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Original Article

Yishendaluo decoction attenuates experimental autoimmune encephalomyelitis by modulating CXCR4 signaling

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Abstract: Yishendaluo decoction, a traditional Chinese medicine formula, has become an effective drug for the treatment of multiple sclerosis (MS). However, the potential mechanism of *Yishendaluo* decoction in attenuating MS was largely unknown; the aim of present study was to detect the influence of *Yishendaluo* decoction on CXCR4 signaling in mouse model of experimental autoimmuneencephalomyelitis (EAE). A total of 80 mice were randomly divided into four groups: normal, model, hormone, and *Yishendaluo* decoction group. The disease incidence, body weight change, and clinical symptoms of EAE mice were monitored. The neuropathology in spinal cord was assessed using hematoxylin-eosin or luxol fast blue staining. The CXCR2 expression and its signaling molecules in spinal cord were examined by western blot. The results showed that *Yishendaluo* decoction administration significantly suppressed the loss of body weight and attenuated clinical symptoms in EAE mice. In addition, *Yishendaluo* decoction dramatically attenuated inflammatory infiltration and demyelination in spinal cords of EAE mice. Furthermore, *Yishendaluo* decoction reduced CXCL12 and CXCR4 expression, down-regulated the expression of STAT1, STAT3 and p65 in spinal cords of EAE mice. These results suggest that *Yishendaluo* decoction is effective for reducing the clinical severity of EAE mice, which may berelated to its inhibitive ability in CXCR4 signaling via regulation of key signaling molecules of JAK/STAT and NF-κB pathway. Our findings may be beneficial for developing therapeutic and preventive strategies for MS.

Keywords: Traditional Chinese medicine, multiple sclerosis, chemokine

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory and demyelinating disorder disease, involving white matter with the numerous affected areas of central nervous system possibly due to autoimmunity [1]. MS is one of the leading causes of neurological disability in young adults. The incidence of MS is much higher in Northern Europe and North America than Africa and oriental area [2]. Patients subjected to multiple sclerosis typically show alternating relapse and remission in the early stage of illness. Currently, with the increasing development of diagnosis instrument, the disease incidence of MS recently shows an ascending tendency in China [2].

To date, due to the complexity of multiple sclerosis etiology and pathogenesis, there is no effective treatment for MS. A large body of evidence has indicated that traditional Chinese medicine (TCM) treatment might be advantageous in improving neurological damage and promoting neural function recovery in MS patients [3]. TCM assumed that the pathogenesis of MS is due to deficiency of kidney-essence, function disharmony of entrails, and recurrence of exogenous evil [3]. Therefore, treatment of MS is based on tonifying kidney, removing toxic substance, and eliminating obstruction in collaterals [3]. Based on the feature of compatibility and efficacy of the distinguished ancient prescription-dihuangyin, we bring forward *Yishendaluo* decoction as the treatment prescription for MS.

Yishendaluo decoction is an effective drug for MS, invented by Professor Ying Gao from Beijng University of Chinese Medicine. Previous stud-

ies have revealed that *Yishendaluo* decoction significantly improves neurological function, reduces production of inflammatory mediators, and relieves damage induced by inflammatory reactions during experimental allergic encephalomyelitis in mice [4]. For MS patients in remission, the administration of *Yishendaluo* decoction for more than 9 months reduced the recurrence rate, inhibited new lesions, and decreased neurological deficits and cognitive dysfunction (unpublished data). However, the potential mechanism of *Yishendaluo* decoction in attenuating EAE was largely unknown.

The infiltration of inflammatory cells into the central nervous system (CNS) is acritical step in the neuropathogenesis of MS. The chemokine CXCL12, a potent chemo attractant for monocytes and lymphocytes, and its receptor CXCR4, play important roles inrecruiting circulating lymphocytes into CNS [5]. CXCL12 was significantly up-regulated in the cerebrospinalfluid (CSF) from patients with MS [6]. Recent study showed that CXCL12 play vital role in the pathogenesis of EAE and probably regulates the severity of EAE [7]. Additionally, CXCL12/CXCR4 signaling regulates the migration of oligodendrocyte progenitors (OPs) into the corpus callosum during EAE [8]. Therefore, the aim of present study was to detect the influence of *Yishendaluo* decoction on CXCR4 signaling in mouse model of experimental autoimmuneencephalomyelitis (EAE), and explore its molecularmechanism.

