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Original Article

Efficacy of Baishao Luoshi decoction (白芍络石方) on synaptic plasticity in rats with post stroke spasticity

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Abstract

OBJECTIVE: To evaluate the efficacy of Baishao Luoshi decoction (白芍络石方, BD) on synaptic plasticity in rats with post stroke spasticity (PSS), and to study the mechanism behind the action.

METHODS: The PSS model of rat was established by middle cerebral artery occlusion (MCAO). The neurological deficit symptoms were evaluated by modified neurological deficit score (mNSS). Muscle tension were evaluated by Modified Ashworth score (MAS). Transmission electron microscopy (TEM) was used to observe the synaptic ultrastructure. The expression of synaptic plasticity-related protein brain derived neurotrophic factor (BDNF), growth associated protein-43 (GAP43), synaptophysin (p38) and microtubule-associated protein 2 (MAP2) in the brain tissue around the infarct were detected by Western blotting.

RESULTS: We found that mNSS were significantly improved and limb spasticity was ameliorated treated by BD. The thickness of postsynaptic density and the synaptic curvature increased significantly. The expression of synaptic plasticity-related protein BDNF, GAP43, p38, MAP2 in the brain tissue around the infarct were raised remarkably after treated by BD.

CONCLUSIONS: Alleviating PSS by BD may be related to rescuing the synaptic plasticity, which provides a probable new therapeutic method for PSS.

Keywords: infarction, middle cerebral artery; neuroprotection; post stroke spasticity; synaptic plasticity; Baishaoluoshi decoction

1. INTRODUCTION

Stroke is the second leading cause of disability and death all over the world, and the most common cause of disability and death in China, where resided one fifth of the world's population.[1] Post stroke spasticity (PSS) is one of the most common damage after acute stroke, which is clinically characterized by increased muscle tone and tendon hyperreflexia, with an incidence account for 30%-80%.[2,3] Pain, muscle stiffness, depression and abnormal posture caused by PSS are great barrier in the rehabilitation of stroke. What's more, the cost of PSS is fourfold as high compared to patients without limb spasticity, which brings heavy caregiver and financial burden on families and societies.[4]

Therefore, it is urgent and imperative to clarify the pathogenesis of PSS and critical to find effective drugs for alleviating spasticity. Antispasmodic oral drugs (such as baclofen, dantrolene, tizanidine, and benzodiazepines) can relieve spasm of hemiplegia limbs to a certain extent. But their use is restricted due to fatigue, drowsiness, dizziness, dry mouth and nausea side effects.[5,6] Local injection of botulinum toxin type A (BoNT-A) has been

shown to relieve muscle tone and pain, as well as improve motor function.[7] However, the therapeutic efficacy varies by BoNT-A and with short duration of action. Common side effects reported by BoNT-A include muscular weakness, pain, dysphagia, peripheral edema and dry mouth.[8] Most importantly, the local injection of BoNT-A must be guided by electromyography or ultrasound, and with high technical requirements for the operator,[9] which leads to the limitation of clinical application in most hospital in China.

So natural products with slight side effect is turning to be the focus of research hotspots in PSS prevention and treatment, and Traditional Chinese Medicine (TCM) is one of them.[10,11] Baishao Luoshi decoction (白芍络石方, BD) is an excellent representative of TCM which can prevent and treat PSS. Our previous study found that BD can ameliorate spasticity and enhance the motor function of hemiplegic upper limb, improve the daily living ability in PSS patients, and with a reliable security.[12] To further clarify its therapeutic mechanism, the PSS model of rat was established by middle cerebral artery occlusion (MCAO), aiming to elucidate the molecular mechanism of BD in the treatment of PSS from the aspect of synaptic plasticity.

2. MATERIALS AND METHODS

2.1. Materials and reagents

Positive reference drug Baclofen tablets were purchased from Fuan Pharmaceutical (Group) Co., Ltd. (Ningbo, China). Chloralic hydras was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Paraformaldehyde was purchased from Biosharp Co., Ltd. (Hefei-China). BCA Protein Assay Kit, RIPA Lysis Buffer, and QuickBlock™ Primary Antibody Dilution Buffer for western blot were purchased from Beyotime Company (Shanghai, China). MCAO monofilament was purchased from Beijing Cinontech Biotech Co., Ltd. (Beijing, China). Ethyl alcohol, acetone, glutaraldehyde, osmic acid, lead citrate, and uranyl acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Ningbo, China). Microtome was obtained from Leica Co., Ltd. (Heidelberg, Germany). Diamond knife was obtained from Daito Me holdings Co., Ltd. (Aichi, Japan). Microplate reader (EIX808U) was obtained from BioTek Instruments, Inc. (Winooski, VT, USA). Electrophoresis Apparatus (SE300) was obtained from Hoefer Inc. (Holliston, MA, USA). Gel imaging system (G: B0X) was obtained from Syngene International Ltd. (Cambridge, UK).

