, is a traditional Chinese formula. It has been used for many years in the clinic to treat postmenopausal cognitive decline.

The mechanism by which BWS protects learning and memory has not been studied. We have used ovariectomised rat as the estrogen-depleted postmenopausal model[17] to examine the effects of BWS on postmenopausal cognitive disorder. In addition we have, for the first

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time, studied the effects of BWS on synapse density in the hippocampus, using transmission electron microscopy and by measuring synaptophysin P38 (P38) mRNA and protein expression in the hippocampus. P38 is a calcium protein (molecular weight 38 kDa) that is synthesised in neuronal bodies, transported to axonal terminals and specifically expressed on the presynaptic vesicle membrane.[18] The P38 mRNA and position and quantity of protein reflect the distribution and density of synapses, and can be used to determine changes in the number of synapses. P38 is though to be susceptible to estrogens and can reflect the function of nerve transport systems.[19]

Materials and Methods

Herbal materials

BWS consists of four medicinal components, as shown in Table 1. Fresh roots of these herbs were harvested from Jilin, Sichuan, Yunnan and Hebei provinces in China. The herbal farms in these areas have been shown to produce the best quality herbs. Plant materials were collected in September or October after 2 years' cultivation, except for *Panax ginseng*, which was harvested at 6 years. The plants were authenticated by a botanist during field collection.

The roots and sclerote were ground into powder and stored in a desiccated environment before delivery to the laboratory. Voucher specimens (numbers listed in Table 1) were deposited at the Herbarium of Shandong University (Jinan, China).

Herbal extraction

After drying, these herbs were mixed in the proportions shown in Table 1. Forty-eight grams of the mixed material was mixed with 300 ml 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. The pool of the extracts from two boilings and filterings was lyophilised to form a dried powder. The yield of BWS extract was 25% (w/w) of the original herbs. The resulting lyophilised powder was stored at 4°C, and was diluted to the appropriate concentrations with distilled water and filtered before use.

Analysis of Bu-Wang-San by HPLC

BWS (0.5 g) was extracted with 20 ml methanol with ultrasonication for 30 min followed by centrifugation. The supernatant was analysed by an HPLC system equipped with LC-10 AD pumps, a photodiode array detector and a CTO-10A column oven (Shimadzu, Kyoto, Japan) and a Kromosil C18 column (5 μm, 200 × 4.6 mm; Alltech Associates Inc., Columbia, MD, USA). The mobile phase consisted of acetonitrile and H_3PO_4-acidified water (0.1% v/v) delivered at a flow rate of 1.0 ml/min. The column temperature was 25°C. The column effluent was analysed by UV detection at 200–450 nm. Peak analysis and assignment were performed using the system analysis software (CLASS-LC10; Shimadzu). The contents of ginsenoside Rg1, ginsenoside Rb, pachyman, polygalic acid and β-asaricin were estimated from standard calibration curves created using authentic standards.

Animals and treatment

All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals, and were approved by the Shandong University Animal Care and Use Committee.

Ten-week-old virgin female Wistar rats weighing 260–300 g were purchased from Shandong University Laboratory Animal Shelter (Shandong, China). The rats were divided randomly into four groups.

Anaesthesia was induced with 3% sodium pentobarbital (30 mg/kg i.p.). The first group of rats underwent a sham operation (sham group) via a dorsal incision. The remainder underwent bilateral ovariectomy. The ovariectomised rats were then divided randomly into three groups of eight for treatment, starting on the day after surgery. The sham group and the control ovariectomised group were given distilled water. One group of ovariectomised rats were given 17β-estradiol (100 μg/kg per day); the other ovariectomised group were given BWS (1.2 g/kg per day) by oral gavage, daily for 3 months. The dose of BWS was determined by conversion of the regular dose for humans to that for the rat and from a pilot experiment with different doses of BWS in rats.

Morris water maze

Twenty-four hours after the last drug treatment, rats were put into the Morris water maze to assess learning and memory performance on a spatial orientation task.[20] A circular 180 cm diameter swimming pool made of black polyethylene was filled 32 cm deep with water at 25 ± 2°C. The water was made opaque by the addition of pure milk powder (Inner Mongolia Yili Industrial Group Co. Ltd, Neimonggu, China). A round transparent platform (10 cm diameter, 30 cm high) was hidden below the surface of the 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. Chengdu, China) which recorded the swimming pattern, including the length of the swim path on each trial.