Materials and methods

Animals and reagents

A total of 80 female C57BL/6 mice (6-8 week old and weighing 20-22 g) were purchased from animal centre of Henan University of Traditional Chinese Medicine and maintained in a specific pathogen-free room with a temperature of 24°C, 55%-65% relative humidity, and a 12-h light-dark cycle, with standard chow and water ad *libitum* at Animal Facilities of Henan University of Traditional Chinese Medicine. The experimental procedures were approved by the Laboratory Animal Care Committe eat Medical College of Henan University of Traditional Chinese Medicine. All animals received care according to the Guide for the Care and Use of Laboratory Animals (NIH, Bethesda, MD). The MOG35-55 peptide (MEVGWYRSPFSRVVHLYRNGK) was synthesized by GenScript (Piscataway, NY, USA). Complete Freund's adjuvant (CFA) and pertussis toxin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mycobacterium tuberculosis H37RA was purchased from BD Biosciences (Franklin Lakes, NJ, USA). Monoclonal anti-CXCR4, monoclonalanti-CXCL-12, monoclonal anti-NF-κB p65, monoclonal anti-phospho-STAT1 (Tyr701), monoclonal anti-phospho-STAT3 (Tyr705), and monoclonalanti-glyceraldehyde phosphate dehydrogenase (GAPDH) were purchased from Cell Signaling Technology (Danvers, MA, USA).

Yishendaluo decoction preparation

Yishendaluo decoction, consisting of 20 g prepared rehmannia root, 10 g collacoruscervi, 10 g cape jasmine fruit, 10 g grass leaf sweet flag rhizome, and 6 g licorice roots, was supplied by pharmaceutical preparation section of Henan Province Hospital of Traditional Chinese Medicine (Zhengzhou, China). In brief, the chopped herbs were immerged in 40-60°C water for 60 min, then decocted at low boiling temperature for 30-60 minutes, the poaching liquid was filtered and concentrated as decoction of 2 g crude drug/mL, and stored at 4°C before administration to mice.

Experimental protocol and administration of Yishendaluo decoction

The mouse EAE model was established as described previously [9]. Eighty mice were randomly divided into four groups (n = 20 per group): normal group, model group, hormone group, and *Yishendaluo* decoction group. Mice (except normal control group) were immunized subcutaneously at four sites in the hind flanks with anemulsion of 100 μL MOG33-35 (2 mg/mL) and 100 μL CFA supplemented with 5 mg/mL Mycobacterium tuberculosis H37RA on day 0. In addition, each mouse was intraperitoneally injected with pertussis toxin (25 mg/kg) on the day of immunization and the day after immunization, an additional injection of MOG33-35 in CFA was delivered on day 7. Mice in the normal group were injected with an equal volume (0.2 mL) of PBS. Mice were weighted and clinically scored for EAE daily by the same investigator for 35 days after immunization. On day 1 after immunization, mice in *Yishendaluo* decoction group were treated with a daily oral-gavage of *Yishendaluo* decoction (2 g/kg, 0.2 mL) for 35 consecutive days. The normal group

Table 1. Effect of Yishendaluo decoction on body weight (g) of experimental autoimmune encephalomyelitis mice

Group	After immunization (day)					
	0	7	14	21	28	35
Normal	19.52±1.56	20.12±1.69	20.51±1.89	21.22±2.01	20.97±2.14	20.54±1.79
Model	20.04±1.75	19.24±1.36	15.49±0.97[a]	16.27±1.22[a]	19.75±2.04	20.11±1.34
Hormone	20.12±1.49	19.39±1.04	16.32±0.74[a]	17.03±1.01[a]	20.01±1.64	20.34±1.52
Yishendaluo decoction	19.82±1.26	20.17±1.72	20.67±1.37[b,c]	21.04±2.13[b,c]	21.39±2.41[b]	21.22±1.76

Data are expressed as mean ± SD. N = 10 per group. [a]P<0.05, vs. normal group; [b]P<0.05, vs. model group; [c]P<0.05, vs. hormone group.