2.2. Preparation of BD

The BD comprised: Baishao [Baishao (*Radix Paeoniae Alba*) and Luoshiteng (*Caulis Trachelospermi Jasminoidis*)] at a ratio of 1∶1. Formula granules of Baishao (*Radix Paeoniae Alba*) (No. 16050101, 15090121, 16010069) and Luoshiteng (*Caulis Trachelospermi Jasminoidis*) (No. 15110003, 16082053, 16050059) were purchased from Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd. (Sichuan, China).

2.3. Animals

Male specific pathogen free Sprague-Dawley rats (weight, 250-280 g) were obtained from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China; No. 43004700027199) and were housed 3-5 to a cage in standard laboratory conditions. The use of rats and their treatments was approved by the Laboratory Animal Welfare and Ethics Committee, Hunan Normal University (Changsha, China; No. 14-XL 1), and all the experiments were carried out in strict compliance with their regulations.

2.4. Quality analysis of BD with HPLC

To ensuring the quality of stability, reliability and therefore warrant the safety and effectiveness of BD. The chromatograms of BD were established by using high performance liquid chromatography (HPLC). A Shimadzu LC-20AT series HPLC system (Shimadzu Corporation, Kyoto, Japan) and a WondaSilC 18 for herbal medicine (250 mm × 4.6 mm × 5 μm) were used for HPLC analysis. The reference substance paeoniflorin (110736), and tracheloside (111858) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The reference substance albiflorin (PR141015-1) was purchased from ChromaDex Co. (Suite G Irvine, CO., USA). The separation was performed with a mobile phase consisted of 0.1% phosphate aqueous solution and acetonitrile as a gradient procedure. The detection was conducted at the wavelength of 230 nm with a flow rate of 1.0 mL/min at 25 ℃.

2.5. Establishment of rat model about PSS

The PSS rat model was established by MCAO as previously described.[13] Briefly, the rats were anesthetized with 10% chloral hydrate (0.35 mL/100 g) through intraperitoneal injection after fast 4-6 h. No symptoms of peritonitis or pain after intraperitoneal injection of chloral hydrate were observed. Heating pad was used to maintain the rat body temperature throughout the process. After skin disinfection, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed carefully. Then the ECA was ligated with 4-0 surgical suture and the ICA was clamped temporarily by micro-arterial clip. Next, the proximal and distal ends of CCA were ligated temporarily separately. The right middle cerebral artery (MCA) was occluded with a MCAO monofilament (Beijing Cinontech Biotech Co. Ltd., Beijing, China, 0.26 mm in diameter) by inserting it from the right CCA and advancing into the ICA until it blocked the origin of the MCA. At last, the monofilament was trimmed and the neck skin was disinfected and sutured. The sham group share same surgical procedures except inserting the monofilament. Three days after

model established, the rats were randomly divided into six groups as follows: high dose BD group (H), low dose BD group (L), baclofen group (B) (positive reference drug), model group (M), sham-operated group (S), and control group (C). Rats were daily treated orally with BD (10.8 g/kg in H and 5.4 g/kg in L) or baclofen (5.4 mg/kg in B), or normal saline (M, S, C) by gavage for 4 weeks. The rats were sacrificed with excessive anesthesia with chloral hydrate 4 weeks following treatment. The brain was separated and reserved for transmission electron microscopy (TEM) and Western blot detection.

