Influences of Bushen Xingnao Decoction on expression of vascular endothelial growth factor, IL-1β and tumor necrosis factor-α in vascular dementia rats

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Abstract: To observe influences of Bushen Xingnao Decoction (BSXND) on expression of vascular endothelial growth factor (VEGF), IL-1β and tumor necrosis factor-α (TNF-α) in brain tissues and serum level of vascular dementia rats and to investigate neuroprotective mechanism of BSXND for vascular dementia. Wistar rats were randomly divided into normal group (N group), sham operation group (S group), dementia model group (M group) and Bushen Xingnao decoction treatment group (MT group). After the model was successfully established, 2, 4, 6 weeks were regarded as observation point. Expressions of VEGF, IL-1β and TNF-α in serum and brain of rat brain were measured by enzyme linked immunosorbent assay. The expressions of IL-1β and TNF-α in MT group were lower than those in M group ($P< 0.05$), the expression of VEGF in MT group was higher than that in M group ($P< 0.05$); the expressions of VEGF, IL-1β and TNF-α in MT and M groups were higher than those in N and S groups ($P<0.01$). BSXND can reduce the levels of IL-1β and TNF-α in brain tissues and serum of vascular dementia rats and increase the expression of VEGF. BSXND can play cerebral protective role by suppressing the neuroinflammation response of vascular dementia rats and enhancing vascular repair.

Keywords: Vascular dementia; Bushen Xingnao decoction; vascular endothelial growth factor; tumor necrosis factor-α; Interleukin-1β.

INTRODUCTION

Vascular dementia (VD), one of the main types of senile dementia is a dementia syndrome produced by cerebrovascular disease induced brain tissue damage. In China and Japan, VD is accounting for about 50%-70% of all dementia patients. Traditional Chinese medicine has a better effect for the clinical treatment on vascular dementia. Bushen Xingnao decoction, on the basis of long-term clinical practice, is the effective preparation to treat VD, involving in nourishing kidney and qi, removing blood stasis and reducing phlegm and activing brain and regaining consciousness. This study, through adopting Bushen Xingnao decoction on rats vascular dementia model for treatment intervention and detecting vascular endothelial growth factor (VEGF), IL-1β and tumor necrosis factor-α (TNF-α), was to discuss the brain protective mechanism.

MATERIALS AND METHODS

Animals

Wistar rats, female, weight in 200-250g, were provided by Animal Experimental Center of Zheng zhou University, Certification number: Medicinal animal No. 20-012.

Drugs and reagents

Bushen Xingnao decoction was made up by 9g ginseng, 12g prepared rehmannia root, 12g glossy privet fruit, 12g prepared fleece flower root, 9g salvia miltiorrhizae, 9g ligusticum chuanxiong hort, 9g red peony root, 6g acorus tatarinowii, 6g polygala tenuifolia, 6g gastrodia elata and 2g borneol (The above constituents are nontoxic and can be applied to food items).

After purchased, it was authenticated by Professor Heling Jiao in the Traditional Chinese Medicine Teaching-research Office of National Hospital of Zhang Zhongjing of Nanyang Institute of Technology. The drug was cold soaked for 3h with a 5-time volume of water in total volume, with slightly boiled in 1h x 2 time and the filtration was combined. The concentrated solution containing $1.5g \cdot ml^{-1}$ crude drug was concentratedly made through slow fire, bottled in aseptic condition and placed in refrigerator at 4^{o} for standy application; Interleukin-1β (IL-1β) detection kit (batch number 20081221) tumor necrosis factor-α (TNF-α) detection kit (batch number: 20080605) was purchased from Fuzhou Maixin Biotech. Co., Ltd.; VEGF detection kit (batch number: 20080916) was peruchased from Beijing Biosynthesis Biotechnology Co., Ltd.; Rats shuttle box was produced by Henan Province Chinese medicine research institute.

Screening on animals

After adaptive feed for 7 d, rats were took shuttle-training screening by adopting active avoidance response system, which took light as conditioned stimulus and foot shock as unconditioned stimulus. If the rat could immediately complete the shuttle movement after the light stimulus, it

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was called active avoidance reaction (AAR), while that the rat could accomplish the shuttle movement with the assistance of electrical stimulus was called passive avoidance response (PAR). The learning-memory ability of rat, namely the AAR acquisition rate, was represented with the ratio between accomplished times of AAR and tested total times (20 times). Before the model making, all rats were trained with shuttle box, from which those with AAR acquisition rate more than or equal to 80% were enrolled.

Preparation of vascular dementia model

In accordance with literature (Tayebati 2006 and Sarti *et al.,* 2002), the dementia model was produced. With the utilization of 3.5% chloral hydrate, after anesthesia on rat ip with the weight in 10 $mbkg^{-1}$, the rats was fixed on their backs, with incision in the center neck, and the bilateral common carotid artery was separated. Then permanent ligation on bilateral common carotid artery was conducted, in which the rat body temperature was kept at 36.5□. As to the rats in control group, except for not ligating the bilateral common carotid artery, the rest treatment was the same with that for the rats in model group. After the operation, penicillin (16 $U{\cdot}g^{-1}$) and gentamicin ($8U{\cdot}g^{-1}$) was injected for preventing infection.

Grouping and dosing

The rats with successful molding, according to random principle, were divided into 8 groups: normal group, sham operation group, dementia modeling group (3 subgroups), Bushen Xingnao decoction treatment group (3 subgroups), 10 rats in every group. The 2nd, 4th and 6th week after successful modeling were selected as observation point. At the beginning of the second modeling day, ig was administrated on the equivalent volume continuously and the administration volume was equally $10mL.kg^{-1}$, while normal saline in same volume was administrated in normal group, modeling group and sham operation group.