Table 2. Effect of Yishendaluo decoction on clinical score of experimental autoimmune encephalomyelitis mice

Group	After immunization (day)				
	7	14	21	28	35
Model	0.52±0.44	6.42±2.11	7.81±2.33	6.78±2.09	6.44±1.01
Hormone	0.51±0.31	4.65±1.72[a]	5.37±1.83[a]	2.56±0.68[b]	1.78±0.92[b]
Yishendaluo decoction	0.25±0.30[a]	3.12±1.50[b,c]	4.11±1.67[b,c]	2.78±0.79[b]	1.56±0.50[b]

Data are expressed as mean ±SD. N = 10 per group. [a]P<0.05, [b]P<0.01, vs. model group; [c]P<0.05, vs. hormone group. Clinical score of EAE was calculated according to weaver's 15-point neurological function scale, tail activity: 0 point, asymptomatic; 1 point, tail tension reduces or tail distant paralysis; 2 points, tail complete paralysis; limb activity: 0 point, asymptomatic; 1 point, gait instability; 2 points, limbhemiparesis, limbs drag when walking; 3 points, completeparalysis of limbs, limb valgus when walking. The tail andlimb paralysis was evaluated and the total scores wereobtained by accumulating scores. Death was recorded as 15 points.

and model group were administered with an equal volume of PBS. The hormone group was treated with a daily oralgavage of (0.078 mg/kg, 0.2 mL) prednisone acetate (Tianjin LishengPharmaceutical Co., Ltd., Tianjin, China; Drug ApprovalNo. 0407018).

Histological analysis

Spinal cords were detached from the mice on day 22 after immunization (at the peak of neurological impairment). After anesthetized by intraperitoneal injection of 10% chloral hydrate, mice were perfused intracardially with PBS, followed by 4% paraformaldehyde, the spinal cord was carefully removed and fixed with 10% formalin overnight, the fixed tissues were embedded in paraffin and cut into 4 μm sections with a microtome (RM2255, Leica, Nussloch, Germany), placed on glass sides, and deparaffinized. Sections were stained with hematoxylin-eosin (H&E) or luxol fast blue (LFB) to assess inflammatory infiltration or demyelination, respectively. The cells in the infiltrates and the demyelination region were observed under an optical microscope (Nikon, Tokyo, Japan), and quantified with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Western blot analysis

22 days after immunization, mice were intraperitoneally anesthetized with 10% chloral hydrate, and perfused intracardially with PBS. The spinal cords of EAE mice were immediately collected and homogenized by sonicationin cold RIPA buffer with protease inhibitoron ice. The supernatants were obtained by centrifugation at 10000 g at 4°C for 30 min. The concentration of protein was measured by bicinchoninic acid protein assay kit (Beyotime, Shanghai). 50 μg proteins were separated by 10% SDS-PAGE, transferred onto polyvinylidene fluoride membranes. The primary antibodies to CXCR4, CXCL12, pSTAT1, pATAT3, NF-κB p65 (1:500, CST, USA) and GAPDH (1:2000; CST USA) were incubated with the blot overnight at 4°C. After being extensively washed with PBS containing 0.1% Triton X-100, the membranes were incubated with HRP-conjugated goat anti-mouse antibody for 30 min at room temperature. The bands were visualized using the ECL system (Millipore, Billerica, WI, USA).