2.6. Modified neurological severity score (mNSS)

MNSS was used to evaluate neurological deficits showing in Table 1, which was composed of motor function, sensory function and reflex deficiency. One point is awarded when the rat cannot carry out the test or lack the tested reflex. The higher the score, the more severe the neurological damaged.[14]

2.7. Spasticity evaluation

The Modified Ashworth Scale (MAS) was used to estimate spasticity in the hemiplegia limb. MAS scores are as follows: scoring 0 means no increase in muscular tension; scoring 1 means a slight resistance at the end of passive movement in hemiplegia limb; scoring 1^+ means a slight resistance throughout less than half of the passive movement in hemiplegia limb; scoring 2 means a resistance throughout most of the passive movement in hemiplegia limb; scoring 3 means a resistance throughout all of the passive movement in hemiplegia limb which leading to dyskinesias; scoring 4 means the hemiplegia limb is stiff in flexion or extension.[15] MAS scores (0, 1, 1^+, 2, 3, and 4) were assigned numerical values (0, 1, 2, 3, 4, and 5, severally).[16]

2.8. Ultrastructure observed by transmission electron microscopy (TEM)

To observe the ultrastructure of synapse with TEM, brain tissue around the infarct was cut into 1 mm × 1 mm × 1 mm, postfixed in 2.5% glutaraldehyde for 2 h and 1% osmic acid, the brain tissue was dehydrated in graded alcohol and embedded in epoxy resin. Sections (60-80 nm thick) were sliced and double stained by uranium acetate and lead citrate for 15 min. The brain tissue was observed by TEM (HITACHI Corporation, Hitachi, Japan) and the morphology of Gray 1 synapse was analyzed by image-pro Plus 6.0.

2.9. Western blot

Ischemic brain tissue was homogenized in lysis buffer and centrifuged at 12000 rpm for 5 min at 4 ℃. Supernatant liquid was collected and stored at －80 ℃ until use. Protein concentration of the supernatant liquid was detected using a BCA protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Every sample (50 μg) was separated by electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated in 5% non-fat dry milk at room temperature for 1 h and then incubated at 4 ℃ overnight with the following primary antibodies: rabbit monoclonal anti-brain derived neurotrophic factor (BDNF) antibody (1:1000; catalog # ab108319; abcam, Cambridge, UK), rabbit monoclonal anti-growth associated protein-43 (GAP43) antibody (1:1000; catalog # ab75810; abcam, Cambridge, UK), rabbit monoclonal anti- p38 antibody (1:1000; catalog # ab32142; abcam, Cambridge, UK), rabbit polyclonal anti-microtubule-associated protein 2 (MAP2) antibody (1:1000; catalog # ab32454; abcam, Cambridge, UK). After washing 3 times with tris-buffered saline and tween, membranes were incubated on a shaker for 2 h at room temperature with horseradish peroxidase-labeled Goat Anti-Rabbit antibody (1:1000; catalog # A0208; Beyotime Institute of Biotechnology, Shanghai, China). Enhanced chemiluminescence was used to detect the proteins and image J software (National Institute of Health) was used to quantitative analysis.

Table 1 Modified neurological severity score

Test item	Score
Motor function	
Raising the rat by the tail:	
Flexion of forelimb or hindlimb	1
Head moved more than 10 ℃ to the vertical axis within 30 s	1
Walking on the ground	
Normal walking	0
Circling toward the paretic side	1
Fall down to the paretic side	2
Abnormal movement	
Immobility, staring, tremor and pilo-erection	1
Myodystony and irritability	1
Sensory function	
Ipsilateral forelimb retracts after acupuncture	0
Ipsilateral forelimb does not retract after acupuncture	1
Reflex deficiency	
Pinna reflex (head shake when touching the external auditory meatus)	1
Corneal reflex (blink when lightly touching the cornea with cotton)	1
Startle reflex (Motor response to sudden appearance of the noise)	1
Total score	11

2.10. Ethics approval and consent to participate

The present study was approved by the Laboratory Animal Welfare and Ethics Committee, Hunan Normal University (Changsha, Hunan, China).

2.11. Statistical analysis

Data were expressed as the mean ± standard deviation. Statistical analysis was performed by SPSS 20.0 (IBM Corp., Armonk, NY, USA). Statistical significance was estimated by one-way analysis of variation, followed by Dunn's post-hoc test. $P < 0.05$ was considered to be statistically different.

3. RESULTS

3.1. Chromatogram of BD established by HPLC

The main components of BD are paeoniflorin, albiflorin and tracheloside. HPLC chromatogram of the reference sample demonstrated that albiflorin was at 33.652 min (peak 1), paeoniflorin at 37.253 min (peak 2), and tracheloside at 61.644 min (peak 3) (Figure 1A). There were 8 main characteristic peaks in the HPLC chromatogram of BD, and peak 1 belonged to albiflorin, peak 2 belonged to paeoniflorin, and peak 3 belonged to tracheloside. Chromatogram of 3 batches of BD were established, showing a high degree of similarity, representing that BD from different batches were stable and consistent (Figure 1B).