Table 1 Composition of the Bu-Wang-San formulation

Components	Province	Voucher specimen	Part used	Amount used (g)
Panax ginseng C.A. Mey	Jilin	SDU0810	Root	6
Acorus gramineus Soland	Sichuan	SDU0156	Root	12
Poria cocos (Schw.) Wolf	Yunnan	SDU0123	Dried sclerote	20
Polygala tenuifolia Willd	Hebei	SDU0241	Root	10

Behavioural test

Spatial acquisition testing was conducted between 8:30 and 11:30 am and 1:30 and 4:30 pm. Rats were trained for 4 days, with eight trials per day, and a 2-min break between trials. On the first day rats were 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 more times. Finally, they were placed at the edge of the pool and allowed to swim to the 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 the following way: rats were placed at the edge of the pool, at the midpoint of each of the four quadrants in turn. 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 guided to it. We recorded the swim time (s), swim distance (cm) and swim speed (cm/s) on each trial.

Spatial retention

The spatial retention trial was conducted 24 h after the spatial acquisition phase to determine long-term memory. Each rat was allowed to swim for 60 s. The platform was removed from the pool for this trial. We measured the parameters including quadrant time (percentage of time spent in the quadrant in which the platform was located in the spatial acquisition phase) and platform crossings (the number of times the rat crossed the exact location of the platform).

Ultrastructural observation and spine synapse density

One hour after the Morris water maze test, six rats in each group were deeply anaesthetised with 3% sodium pentobarbital (30 mg/kg i.p.) and perfused through the ascending aorta with 100 ml 0.1 M phosphate-buffered saline (pH 7.4) followed by 60 ml 3% glutaraldehyde. The region of the forebrain containing the hippocampus was removed and cut into blocks. Blocks were then trimmed into 1 mm^3 cubes and fixed in 3% glutaraldehyde overnight. The next day, the blocks were washed three times with 0.2 M phosphate buffer, fixed with 1% osmium tetraoxide, washed with 0.2 M phosphate buffer again, and dehydrated by different concentrations of ethanol. The sections were immersed in fresh Spon812 resin/acetone (1:1) for 30 min, embedded and left overnight at 70°C for polymerisation. Semi-thin sections were obtained and stained with toluidine blue for light microscopic examination to locate the pyramidal cells. Thin sections (50 nm) were made with an ultramicrotome and stained with 2% uranyl acetate and lead citrate. The synaptic density was observed using a H-7000FA transmission electron microscope (Hitachi Co. Ltd, Tokyo, Japan). Photographs were taken at a magnification of 20 000. Synaptic density was determined by counting all the synapses in each photo. We used an indirect counting technique based on geometrical assumptions to count the number of synapses. N_v is based on the relationship between the number of profiles of objects per unit area of the section, Q_A (total number synapses/photo), the caliper height of the objects in a direction normal to the plane of the section, H (rate of synapses/photo), and the thickness of the sections, h (50 nm): $N_V = Q_A/(H + h)$. The number of synapses (N) defined as having both a postsynaptic density and at least two vesicles in the presynaptic density or at least two vesicles in the presynaptic terminal no more than 0.2 μm from the synaptic cleft was counted.

RNA isolation and real-time PCR assay

Frozen specimens of 50–100 mg of hippocampus were homogenised and the total RNA was sequentially extracted using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA was treated with DNase in order to remove genomic DNA. Total RNA was used for cDNA synthesis with M-MLV reverse transcriptase (Promega, Madison, WI, USA) and oligo-dT primers (Invitrogen) in a volume of 20 μl at 37°C for 60 min and 95°C for 5 min. Quantitative real-time PCR was performed on a LightCycler apparatus (Roche Diagnostics, Mannheim, Germany) using a SYBR RT-PCR kit (Takara Bio Inc., Kyoto, Japan). The primers for synaptophysin P38 were 5′-TCCCGGAATACTTGGAGGCT-3′ (sense) and 5′-AAGAGGGAGGGACCACAGTCA-3′ (antisense); the primers for β-actin were 5′-CGTTGACATCCGTAAAGACC-3′ (sense) and 5′-TAGAGCCACCAATCCACACA-3′ (antisense). The PCR reaction for P38 was produced in an initial denaturation at 95°C for 10 s, followed by 50 cycles of 57°C for 5 s, 72°C for 10 s, and terminated by a cooling step for 30 s at 65°C. β-actin was used as an internal control. The threshold cycles (Ct) were used to quantify the mRNA levels of the target genes. Relative mRNA was calculated as $2^{-\Delta\Delta Ct}$, where ΔCt is the difference in threshold cycles for P38 and β-actin genes.

