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# Bu Shen Yi Sui capsule promotes remyelination correlating with Sema3A/NRP-1, LIF/LIFR and Nkx6.2 in mice with experimental autoimmune encephalomyelitis

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ABSTRACT

*Ethnopharmacological relevance:* Bu Shen Yi Sui capsule (BSYSC), based on traditional Chinese formula Liu Wei Di Huang pill, is effective for the treatment of multiple sclerosis (MS) in clinical experience and trials. Our previous studies confirmed that BSYSC had the neuroprotective effect in MS and its animal model, experimental autoimmune encephalomyelitis (EAE); however, its mechanism of action was not clear. Thus, the effect of BSYSC on remyelination and the underlying mechanisms were investigated in the EAE mice.

*Materials and methods:* The EAE model was established by injecting subcutaneously myelin oligodendrocyte protein (MOG) $_{35–55}$ in mice. Mice were treated with BSYSC (3.02 g/kg) or vehicle daily by oral gavage for 40 days. The body weight and clinical score of mice were evaluated. Brain was observed by magnetic resonance imaging. The inflammation infiltrate of brain and spinal cord was determined by hematoxylin-eosin staining, while the structure of myelin sheath was visualized by transmission electron microscopy on days 23 and 40 post immunization (dpi), respectively. The protein and mRNA levels of platelets-derived growth factor receptor (PDGFR) α and 2′, 3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) were measured by immunohistochemistry, western blot and quantitative real-time polymerase chain reaction. The protein expressions of semaphorins (Sema) 3A, Neuropilin (NRP) − 1, leukemia inhibitory factor (LIF), LIF receptor (LIFR) and Nkx6.2 were further investigated by western blot.

*Results:* BSYSC treatment improved the body weight and clinical score of EAE mice, alleviated inflammatory infiltration and nerve fiber injuries. It also protected the ultrastructural integrity of myelin sheath. BSYSC significantly increased expressions of PDGFRα and CNPase in mice with EAE on 40 dpi. Furthermore, BSYSC treatment increased the expressions of LIF, LIFR and Nkx6.2 and reduced Sema3A and NRP-1 in EAE mice on 40 dpi.

*Conclusions:* The data demonstrated that BSYSC exhibited the neuroprotective effect against EAE by promoting oligodendrocyte progenitor cells (OPCs) proliferation and differentiation, thus facilitating remyelination. Sema3A/NRP-1, LIF/LIFR and Nkx6.2 are likely contributed to the effects of BSYSC on OPCs.

## 1. Introduction

Multiple sclerosis (MS) is a central nervous system (CNS) disorder mediated by immunity. It is one of the main causes of neurological dysfunction in young adults. The pathological hallmark of MS is focal demyelinated plaques within the CNS (Fitzner and Simons, 2010).

*Abbreviations:* BSYSC, Bu Shen Yi Sui capsule; CNPase, 2′,3′-cyclic nucleotide-3′-phosphodiesterase; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; LIF, leukemia inhibitory factor; LIFR, leukemia inhibitory factor receptor; MOG, myelin-oligodendrocyte glycoprotein; MS, multiple sclerosis; NRP-1, neuropilin-1; OPCs, oligodendrocyte progenitor cells; OL, oligodendrocyte; PA, prednisone acetate; PDGFRα, platelets-derived growth factor receptor α; SC, spinal cord; Sema3A, semaphoring 3A; TCM, traditional Chinese medicine

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Spontaneous remyelination is usually present after the onset of the disease, but the process is inadequate and eventually fails, especially during remission stage, leading to progressive disability (Patani et al., 2007). Thus, the promotion of remyelination is one of the important tactics for the recovery of neurological function in MS. The study found that remyelination involves the recruitment of oligodendrocyte precursor cells (OPCs), which including OPCs proliferation and migration to the damaged region, followed by differentiation into mature oligodendrocytes (OLs), then wrapping axons to form new myelin sheaths (Alizadeh et al., 2015). It has shown that after demyelination, OPCs undergo proliferation, which is characterized by the presence of the early marker platelets-derived growth factor receptor (PDGFR) α, and transform into mature OLs that acquire the marker 2′, 3′-cyclic nucleotide-3′-phosphodiesterase (CNPase) (Aharoni et al., 2008). Experimental evidence has suggested several neuroprotective cytokines, guidance molecules and transcription factors, notably leukemia inhibitory factor (LIF), semaphorin (Sema) 3A and Nkx6.2 have been shown to regulate the myelination process of OLs. LIF improves remyelination by promoting OPCs to proliferate (Deverman and Patterson, 2012), Sema3A exerts repulsive effects on OPC migration (Syed et al., 2011), and Nkx6.2 involves in the regulation of OLs maturation (Cai et al., 2010).

Clinical experience accumulated from immunotherapies (such as prednisone acetate, etc.) in MS has demonstrated the benefits of reducing nerve injury and relapse rate on MS patient (Dendrou and Fugger, 2017). However, after long-term use, drug efficacy can be offset by an increased risk of serious side effects. No safe and effective treatment has been proved to improve demyelination of MS. In recent years, traditional Chinese medicine (TCM) has been found to have potential advantages and value in the treatment of MS.