3.2. Neuroprotective effects of BD on PSS rats

Weight of the rats dropped dramatically after the MCAO surgery for about 10 d, as it can be a roughly reflection of health condition on rats, indicating a seriously trauma of ischemic stroke. Then the weight increased gradually, furthermore, the weight of rats in high dose and low dose of BD rebounded and increased faster than baclofen group and model group ($P < 0.01$), remainding us that BD can improve survival quality of PSS rats (Figure 2A). Rats treated with high dose or low dose of BD had significant improvement on mNSS at 2 and 3 weeks compared to model group ($P < 0.01$), but no obvious statistically difference at 1 and 4 weeks after the intervention ($P > 0.05$) (Figure 2B).

3.3. Spasticity ameliorated treated by BD on PSS rats

MAS score showed that the spasticity of hemiplegic limbs on PSS rats treated with positive reference drug baclofen had been ameliorated obviously. Fortunately, the spasticity of PSS rats being treated with high dose or low dose of BD had also been relieved at 3 and 4 weeks after the intervention ($P < 0.05$), but there was no statistically difference at 1 and 2 weeks after the intervention when estimated by one-way analysis of variation ($P > 0.05$) (Figure 2C).

3.4. Rescuing synaptic structure by BD on PSS rats

The TEM showed that the thickness of postsynaptic

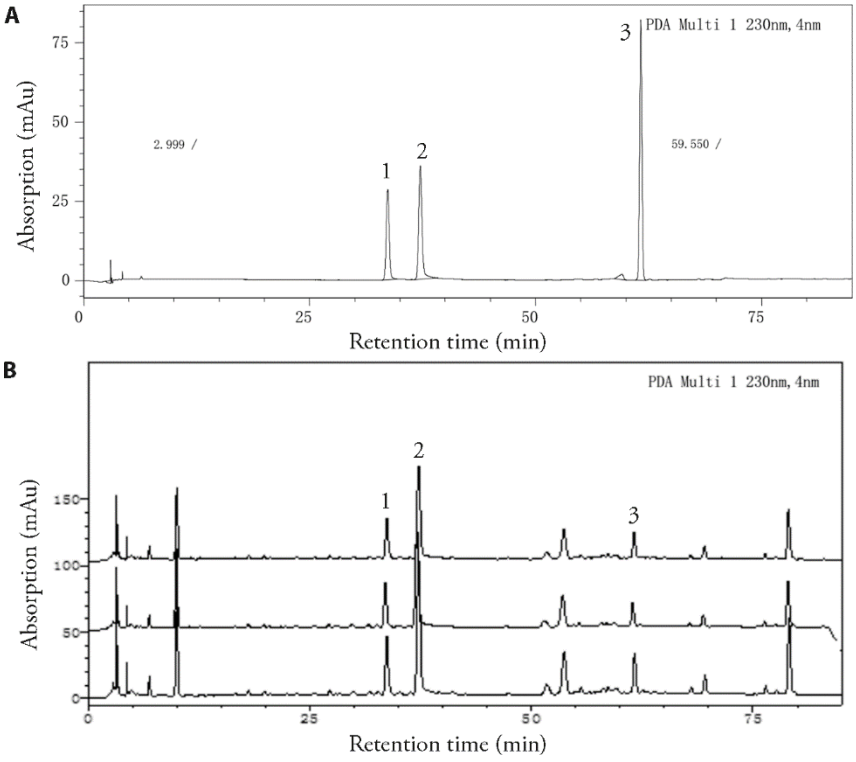

Figure 1 Chromatogram of BD established by HPLC

A: reference sample of paeoniflorin, albiflorin and tracheloside, the main component of BD [Baishao (Baishao (*Radix Paeoniae Alba*) and Luoshiteng (*Caulis Trachelospermi Jasminoidis*) at a ratio of 1∶1]. Peaks 1-3 represented paeoniflorin, albiflorin and tracheloside respectively; B: HPLC chromatogram of three different batches of BD formula granules [Baishao (*Radix Paeoniae Alba*) (No. 16050101, 15090121, 16010069) and Luoshiteng (*Caulis Trachelospermi Jasminoidis*) (No. 15110003, 16082053, 16050059)]. BD: Baishaoluoshi decoction; HPLC: high performance liquid chromatography.