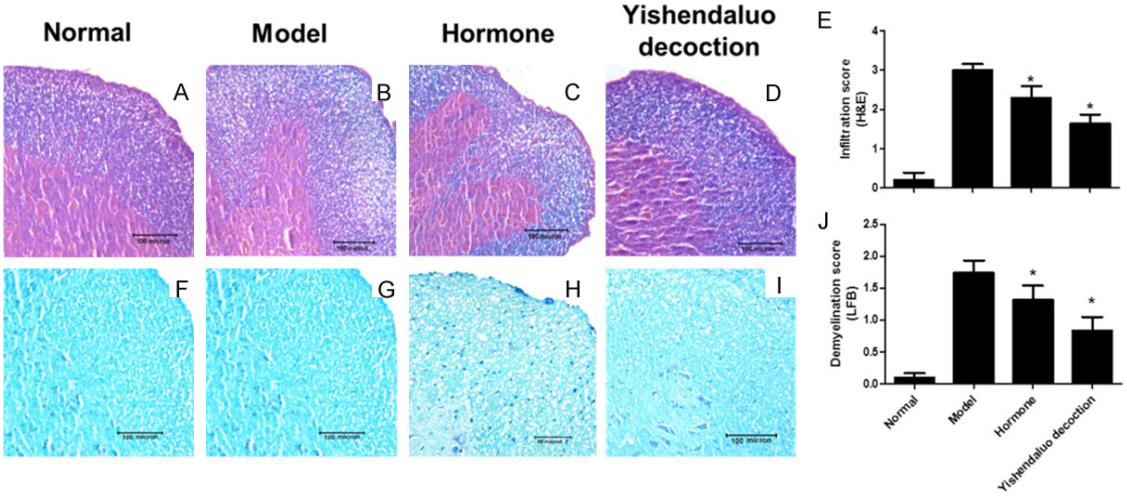

Figure 1. *Yishendaluo* decoction administration attenuates neuropathology in spinal cord in EAE mice. Spinal cords were isolated from the mice on day 22 after immunization (at the peak of neurological impairment). The spinal cord was fixed, imbedded in paraffin and cut into 4 μm sections. Sections were stained with hematoxylin-eosin (H&E) or luxol fast blue (LFB) to assess inflammatory infiltration or demyelination, respectively. (A-D) (H&E) staining. Inflammatory cell infiltration decreased remarkably in the *Yishendaluo* decoction group compared with the model group (bars = 100 μm). (F-I) LFB staining. Demyelination was significantly decreased by *Yishendaluo* decoction treatment (bars = 100 μm). The cells in the infiltrates (E) and the demyelination region (J) were quantified with ImageJ software. Values represent the means ± SD. $^{*}P<0.05$ compares with model group.

Statistical analysis

Results were expressed as mean ± SD. The data were analyzed using ANOVA and $P<0.05$ was regarded as statistically significant.

Results

Yishendaluo decoction suppresses the loss of body weight and attenuates clinical symptoms in EAE mice

To investigate the effect of *Yishendaluo* decoction on EAE, eighty mice were randomly divided into four groups (n = 20 per group): normal group, model group, hormone group, and *Yishendaluo* decoction group. Mice from *Yishendaluo* decoction group were treated with a daily oralgavage of *Yishendaluo* decoction (2 g/kg) at day 1 after immunization for 35 consecutive days. The incidence of EAE was 100% in the model, and hormone group, whereas only 75% in the *Yishendaluo* decoction group. Moreover, mice treated with *Yishendaluo* decoction gained weight normally, whereas the body weight in model group and hormone group significantly decreased day 14 and 21 after immunization (**Table 1**). On day 35, mice in four groups gradually recovered bodyweight to baseline levels (**Table 1**). In addition, neurological dysfunction was observable in mice on day 9 and peaked on day 21 after immunization. *Yishendaluo* decoction group displayed significantly lower clinical scores compared to the model group and hormone group day 14 and 21 after immunization (**Table 2**).

Yishendaluo decoction attenuates neuropathology in spinal cord in EAE mice

To further explore the protection effect of *Yishendaluo* decoction on EAE, H&E and LFB staining were performed to examine the level of cellular infiltration and demyelination insections of the lumbar spinal cord. From H&E staining, a massive inflammatory distribution was observed in the model group compared with normal mice (**Figure 1A** and **1B**). However, *Yishendaluo* decoction treatment significantly reduced the severity of inflammatory cell infiltration in spinal cord compared with model group and hormone group (**Figure 1B-D**). The infiltrated cells in the spinal cord were quantified with ImageJ software (**Figure 1E**). In addition, the level of demyelination was assessed using LFB staining. As shown in **Figure 1F**, **1G**, visible myelin loss in the spinal cord was found