Western blot analysis

Proteins were extracted for Western blot analysis as reported previously.[21] Then 50 μg protein samples were separated on a 10% SDS-PAGE gel and electrophoretically transferred to nitrocellulose membrane. The membrane was washed, blocked and then incubated with mouse monoclonal anti-P38 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C and then with an anti-mouse horseradish peroxidase-conjugated secondary antibody (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 60 min at room temperature. The bands were detected with an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Uppsala, Sweden) and exposed to an X-ray film. The intensity of the bands was quantified using a densitometer.

Statistical analysis

All statistical analysis was performed using SPSS software (Version 10.0, SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± SD. Statistical comparisons were performed by one-way analysis of variance. If only two groups were compared, an unpaired Student's *t*-test was applied. The accepted level of significance was $P < 0.05$.

Results

Spatial acquisition

The Morris water maze is a well-established paradigm for evaluating deficits in hippocampal-dependent memory. In particular, learning and memory deficit is demonstrated by extended time in acquisition and retention. The Morris water maze has been used to test spatial memory in many studies. For example, one study found that estrogen given to ovariectomised rats improved spatial memory.[22] Others have found that chronic exogenous estrogen can impair spatial memory in the water maze in both rats and mice.[23,24]

In the spatial acquisition phase of testing, the ovariectomised rats had significantly longer swim times than the sham and BWS-treated groups, although animals in these two groups did achieve shorter swim times at the end of the whole trial. Comparison of the four groups at the end of the experiment showed that untreated ovariectomised rats had significantly longer swim times than other groups; ovariectomised rats treated with BWS showed reduced swim times, which were close to those of the sham rats and estradiol-treated rats. Moreover, there was a significant decrease in swim time in the sham group, estradiol-treated and BWS-treated groups over the 3 days ($P < 0.01$), but not in the ovariectomised group (Figure 1a).

BWS also showed a dramatic positive effect on swim distance. Control ovariectomised rats had significantly longer swim distances than the other groups; the BWS-treated group had significantly shorter swim distances, similar to that of the sham group. There was also a trend for decreasing swim distance in the sham, estrogen and BWS groups over the 3 days ($P < 0.01$), but not in the control ovariectomised group (Figure 1b).

Neither ovariectomy nor treatment had an effect on swim speed (Figure 1c).

