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Protective effect of Shouwu Yizhi decoction against vascular dementia by promoting angiogenesis

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[ABSTRACT] *Shouwu* is a traditional Chinese medicine (TCM) with neuroprotective effect. Shouwu Yizhi decoction (SYD) was designed based on TCM theory. However, little is known about the roles of SYD in Vascular dementia (VaD). The present study aimed to evaluate the potential effects of SYD on the vascular cognitive impairment and explore the underlying mechanism by establishing focal cerebral ischemia/reperfusion (I/R) rat model to induce VaD. SYD administration (54 mg·kg^{-1}) for 40 days obviously improved the vascular cognitive impairment in the middle cerebral artery occlusion (MCAO) rats as evidenced by the declined neurological deficit score and shortened escape latency via neurological deficit assessment and Morris water maze test. Moreover, SYD decreased neuron damage-induced cell death and ameliorated the ultrastructure of endothelial cells in the MCAO rats, thereby alleviating VaD. Mechanistically, SYD caused increases in the expression of vascular endothelial growth factor (VEGF), CD34 and CD31, compared with the MCAO rats in coronal hippocampus. Simultaneously, the expression level of miR-210 was elevated significantly after SYD administration, compared with the vehicle rats ($P < 0.01$). The expression of Notch 4 at both mRNA and protein levels was upregulated remarkably along with the notably downregulated DLL4 expression under SYD administration compared with the vehicle rats ($P < 0.05$). Overall, the above results indicated that SYD promoted angiogenesis by upregulating VEGF-induced miR210 expression to activate Notch pathway, and further alleviated neuron damage and ameliorated the ultrastructure of endothelial cells in the MCAO rats, ultimately enhancing the cognition and memory of MCAO rats. Therefore, our findings preliminarily identified the effect and the mechanism of action for SYD on VaD in rats. SYD could be a potential candidate in treatment of VaD.

[KEY WORDS] Shouwu Yizhi decoction; Vascular dementia; Angiogenesis; VEGF-Notch signaling pathway

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Introduction

Vascular dementia (VaD) or vascular cognitive impairment (VCI) is considered the second-most-common cause of dementia worldwide [1]. Approximately 25%–30% of ischemic stroke survivors develop delayed or immediate VaD with impaired cognition, memory and linguistic ability caused by ischemic, hypoperfusive, or hemorrhagic brain lesions [2-4]. To the best of our knowledge, there is no definitive medical or surgical treatment available for VaD. Theoretically, promoting angiogenesis, especially in functional blood vessels, is an approach to reducing the extent of ischemia and improving cognition in patients with VaD [5]. Hence, effective therapeutic approaches to targeting angiogenesis are expected to reduce mortality of this disease.

Shouwu Yizhi decoction (SYD) is a patented traditional Chinese medicine (TCM) composed with seven herbs (Zhi Shou Wu, *Alpinia oxyphylla, Astragalus membranaceus, Gastrodia elata, Salvia miltiorrhiza, Polygala tenuifolia* and *Ligusticum chuanxiong*), two medicinal animals (Hirudo and

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Geosaurus) and one herb extract (Ginkgo extract). Among them, *Shouwu* and *Alpinia oxyphylla* are the dominating components: *Shouwu* is widely used in patients with yang-deficiency of spleen or kidney and possesses neuroprotective effect. Wan *et al*. have reported that Renshen Shouwu capsule (RSSW) improves the learning ability and memory of rats with brain ischemia [6]. Liu *et al*. have pointed out that *Alpinia oxyphylla* could improve cognitive impairment in Aβ 1-42 mouse model of Alzheimer's disease [7]. Apart from that, *Astragalus membranaceus* is a famous TCM capable of inducing angiogenesis and proliferation of endothelial cells [8]. *Salvia miltiorrhiza* and its extracts all exhibit protective effects against learning and memory impairments and may become a promising candidate for AD therapy [9-11]. Moreover, the peptide isolated from hirudo and geosaurus presented positive physiological functions, particularly with pro-angiogenic properties [12-13]. Cai *et al*. have found that the rhizomes of *Chuanxiong* is safety and effective in the treatment of patients suffered from a stroke [14]. However, the effects of SYD on VaD and related mechanisms have rarely been reported. According to the neuroprotective effect of Shouwu and Alpinia oxyphylla, accompanied by the invigorating Qi and promoting blood effects of other subsidiary drugs, we speculated that SYD could improve cognitive impairment and stimulate angiogenesis.