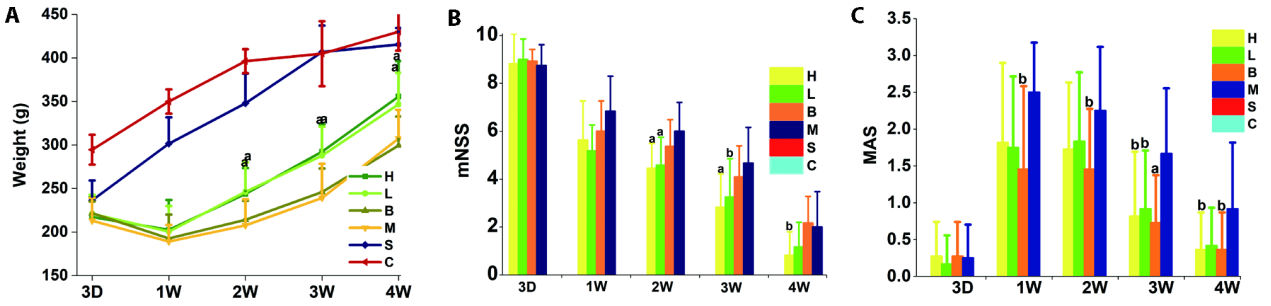

Figure 2 Neuroprotective effects of BD on PSS rats

A: comparison of weight in each group at different time. Weight of rats in each group were detected at different time, 3 d after model established, 1, 2, 3, and 4 weeks after intervention; B: comparison of mNSS in each group at different time. mNSS was used to evaluate neurological deficits at different time, 3 d after model established, 1, 2, 3, and 4 weeks after intervention; C: comparison of MAS in each group at different time. MAS was used to evaluate spasticity of the affected fore limb 3 d after model established, 1, 2, 3, and 4 weeks after intervention. The PSS model was established by MCAO, and intervention began 3 d after model established for 4 weeks. Rats were daily treated orally with BD (10.8 g/kg in H and 5.4 g/kg in L) or baclofen (5.4 mg/kg in B), or normal saline (M, S, C) by gavage for 4 weeks. H: high dose BD group (10.8 g/kg); L: low dose BD group (5.4 g/kg); B: baclofen group (5.4 mg/kg); M: model group (normal saline); S: sham-operated group (normal saline); C: control group (normal saline). BD: Baishaoluoshi decoction; PSS: post stroke spasticity; mNSS: modified neurological deficit score; MAS: Modified Ashworth score; MCAO: middle cerebral artery occlusion. $^{a}P < 0.01$ and $^{b}P < 0.05$ as compared to model group ($n = 12$).

density (PSD) increased significantly after being treated by high dose or low dose of BD compared with model group ($P < 0.01$) (Figure 3A, 3B). Same results were shown in the synaptic curvature (Figure 3A, 3C). But the width of the synaptic gap had no difference in each group ($P > 0.05$) (Figure 3A, 3D). Therefore, the synaptic structure injured by ischemia can be rescued by BD.

3.5. Synaptic plasticity associated protein BDNF, GAP43, p38 and MAP2 increased treated by BD on PSS rats

Abundant proteins that regulate synaptic plasticity accumulated in presynaptic and postsynaptic, such as BDNF, GAP43, p38, MAP2 and so on, which play an important role in the development, maturation, and plasticity of synapse. Here we find that the expression of synaptic plasticity associated protein BDNF, GAP43, p38, MAP2 in the brain tissue around the infarct raised remarkably after treated by high dose or low dose of BD compared with model group ($P < 0.01$) (Figure 4A-4E), suggesting that BD can promote nerve growth and improve synaptic plasticity.

4. DISCUSSION

Neurons that die after stroke cannot be regenerated. Nevertheless, the connections of synapses are reproducible, which can promote the recovery of neurological function by adjusting its structure and function. Repeated activities of the synapse connection cause changes in the morphology and number of synapses, which called synaptic plasticity, in terms of dendrite, axon, synapse size and number in structural forms.[17,18] The main pathology of PSS is that the inferior nerve loses the innervation of the upper nerve, so the synaptic plasticity can re-establish the innervation of the upper nerve, promoting the rehabilitation of PSS.[19] The present study demonstrated that BD ameliorated hemiplegic limb spasm by increasing the thickness of PSD, the synaptic curvature and raising the expression of synaptic plasticity-related protein BDNF, GAP43, p38, MAP2 in the brain tissue around the infarct, indicating that synaptic plasticity may be an important mechanism in the rehabilitation of PSS treated by BD.