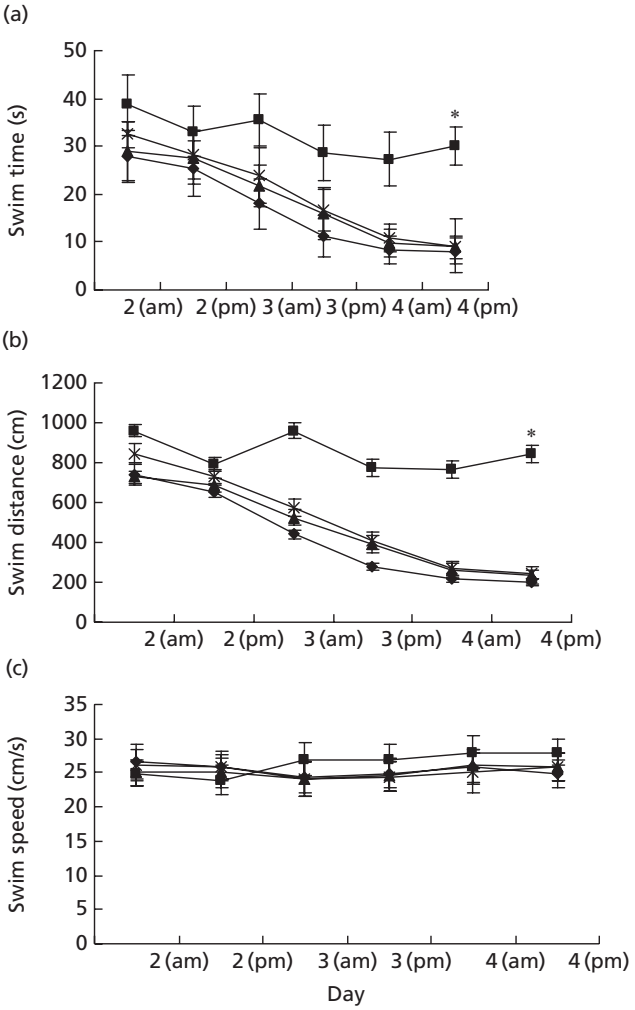

Figure 1 Performance in the spatial acquisition task as illustrated by (a) swim time, (b) swim distance and (c) swim speed, measured morning and afternoon for 3 consecutive days. In the ovariectomised group there were no significant differences between findings at day 2 am and day 4 pm. ◆, sham rats; ■, ovariectomised controls; ▲, estradiol treated; ×, Bu-Wang-San (BWS) treated. Each symbol represents the mean ± SD ($n = 8$). *$P < 0.01$ BWS vs ovariectomised rats on day 4.

Spatial retention

In the spatial retention trial, both quadrant time and platform crossings were significantly affected by BWS (Table 2). BWS treatment protected against the ovariectomised-induced decrease in the retention for the target quadrant during the trial. In the spatial retention trial, quandrant time in ovariectomised rats was significantly reduced compared with the sham group, but BWS treatment prevented this decline in ovariectomised rats. Rats in the BWS group had significantly more platform crossings than those in the ovariectomised group.

Table 2 Quadrant time and platform crossings

Treatment	Quadrant time (%)	Platform crossings (n)
Sham	31.70 ± 4.58	3.75 ± 1.04
Control OVX	18.40 ± 4.71*	2.25 ± 0.71
OVX + estradiol	28.10 ± 5.02	3.68 ± 0.92
OVX + BWS	29.30 ± 5.02	3.63 ± 0.92†

Values are mean ± SD.
BWS, Bu-Wang-San formulation; OVX, ovariectomised.
*$P < 0.01$ vs sham rats; †$P < 0.01$ vs OVX group.

Spine synapse density

Ultrastructural observation of the hippocampi of the ovariectomised rats showed that the number of spine synapses was apparently decreased. The number was significantly increased by BWS treatment. The number in the BWS group was similar to that in the sham and estradiol groups (Figure 2).

The average spine synapse density per μm^3 is shown in Figure 3. Synaptic density in the CA1 region of the hippocampus was significantly affected by BWS treatment. The density was reduced in the ovariectomised group relative to both other groups ($P < 0.001$). There was no significant difference between the BWS group and the sham group.