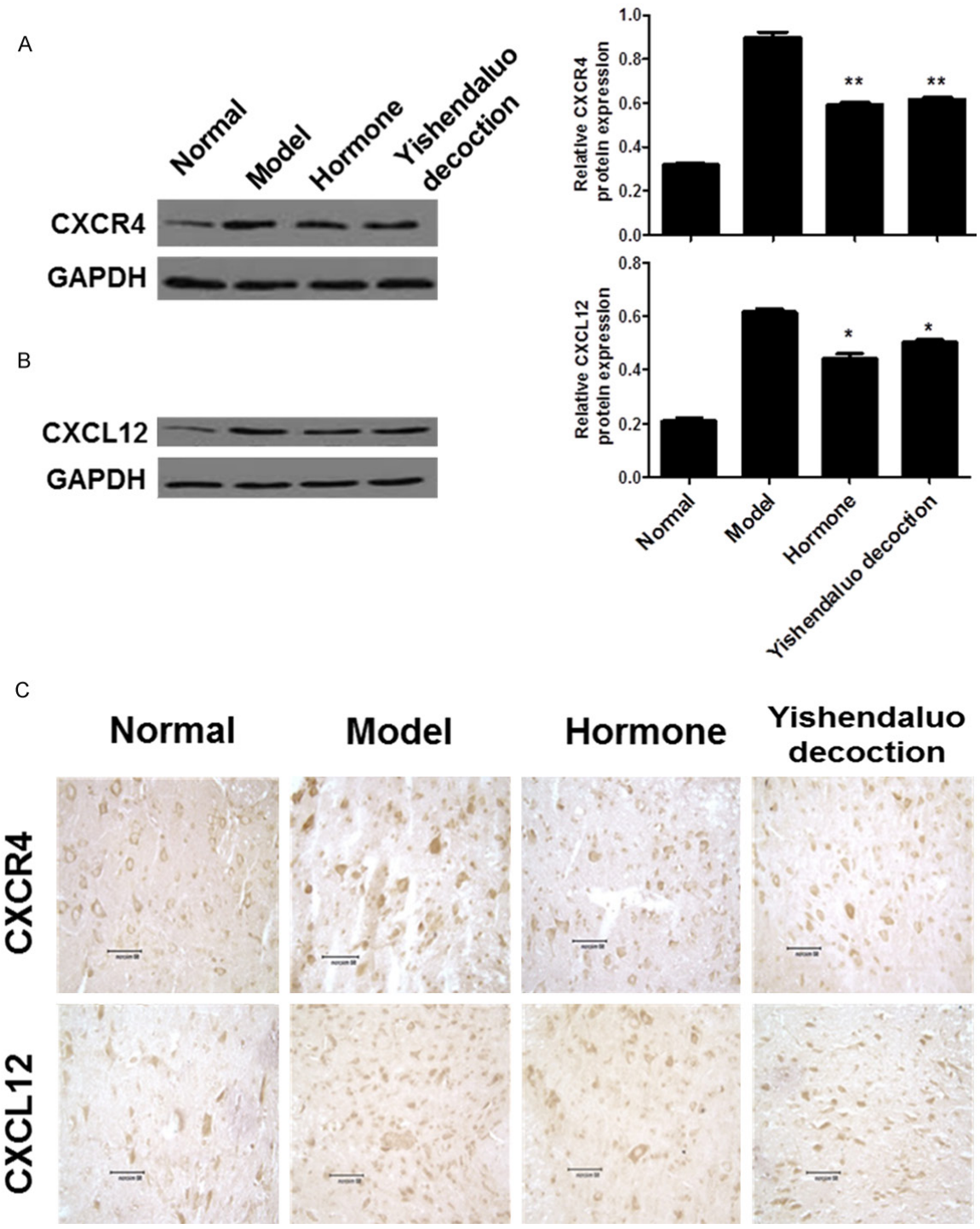

Figure 2. *Yishendaluo* decoction administration reduces CXCL12 and CXCR4 expression. Spinal cords were isolated from EAE mice 22 days after immunization. Total protein was extracted and the CXCR4 (A) and CXCL12 (B) expression were examined by western blotting. GAPDH was used as an internalcontrol. Data shown are the representative bands. (C) The spinal cord was fixed, imbedded in paraffin and cut into 4 μm sections. The CXCR4 and CXCL12 expression in spinal cord was examined by immunohistochemistry (bars = 50 μm). Data shown are the representative images. Values represent the means ± SD. $^{*}P<0.05$, $^{**}P<0.01$ compares with model group.

in the model group compared with normal mice. However, *Yishendaluo* decoction treatment significantly reduced the density of demyelination compared with model group and hormone group (**Figure 1G-I**). The demyelination region was quantified with ImageJ software (**Figure 1J**).