The key role of synaptic plasticity is PSD, an approximately 30-50 nm thick high electron density structure attached to the postsynaptic membrane, where settled abundant of cytoskeletal protein (tubulin, actin, and neurofilament protein), signal molecule (CaMKII), membrane receptors (NMDAR) and PSD-95, SAP90, SAP97 and so on.[20,21] The present study showed that the thickness of PSD decreased after MCAO surgery observed by TEM, and recover to its previous thickness whether treated by high dose of BD or low dose of BD, suggesting that BD can restore the PSD damage in PSS rat. Synaptic curvature is another important parameter of synaptic plasticity. The greater the curvature, the stronger the effectiveness, which has a larger contact surface for more efficient binding of neurotransmitters to their receptors.[22] We observed that the synaptic curvature increased significantly after intervened by high dose or low dose of BD. These results indicated that BD has a protective effect on synaptic plasticity from the aspect of morphology.

Many signal molecules regulating synaptic plasticity accumulate in the presynaptic and postsynaptic membrane, such as BDNF, GAP43, p38, MAP2 and so on.[23,24] As a key regulator of synaptic plasticity, BDNF works through both presynaptic and postsynaptic mechanisms, on one hand BDNF can facilitate the release of presynaptic neurotransmitter by promoting the phosphorylation of presynaptic vesicle protein, and on the other hand, it can enhance the activity of postsynaptic by increasing the calcium concentration in dendrites temporarily.[25]

MAP2 has been identified as a filamentous neuro-specific protein enriched in the soma, axons and dendrites of neurons. It is essential in modulating