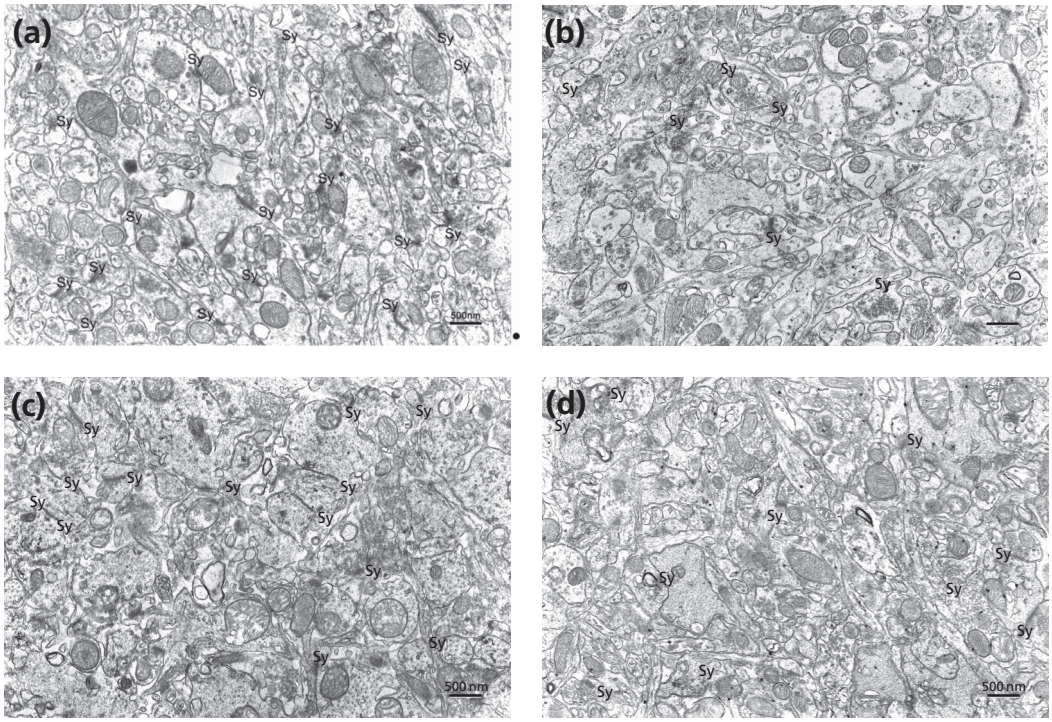

Figure 2 Ultrastructural appearance of the hippocampus in the four groups. (a) The number of synapses (Sy) in the neuropil in the sham group was normal. (b) The number in the ovariectomised group was apparently decreased. (c) The number in the estradiol-treated group was similar to that in the sham group. (d) The number in the Bu-Wang-San group increased compared with the ovariectomised group.

Expression of P38 mRNA in the hippocampus

Estrogen-induced increases in hippocampal plasticity enhance memory function. Estrogen may prevent impairment of transport systems that maintain ion homeostasis and energy metabolism, and thereby forestall excitotoxic synaptic degeneration and neuronal loss in disorders such as Alzheimer's disease and ischaemic stroke.[19]

Our data show that P38 gene expression in the hippocampus of ovariectomised rats (0.46 ± 0.18 fold) was down-regulated compared with that in sham rats (0.97 ± 0.06 fold) ($P < 0.01$). A significant increase in P38 expression was observed in the BWS group (0.74 ± 0.31 fold) compared with the ovariectomised group ($P < 0.01$) (Figure 4).

P38 protein expression

Western blot analysis showed that expression of P38 protein was significantly lower in the control ovariectomised group than in the sham group ($P < 0.01$). A significant increase in P38 protein expression was observed in the BWS treatment group compared with the ovariectomised group ($P < 0.01$) (Figure 5).

Discussion

Ovariectomy of young animals has been shown to result in synaptic loss after 6 days.[25] Interactions of the estrogen receptor system with various growth factors is important for

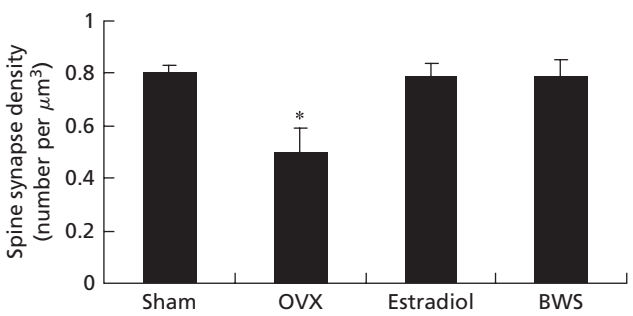

Figure 3 Spine synapse density in the hippocampus. Each bar represents the mean ± SD ($n = 6$). *$P < 0.001$ vs sham group. OVX, ovariectomised; BWS, Bu-Wang-San.