In the present study, we successfully established focal cerebral ischemia/reperfusion (I/R) model in rats by transient middle cerebral artery occlusion (MCAO) to induce VaD and subsequently evaluated whether SYD could ameliorate the vascular cognitive impairment in MCAO rats and explore the related mechanism. Our results showed that SYD alleviated neuron damage and improved the ultrastructure of endothelial cells to enhance the cognitive ability in the MCAO rats by promoting Notch pathway-dependent angiogenesis.

Materials and Methods

Preparation of SYD

The prescription of SYD is shown in Table 1. First, the powder of processed *Polygonum multiflorum* Thunb. (Zhi Shou Wu, ZSW), *Salvia miltiorrhiza* Bge. (Dan Shen, DS) and hirudo (Shui Zhi, SZ) were mixed and refluxed for 3 h using 85% ethanol thrice. The extract was filtered; and the suspension was concentrated with a relative density of 1.30–1.35 (50 °C) for later use. TSecond, *Alpinia oxyphylla* Miq. (Yi Zhi, YiZ) and *Ligusticum chuanxiong* Hort. (Chuan Xiong, CX) were distillated for the volatile oil, which was then encapsulated by β-cyclodextrin (β-CD). The residues and the extracted solution were collected respectively. Third, *Astragalus membranaceus* (Fisch.) Bge. Var. *mongholicus* (Bge.) Hsiao (Huang Qi, HQ), *Polygala tenuifolia* Willd.(Yuan Zhi, YuanZ), geosaurus (Di Long, DL), *Gastrodia elata* Bl.(Tian Ma, TM) and the above two residues were extracted by water for 2 h twice. Fourth, the extracted solution from Steps 2 and 3 were mixed and concentrated to 1.06–1.10 (50 °C) by a rotary evaporator (SY2000, Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China). Then, the extraction (with alcohol content of 65%) was set at 4 °C overnight, and concentrated into 1.30–1.35 (50 °C). Fifth, the resultant extracts (density: 1.30–1.35) from Steps 1 and 4 were mixed with starch, and dried in vacuum (DZF-6050, Beijing ZKHS Instrument Co., Ltd., Beijing, China) at 60–65 °C. Finally, the dried extracts mixed with Ginkgo extract and volatile oil (in β-CD) in Step 2.

Table 1 Prescription of SYD

Chinese name	Latin Name	English name	Voucher No.	Weight (g)	Medicinal parts
Herbs					
Zhi Shou Wu (ZSW)	*Polygonum multiflorum* (Thunb) Harald.	Fleeceflower root	TCM-ALD02	500	Root
Yi Zhi (YiZ)	*Alpinia oxyphylla* Miq.	Sharpleaf Galangal fruit	TCM-GAM03	300	Seed
Huang Qi (HQ)	*Astragalus membranaceus* (Fisch.) Bunge	Milkvetch root	TCM-AMB03	350	Root
Tian Ma (TM)	*Gastrodia elata* Bl.	Tall Gastrodia Tuber	TCM-PSD01	250	Rhizoma
Dan Shen (DS)	*Salvia miltiorrhiz* Bge.	Danshen root	TCM-CJF04	350	Root and rhizoma
Yuan Zhi (YuanZ)	*Polygala tenuifolia* Willd.	Thinleaf Milkwort root	TCM-LOP02	200	Root
Chuan Xiong (CX)	*Ligusticum chuanxiong* Hort.	Szechwan Lovage Rhizome	TCM-LCH02	300	Root and rhizoma
Medicinal animals					
Shui Zhi (SZ)	Whitmania pigra Whitman	Hirudo	TCM-GAF01	180	Body
Di Long (DL)	Pheretima	Geosaurus	TCM-YXF01	250	Body
Herb extracts					
Ginkgo extracts	Extractum *Ginkgo Siccum*	Ginkgo Biloba Extract powder	TCM-PBF04	20	Leaf

Quality evaluation of SYD

The emodin from Shouwu in SYD was determined by high-performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) [15]. The chromatographic column was an Agilent Eclipse XDB-C_{18} column (Agilent, Santa Clara, CA USA) (250 mm × 4.6 mm i.d. 5 μm particle size). The mobile phase was consisted of 0.1% phosphoric acid in methyl alcohol at a flow rate of 1 mL·min^{-1}. The detective wavelength