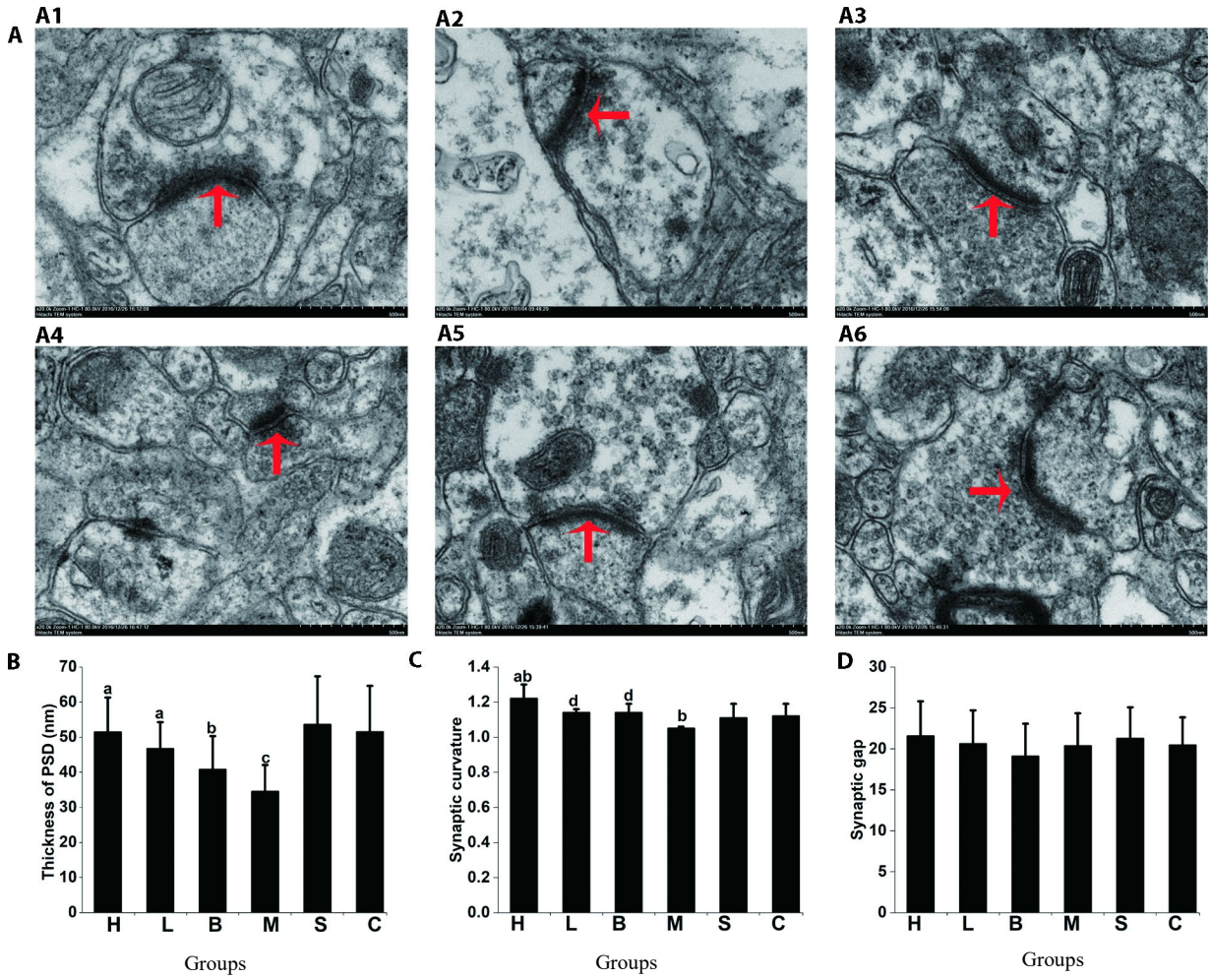

Figure 3 Effects of BD on the synaptic structure in PSS rats

A: synaptic structure observed by TEM (×20 000). The red arrows represent synapse; B: comparison of PSD in the brain tissue around the infarcts; C: comparison of synaptic curvature in the brain tissue around the infarcts; D: comparison of synaptic gap in the brain tissue around the infarcts. Brain tissue around the infarct was stained by uranium acetate and lead citrate, and observed by TEM and the morphology of Gray 1 synapse was analyzed by image-pro Plus 6.0, 4 weeks after intervention. A1: high dose BD group (10.8 g/kg); A2: low dose BD group (5.4 g/kg); A3: baclofen group (5.4 mg/kg); A4: model group (normal saline); A5: sham-operated group (normal saline); A6: control group (normal saline). BD: Baishaoluoshi decoction; PSS: post stroke spasticity; TEM: transmission electron microscopy; PSD: postsynaptic density. $^{b}P < 0.05$ and $^{c}P < 0.01$ as compared to control group; $^{d}P < 0.05$ and $^{a}P < 0.01$ as compared to model group ($n = 3$).

microtubule networks in the dendrites and axons of neurons as well as the assembly and stability of microtubules.[26] The expression of MAP2 decreases after cerebral ischemia injury, and then retrieve upward progressively with nerve regeneration.[27] Similarly, MAP2 also down regulated in cerebral hemorrhage rats, and exercise training can improve the mRNA and protein levels of MAP2 in sensorimotor cortex and promote the recovery of limb function.[28] We also found that MAP2 in the brain tissue around the infarct was raised remarkably after being treated by high dose or low dose of BD, suggesting that BD can promote nerve growth and improve synaptic plasticity.

GAP43 is a phosphoprotein related to neural development, synaptic plasticity, and neural regeneration which highly expressed in neuronal growth cones and presynaptic structure.[29] The prime influence of GAP43 on synapses is to promoting the growth of axons, such as neurite sprouting and axonal sprouting/branching.[30] After cerebral infarction, new axons around the lesion can be observed, and they are rich in mitochondria, endoplasmic reticulum, and abundant of vesicles. When down-regulating GAP43 by gene silencing technology, stability of the synaptic structure around the infarct and the sprouting of axons are significantly adversely affected, hinting that GAP43 plays an indispensable role in synaptic plasticity.[31] Our results showed that the expression of GAP43 increased significantly in high dose and low dose of BD group compared to model group, implicating BD could increase GAP43 to promote nerve regeneration in PSS rats.

P38, also known as synaptophysin, a glycoprotein attached to the outer membrane of synaptic vesicles, accurately reflects the distribution, number, efficiency, and density of synapses.[32] P38 affects the synaptic structure and plasticity by regulating the differentiation, growth of axons and dendrites.[33] It is also an indispensable factor in the release of presynaptic