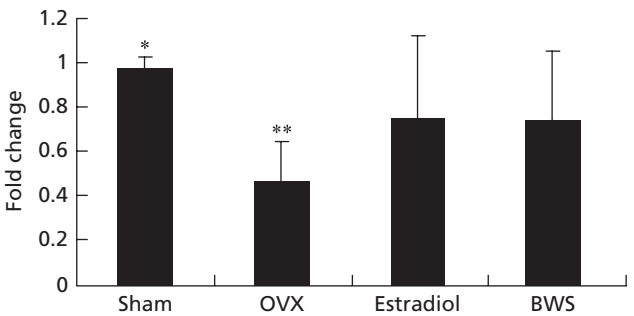

Figure 4 Effect of Bu-Wang-San (BWS) on the expression of P38 mRNA analysed by real-time PCR. Bars are mean ± SD ($n = 8$). *$P < 0.01$ vs ovariectomised (OVX) group; **$P < 0.01$ vs BWS group.

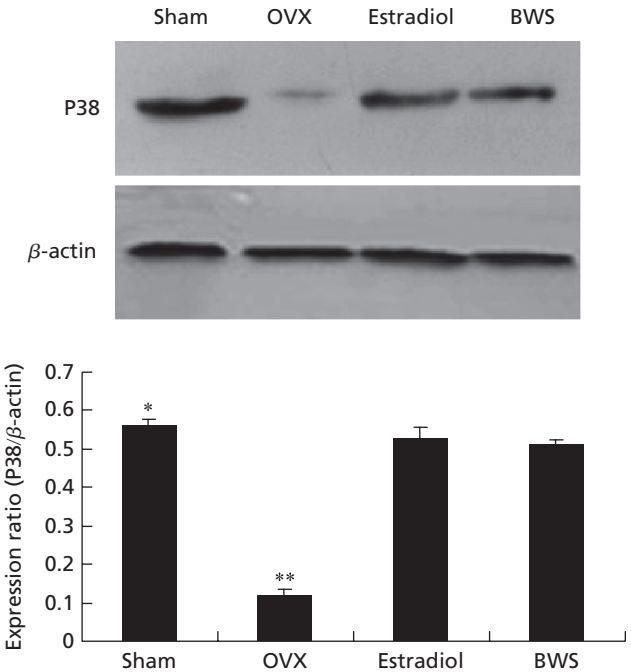

Figure 5 Effect of Bu-Wang-San (BWS) on P38 protein expression, determined by Western blot. $*P < 0.01$ vs ovariectomised (OVX) group; $**P < 0.01$ vs BWS group.