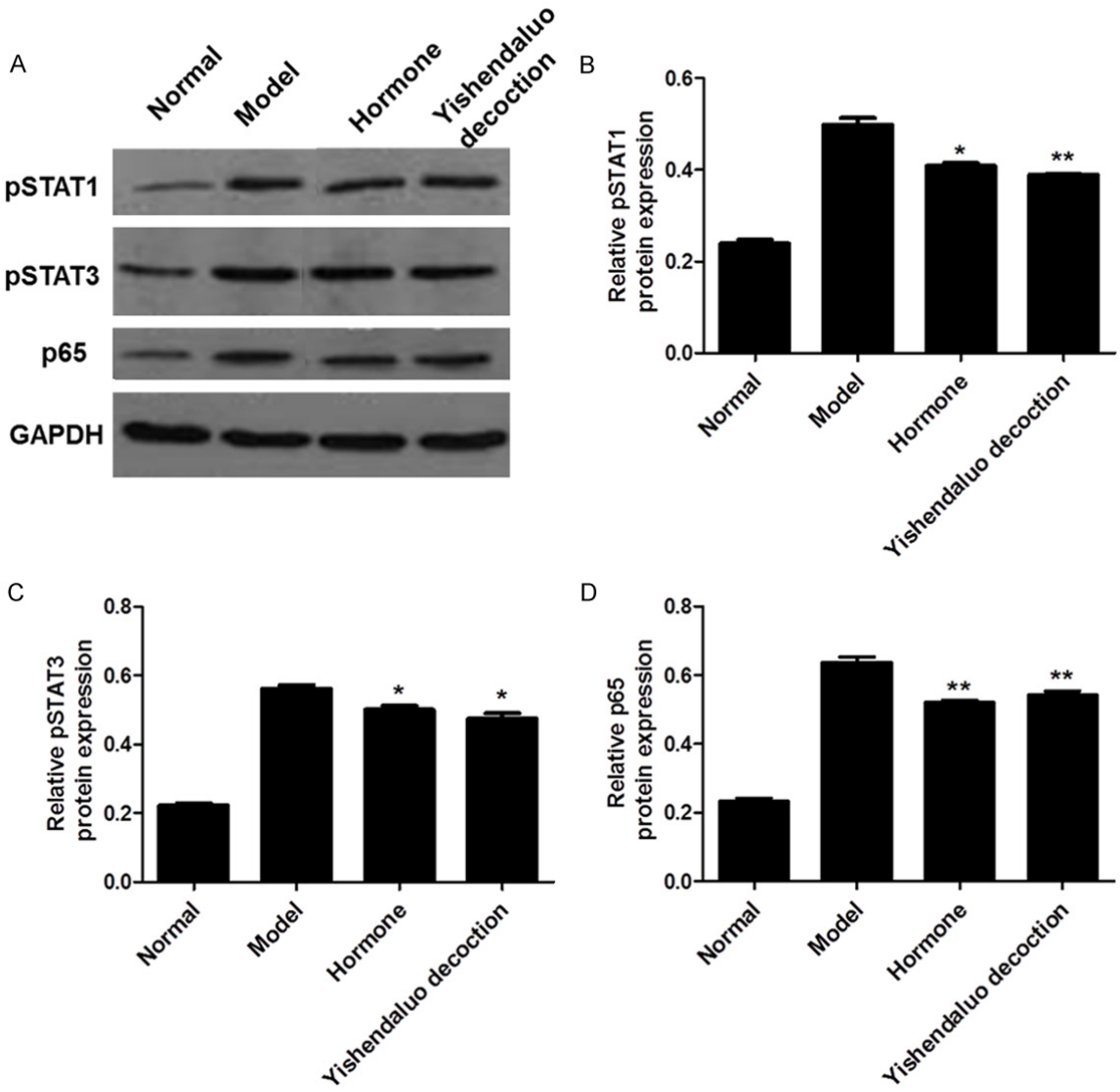

Figure 3. Expressions of pSTAT1, pSTAT3 and p65 were reduced in *Yishendaluo* decoction treated EAE mice. Spinal cords were isolated from EAE mice 22 days after immunization. Total protein was extracted and the pSTAT1 (A and B), pSTAT3 (A and C), and p65 (A and D) expression were examined by western blotting. Data shown are the representative bands. Values represent the means ± SD. $^{*}P<0.05$, $^{**}P<0.01$ compares with model group.