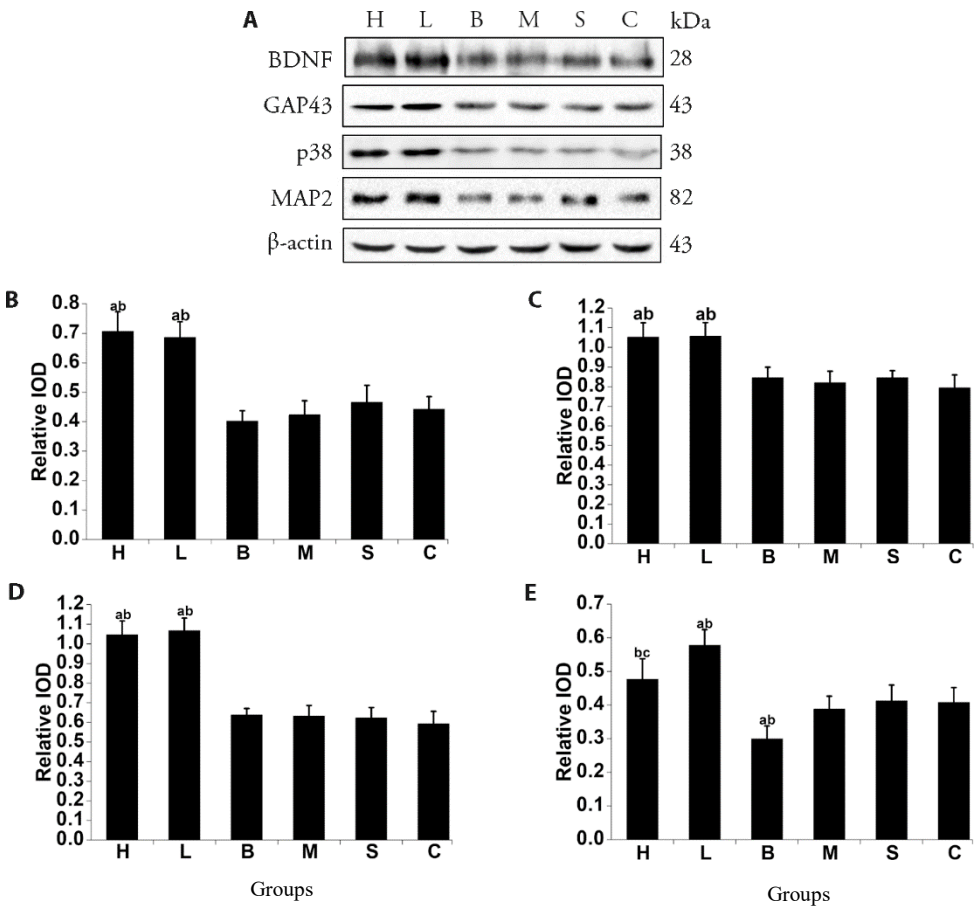

Figure 4 Effects of BD on synaptic plasticity associated protein in PSS rats

A: protein expression levels of BDNF, GAP43, p38 and MAP2 in brain tissue around the infarct were detected by Western blot analysis, 4 weeks after intervention. β-actin was used as the internal control. B: the relative expression of BDNF, C: the relative expression of GAP43, D: the relative expression of p38, E: the relative expression of MAP2: Densitometric analysis. H: high dose BD group (10.8 g/kg), L: low dose BD group (5.4 g/kg), B: baclofen group (5.4 mg/kg), M: model group (normal saline), S: sham-operated group (normal saline), and C: control group (normal saline). BD: Baishaoluoshi decoction; PSS: post stroke spasticity; BDNF: brain derived neurotrophic factor; GAP43: growth associated protein-43; MAP2: microtubule-associated protein 2. $^{a}P < 0.01$, $^{c}P < 0.05$, as compared to control group; $^{b}P < 0.01$ and $^{d}P < 0.05$, as compared to model group (n = 6).

neurotransmitters. Therefore, p38 is a reliable marker of synapse. Immunofluorescent staining of p38 was used to track synaptic plasticity of the corticospinal tract (CST) after stroke, CST axonal density and p38 staining of CST axonal terminals were prominently increased at day 28 in comparison with day 14 after ischemic stroke in mice, suggesting that synaptic plasticity mediated by p38 makes a significant contribution to neurological recovery.[34] Our present results show that BD can also improve synaptic plasticity by increasing the level of p38, and promote the neurological recovery in PSS rats.

The results of the present study suggest that BD alleviates spasticity on PSS may be related to rescuing the synaptic plasticity. Therefore, the BD may be a new treatment strategy and adjuvant therapy for PSS. But further studies are demanded to elucidate the molecular mechanisms of action. And how does BD relieve spasticity through synaptic plasticity is the next research direction that needs to be addressed.

In conclusion, the present study demonstrated that BD improved mNSS and ameliorated limb spasticity, increased the thickness of PSD and the synaptic curvature, and up regulated the expression of synaptic plasticity-related protein BDNF, GAP43, p38, MAP2 in the brain tissue around the infarct in PSS rats. Consequently, alleviating PSS by BD may be related to rescuing the synaptic plasticity, which provides a probable new therapeutic method for PSS.

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