TCM has been widely used for MS in China (Song et al., 2017; Zhou and Fan, 2015). TCM theory thinks that primary pathogenesis of MS is deficiency of liver and kidney yin, accompanying with phlegm and blood stasis. Thus, the treatment of MS follows the basic rules of tonifying kidney, activating blood and resolving phlegm. Liu Wei Di Huang pill (LDP) is a famous classic TCM formula for tonifying kidney yin, which was decribed in “Xiaoer Yaozheng Zhijue” written by Qian Yi of the Song dynasty in the year 1119. Experimental studies showed that the formula exhibited benefit effects in improving cognition deficits and protecting dopaminergic neurons (Liu et al., 2013; Tseng et al., 2014). In our previous study, LDP had a potential role in the treatment of experimental autoimmune encephalomyelitis (EAE), the animal model of MS (Liu et al., 2012). Bu Shen Yi Sui capsule (BSYSC), which was modified based on LDP, added some of herbs for activating blood and resolving phlegm including *Hirudo*, *Leonuri Herba*, *Fritillariae Thunbergii Bulbus*, etc., has been extensively used for many years in Beijing Tian Tan Hospital of China. Randomized clinical trials showed that BSYSC was effective in reducing relapse rate and preventing progression for MS patients (Zhou and Fan, 2015). Beijing Food and Drug Administration have certified this formula as a medicinal preparation (No. 10003).

Our recent experimental reports indicated that BSYSC could regulate Th17/Treg cells and alleviate the injury of axon and myelin sheath in EAE mice (Fang et al., 2017; Zheng et al., 2015). The aim of the current study was to assess the effect of BSYSC on remyelination and the possible mechanism in the myelin oligodendrocyte glycoprotein (MOG) induced mice with EAE.

## 2. Materials and methods

### 2.1. Preparation of BSYSC

BSYSC was prepared from the following herbs: *Rehmanniae Radix Praeparata* (dried root of *Rehmanniaglutinosa (Gaertn.) DC.*, Shudihuang, in *Scrophulariaceae*), *Rehmanniae Radix* (dried root of *Rehmanniaglutinosa (Gaertn.) DC.*, Dihuang, in *Scrophulariaceae*), *Polygoni Multiflori Radix Praeparata* (dried root of *Polygonum multijiorum Thunb.*, Zhiheshouwu, in *Polygonaceae*), *Rhei Radix et Rhizoma* (dried rhizome of *Rheum palmatum* L., Dahuang, in *Polygonaceae*), *Fritillariae Thunbergii Bulbus* (dried bulb of *Fritillaria thunbergii Miq.*, Zhebeimu, in *Liliaceae*), *Hirudo* (dried body of *Whitmania pigra Whitman*, Shuizhi, in *Hirudinidae*), *Scorpio* (dried body of *Buthus martensii Karsch*, Quanxie, in *Buthidae*), *Gastrodiae Rhiazoma* (dried tuber of *Gastrodia elata BI.*, Tianma, in *Orchidaceae*), *Forsythiae Fructus* (dried fruit of *Forsythia suspense (Thunb.) Vahl*, Lianqiao, in *Oleexpertae*) and *Leonuri Herba* (dried aerial part of *Leonuri Herba*, Yimucao, in *Labiatae*). The proportions of these herbs were 10:10:10:2:6:3:2:3:6:10. All the herbs except *Fritillariae Thunbergii Bulbus* were immersed in distilled water for 30 min and boiled for 2 h. The inspissation of the filtered solution was under reduced pressure at 70 °C, and then dried powder was mixed uniformly with *Fritillariae Thunbergii Bulbus* flour. Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) was conducted to identify principal constituents and chemical characteristic fingerprinting of BSYSC. Our previous study has reported the detailed information of BSYSC (Zheng et al., 2015). In the present experiment, we employed the same batch of BSYSC.

### 2.2. Animals

All animal work conformed to the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University (Approval number: AEEI-2015–185). Healthy female C57BL/6 mice (16–18 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing. Mice were kept in standard cages in a controlled environment with a temperature (22 ± 3 °C), humidity (40–50%) and light controlled (in 12 h light/dark cycle) room with a commercial standard solid rodent chow and water ad libitum in the Experimental Animal Center of Capital Medical University.

### 2.3. Induction of EAE

The mice were randomly divided into five groups: (1) normal control (NC); (2) NC + BSYSC 3.02 g/kg; (3) EAE; (4) EAE + prednisone acetate (PA) 6 mg/kg and (5) EAE + BSYSC 3.02 g/kg. EAE mice received subcutaneous injections of 50 μg $MOG_{35–55}$ emulsified in 100 μl complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 0.3 mg of *Mycobacterium tuberculosis* (BD Biosciences, San Diego, CA, USA) followed by 500 ng Pertussis toxin (Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally (i.p.) on days 0 and 2. The EAE mice were treated with water, 6 mg/kg PA, or 3.02 g/kg BSYSC by oral gavage once a day for 40 days. The dosage of BSYSC was determined by our previous study that showed treatment with BSYSC 3.02 g/kg to be more effective in mice with EAE (Fang et al., 2013; Yang et al., 2016). The body weight and clinical score that reflect progression of the disease were determined every day. Parameters for clinical scores using the following criteria (Miller et al., 2010): 0, no symptoms; 1, paralyzed tail; 2, partial hind limb paralysis; 3, totally paralyzed hind limbs; 4, paraplegia with paralyzed forelimbs; 5, death.

### 2.4. Magnetic resonance imaging (MRI) experiments

MRI experiments were performed on a 7.0 T MR scanner (Bruker, Pharma Scan, Germany). Mice were anesthetized with isoflurane in oxygen (5% for induction and 1.5% for maintenance) and scanned on days 23 and 40 post immunization (dpi) throughout the MRI experiments. Respiratory rate was monitored with a pneumatic sensor placed under the abdomen of mice. The acquisition parameters for diffusion tensor imaging (DTI) of the mice brain were TR/TE = 5000/25 ms, 30 diffusion encoding directions, b = 1000 s/mm$^2$. Fractional anisotropy (FA) images were re-establish with paravision version 5.1 software (Bruker, Pharma Scan, Germany) and were measured in the cortex.