Yishendaluo decoction reduces CXCL12 and CXCR4 expression

Extensive evidence supports CXCR4/CXCL12 axis as a key regulator for early development of the MS [10]. The expression of CXCL12 within the CNS was found to be up-regulated in the MS brain, which are likely to attract dendritic cells, macrophages, and T cells to the perivascular areas of the CNS [7]. We therefore examined the CXCR4/CXCL12 levels in CNS of EAE mice after *Yishendaluo* decoction treatment. At day 20 after immunization (acute phase), CXCR4 and CXCL12 protein expression in the brain of EAE mice were significantly increased compared with the normal group (**Figure 2A** and **2B**). However, *Yishendaluo* decoction treatment significantly reduced the CXCR4 and CXCL12 expression (**Figure 2A** and **2B**). In addition, immunohistochemistry analysis confirmed similar results of western blot data (**Figure 2C**).

The regulatory effect of Yishendaluo decoction on CXCR4 signaling relates to NF-κB and JAK/STAT signaling pathways

Given that the binding of CXCL12 to CXCR4 activates multiple signaling pathways including

NF-κB and JAK/STAT [10], the spinal cords were therefore isolated from *Yishendaluo* decoction-treated mice and subjected to analysis of the key signaling molecules of the NF-κB and JAK/STAT pathways by western blot. The results showed that the expression of pSTAT1, pSTAT3 and p65 were dramatically increased in the model group compared to that in normal mice (**Figure 3A-D**). In addition, upregulation of these three signaling molecules were significantly reduced by treatment with *Yishendaluo* decoction (**Figure 3A-D**).

Discussion

Multiple sclerosis is a chronic autoimmune disease, mainly involving the central nervous system of white matter. Nowadays western medicine treatment is still widely used in the world, such as the hormone and interfer on, which not only have great side effects, but also expensive, and bring great psychological and economic burdens to patients, families and society in long-term use of some western medicines [11]. Therefore, studies of TCM treatment on MS are developing frequently, and showed a positive advantage.

In this study, the effects and possible mechanism of *Yishendaluo* decoction on MS were explored using a classical EAE murine model. The results showed that *Yishendaluo* decoction treatment improved clinical symptoms and neurological dysfunction in EAE mice. Moreover, we have demonstrated for the first time that the therapeutic function of *Yishendaluo* decoction may be as a result of reducing the population of inflammatory cells and its key chemokine CXCR4/CXCL12 via regulating NF-κB and JAK/STAT signaling pathway. Therefore, the findings of current study suggest that *Yishendaluo* decoction has therapeutic effect on the disease development of EAE by modulating CXCR4 signaling.

As the complementary and alternative medicine, TCM has been used for treatment of MS for a long history in China [12]. *Yishendaluo* decoction, a formula designed based on the function and feature of the famous prescription-*Dihuangyinzi*, is an effective drug for MS, invented by Professor Ying Gao from Beijng University of Chinese Medicine [4]. *Yishendaluo* decoction consisted of 5 medicinal plants, prepared rehmannia root, collacomuscervi, cape jasmine fruit, grass leaf sweet flag rhizome, and licorice roots. Prepared rehmannia root plays critical role in regulating the organismic hormones function. It could increase circulating cortical hormone level, and maintains cortical hormone level. Cape jasmine fruit and licorice roots have anti-inflammatory properties. Unlike the side effect from using prednisone acetate, *Yishendaluo* decoction attenuates EAE did not induce body weight loss in EAE mice. However, neurological function was significantly improved after treatment with *Yishendaluo* decoction, implying a superior therapeutic effect of *Yishendaluo* decoction on EAE in comparison with prednisone acetate.

In this study, high levels of CXCL12 were found significantly upregulated in the spinal cord of EAE mice in model group, accompanied by a large number of lymphocytes infiltrated into the spinal cord. It has been reported that CXCL12 was a highly effective chemokine for recruitment of inflammatory cells to CNS lesions [10, 13]. CXCL12 binds to its receptor CXCR4 and then induces the activation of the transcription factor nuclear factor-κB (NF-κB), and the phosphoinositide-3 kinase (PI3K)-Akt pathway [14]. Furthermore, CXCL12/CXCR4 also activates the Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway [10, 15]. Therefore, we examined whether *Yishendaluo* decoction could regulate the CXCR4/CXCL12 levels in CNS of EAE mice. The findings showed that *Yishendaluo* decoction administration significantly inhibited the CXCR4 and CXCL12 expressions. In addition, the upregulation of pSTAT1, pSTAT3, and NF-κB p65 was found upregualted in EAE mice of model group, whereas they were dramatically reduced by treatment with *Yishendaluo* decoction. Taken together, our findings indicate that *Yishendaluo* decoction may exert efficacy in EAE by regulating NF-κB and JAK/STAT signal pathway.