neurite growth and differentiation.[26] Some factors can ameliorate the memory disorder of the ovariectomised rat by increasing synaptic sprouting, increasing cholinergic activity in the hippocampus, protecting neurons against amyloid-induced toxicity and other excitotoxic events.[27–29] In behavioural tests, the fact that spatial memory continued to improve significantly in the BWS group during the three training days, but not in the control ovariectomised group, suggests that BWS ameliorated disordered learning of ovariectomised rats. Data from the spatial retention trial demonstrate that BWS protects against the ovariectomised-induced decrease in spatial retention. BWS increased synapse density and the expression of synaptophysin P38 in the hippocampus. In light of the behavioural findings, BWS-treated rats learned better than ovariectomised rats. Ultra-structural observation and real-time PCR in the hippocampus suggest that BWS is capable of protecting neurons from ovariectomy-induced injury and that the protective effect is associated with increased spine synapse density and the expression of synaptophysin P38 in the hippocampus. More work will be needed to elucidate further the effect of BWS on memory in ovariectomised rats, including non-water maze spatial memory tasks and other memory tasks (e.g. inhibitory avoidance). Our present study suggests that BWS could improve cognitive ability and memory in a model of neuronal impairment induced by estrogen depletion. The mechanisms are possibly associated with an increase in synapse density and the expression of synaptophysin P38 in the hippocampus from estrogen-induced injury. BWS may be a beneficial agent for patients with postmenopausal memory disorder.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Notelovitz M. Is routine use of estrogen indicated in postmenopausal women? An opposing view. *J Fam Pract* 1989; 29: 410–415.
2. Sherwin BB. Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology* 1988; 13: 345–357.
3. Ditkoff EC *et al*. Estrogen improves psychological function in asymptomatic postmenopausal women. *Obstet Gynecol* 1991; 78: 991–995.
4. Sherwin BB. Estrogenic effects on memory in women. *Ann NY Acad Sci* 1994; 743: 213–230.
5. Kimura D. Estrogen replacement therapy may protect against intellectual decline in postmenopausal women. *Horm Behav* 1995; 29: 312–321.
6. Sughrue PJ, Merchenthaler I. Estrogen is more than just a "sex hormone": novel sites for estrogen action in the hippocampus and cerebral cortex. *Front Neuroendocrinol* 2000; 21: 95–101.
7. Wise PM *et al*. Minireview: neuroprotective effects of estrogen-new insights into mechanisms and action. *Endocrinology* 2001; 142: 969–973.
8. Toran-Allerand CD *et al*. Novel mechanisms of estrogen action in the brain: new players in an old story. *Front Neuroendocrinol* 1999; 20: 97–121.
9. Roof RL, Hall ED. Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. *J Neurotrauma* 2000; 17: 367–388.
10. Wise PM, Dubal DB. Estradiol protects against ischemic brain injury in middle-aged rats. *Biol Reprod* 2000; 63: 982–985.
11. Hammond CB. Women's concerns with hormone replacement therapy – compliance issues. *Fertil Steril* 1994; 62: 157S–160S.
12. Grady D *et al*. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obstet Gynecol* 1995; 85: 304–313.
13. Anonymous. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer [see comments] [published erratum appears in Lancet 1997 Nov 15; 350 (9089): 1484]. *Lancet* 1997; 350: 1047–1059.
14. Junping K *et al*. Neuroprotective effects of the aqueous extract of the Chinese medicine Danggui-Shaoyao-san on aged mice. *J Ethnopharmacol* 2005; 97: 313–318.
15. Hai-Fa Q *et al*. NT-1, an active constituent extracted from Tiao xin Recipe, enhances long-term potentiation of CA1 subfield in rat hippocampal slices. *Life Sci* 2006; 79: 8–15.
16. Yun YJ *et al*. Neuroprotective effect of Palmul-Chongmyeong-Tang on ischemia-induced learning and memory deficits in the rat. *Biol Pharm Bull* 2007; 30: 337–342.
17. Kalu DN. The ovariectomised rat model of postmenopausal bone loss. *Bone Miner* 1991; 15: 175–192.
18. Franke WW *et al*. Distribution and mode of arrangement of microfilamentous structures and actin in the cortex of the amphibian oocyte. *Cytobiology* 1976; 14: 111–130.
19. Keller JN *et al*. 17Beta-estradiol attenuates oxidative impairment of synaptic Na^+/K^+-ATPase activity, glucose transport,

and glutamate transport induced by amyloid beta-peptide and iron. *J Neurosci* 1997; 50: 522–530.
20. Morris R. Spatial localization does not require the presence of local cues. *Learn Motiv* 1981; 12: 239–260.
21. Sohn EJ *et al*. Anti-atherogenic effects on the methanol extract of Sorbus cortex in atherogenic-diet rats. *Biol. Pharm Bull* 2005; 28: 1444–1449.
22. Markham JA *et al*. Ovarian hormone replacement to aged ovariectomised female rats benefits acquisition of the Morris water maze. *Horm Behav* 2002; 42: 284–293.
23. Holmes MM *et al*. Low levels of estradiol facilitate, whereas high levels of estradiol impair, working memory performance on the radial arm maze. *Behav Neurosci* 2002; 116: 928–934.
24. Fugger HN *et al*. Sex differences in the activational effect of ERα on spatial learning. *Horm Behav* 1998; 34: 163–170.
25. Miller MM *et al*. Estrogen, the ovary, and neurotransmitters: factors associated with aging. *Exp Gerontol* 1998; 33: 729–757.
26. Singh M *et al*. Estrogen-induced activation of the mitogen-activated protein kinase in cerebral cortical explants: convergence of estrogen and neurotrophin signaling pathways. *J Neurosci* 1999; 19: 1179–1188.
27. Gibbs RB. Estrogen and nerve growth factor-related systems in brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. *Ann NY Acad Sci* 1994; 743: 165–199.
28. Morrison JH, Hof PH. Life and death of neurons in the aging brain. *Science* 1997; 278: 412.
29. Stone DJ *et al*. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implication for Alzheimer's disease. *J Neurosci* 1998; 18: 3180–3185.