In summary, the present study demonstrated a novel mechanism of *Yishendaluo* decoction in the treatment of MS. *Yishendaluo* decoction is effective for reducing the clinical severity of EAE mice, which may be related to its inhibitive ability in CXCR4 signaling via regulation of key signaling molecules of JAK/STAT and NF-κB pathway. Our findings may be beneficial for developing therapeutic and preventive strategies for MS.

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Disclosure of conflict of interest

None.

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References

[1] Brownlee WJ, Hardy TA, Fazekas F and Miller DH. Diagnosis of multiple sclerosis: progress and challenges. Lancet 2017; 389: 1336-1346.

[2] Eskandarieh S, Heydarpour P, Minagar A, Pourmand S and Sahraian MA. Multiple sclerosis epidemiology in East Asia, South East Asia and South Asia: a systematic review. Neuroepidemiology 2016; 46: 209-221.

[3] Sun Y and Liu X. A review on traditional Chinese medicine in prevention and treatment of multiple sclerosis. J Tradit Chin Med 1999; 19: 65-73.

[4] Shang X, Gao Y, Yin L and Zhang J. Effects of Yishendaluo decoction on axonal degeneration, inflammatory reaction, and neurological function in a mouse model of experimental autoimmune encephalomyelitis. Neural Regeneration Research 2009; 4: 928-934.

[5] Holman DW, Klein RS and Ransohoff RM. The blood-brain barrier, chemokines and multiple sclerosis. Biochim Biophys Acta 2011; 1812: 220-230.

[6] Noroozi Karimabad M, Khanamani Falahati-Pour S and Hassanshahi G. Significant role(s) of CXCL12 and the SDF-1 3'a genetic variant in the pathogenesis of multiple sclerosis. Neuroimmunomodulation 2016; 23: 197-208.

[7] Calderon TM, Eugenin EA, Lopez L, Kumar SS, Hesselgesser J, Raine CS and Berman JW. A role for CXCL12 (SDF-1alpha) in the pathogenesis of multiple sclerosis: regulation of CXCL12 expression in astrocytes by soluble myelin basic protein. J Neuroimmunol 2006; 177: 27-39.

[8] Banisadr G, Frederick TJ, Freitag C, Ren D, Jung H, Miller SD and Miller RJ. The role of CXCR4 signaling in the migration of transplanted oligodendrocyte progenitors into the cerebral white matter. Neurobiol Dis 2011; 44: 19-27.

[9] Ma K, Chen X, Chen JC, Wang Y, Zhang XM, Huang F, Zheng JJ, Chen X, Yu W, Cheng KL, Feng YQ and Gu HY. Rifampicin attenuates experimental autoimmune encephalomyelitis by inhibiting pathogenic Th17 cells responses. J Neurochem 2016; 139: 1151-1162.

[10] Khorramdelazad H, Bagheri V, Hassanshahi G, Zeinali M and Vakilian A. New insights into the role of stromal cell-derived factor 1 (SDF-1/CXCL12) in the pathophysiology of multiple sclerosis. J Neuroimmunol 2016; 290: 70-75.

[11] Soelberg Sorensen P. Safety concerns and risk management of multiple sclerosis therapies. Acta Neurol Scand 2016; [Epub ahead of print].

[12] Yadav V, Shinto L and Bourdette D. Complementary and alternative medicine for the treatment of multiple sclerosis. Expert Rev Clin Immunol 2010; 6: 381-395.

[13] Zhu Y and Murakami F. Chemokine CXCL12 and its receptors in the developing central nervous system: emerging themes and future perspectives. Dev Neurobiol 2012; 72: 1349-1362.

[14] Wurth R, Bajetto A, Harrison JK, Barbieri F and Florio T. CXCL12 modulation of CXCR4 and CXCR7 activity in human glioblastoma stem-like cells and regulation of the tumor microenvironment. Front Cell Neurosci 2014; 8: 144.

[15] Vila-Coro AJ, Rodriguez-Frade JM, Martin De Ana A, Moreno-Ortiz MC, Martinez AC and Mellado M. The chemokine SDF-1alpha triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. FASEB J 1999; 13: 1699-1710.