Detection on the learning-memory ability

The shuttle-box active avoidance response system was adopted and rats in each group was performed tests before collecting specimems.

Detection on VEGF, IL-1β and TNF-α by enzyme linked immunosorbent assay

The rats were anesthetized by chloral hydrate, thoracic cavity was opened, blood was selected from right ventricular and serum was separated and preserved, waiting for inspection and standby application. The normal saline was rapidly instilled immediately through aorta ascendens after blood collected until the effluent of the right atrium was clear. Afterwards, fresh brain tissues were selected after decapitation, with which the 10% homogenate was made up and supernate was abstracted. The detection on VEGF, IL-1β and TNF-α was strictly performed in accordance with the steps of instruction.

STATISTICAL ANALYSIS

$x \pm s$ was represented for all data. SPSS 14.0 software was adoppted on all data for statistical treatment. Variance analysis was applied for the comparisons on different time points within the same group. T-test was used for the comparisons on the same time point between the two groups. $P<0.05$ was considered as statistically significant difference.

RESULTS

Influence of Bushen Xingnao decoction on the learning-memory ability for vascular dementia rats

From the second modeling week, AAR acquisition rate in modelling group was significantly smaller than that in control group and further reduce was shown at the 4th and 6th week (Compared with sham operation group, $P<0.01$ was shown in three groups); After treatment of Bushen Xingnao decoction, AAR acquisition rate of phase point at each time was distinctly larger than that of phase point at the same time of modelling group (Comparison in the 2nd week showed that $P<0.05$ and that in 4th and 6th week showed that $P<0.01$) (table 1).

Influence of Bushen Xingnao decoction on VEGF expression of vascular dementia rats

From the second modeling week, VEGF value in model group was significantly larger than that in both normal group and sham operation group, moreover, further augment was shown at the 4^{th} and 6^{th} week (Compared with model group and sham operation group, $P<0.01$ was shown in three groups); VEGF value of phase point at each time in Bushen Xingnao decoction treatment group was distinctly larger than that of phase point at the same time of model group (Comparison in the 2^{nd}, 4^{th} and 6th week showed that $P<0.05$) (table 2).

Influence of Bushen Xingnao decoction on IL-1β expression of vascular dementia rats

From the second modeling week, IL-1β value in model group was significantly larger than that in both normal group and sham operation group, moreover, further augment was shown at the 4^{th} and 6^{th} week (Compared with model group and sham operation group, $P<0.01$ was shown in three groups); IL-1β value of phase point at each time in Bushen Xingnao decoction treatment group was distinctly smaller than that of phase point at the same time of model group (Comparison in the 2^{nd} and 4th week showed that $P<0.05$ and that in the 6^{th} showed that $P<0.01$) (table 3).

Influence of Bushen Xingnao decoction on TNF-α expression of vascular dementia rats

From the second modeling week, TNF-α value in model group was significantly larger than that in both normal group and sham operation group, moreover, further

Table 1: Grade changes of shuttle box AAR acquisition rate for rats in every group ($x\pm s, n = 10$)

Group	Dosage /g·kg^{-1}·d^{-1}	AAR acquisition rate/%		
		2 weeks	4 weeks	6 weeks
Normal	-	90.38±8.18$^{2)}$	90.40±7.89$^{2)}$	90.43±8.06$^{2)}$
Sham operation	-	80.17±4.46$^{1)}$	83.61±6.23$^{1)}$	89.33±7.10$^{2)}$
Bushen Xingnao decoction	150	78. 38±3.31$^{1)}$	82.26±5.51$^{1)}$	88.21±7.23$^{2)}$
Model	-	61.24±5.47	58.77±7.61	41.63±8.42

Note: Compared with model group, $^{1)}$ P<0.05, $^{2)}$ P<0.01 (Same with tablse 2 -4)

Table 2: Detection value of VEGF of rats in every group ($x\pm s$, n=10)

Group	Dosage /g·kg^{-1}·d^{-1}	Serum/ng·L^{-1}			Brain tissue/μg·L^{-1}		
		2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
Normal	-	54.1±5.7	54.5±4.8	53.8±4.2	52.4±4.1	52.6±5.3	52.2±3.2
Sham	-	56.2±3.7	55.6±4.4	55.3±5.7	52.9±2.4	53.0±2.8	53.6±8.6
Bushen Xingnao decoction	15.0	80.5±5.8$^{1)}$	83.2±5.4$^{1)}$	91.5±5.4$^{1)}$	6.2±5.3$^{1)}$	104.±6.1$^{1)}$	107.3±4.4$^{1)}$
Model	-	74.4±5.2	76. 6±5.2	81.7±7.8	88.7±8.1	91.8±7.8	97.3±5.1

Table 3: Detection value of IL-1β of rats in every group ($x\pm s$, n=10)

Group	Dosage /g·kg^{-1}·d^{-1}	Serum/ng·L^{-1}			Brain tissue/μg·L^{-1}		
		2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
Normal	-	13.6±5.2$^{2)}$	13.3±4.3$^{2)}$	13.4±4.2$^{2)}$	15.4±3.4$^{2)}$	15.1±5.4$^{2)}$	15.3±4.2$^{2)}$
Sham	-	13.4±4.6$^{2)}$	13.6±4.7$^{2)}$	13.7±5.7$^{2)}$	17.9±5.4$^{2)}$	18.0±2.8$^{2)}$	18.1±5.6$^{2)}$
Bushen Xingnao decoction	15.0	18.5±5.6$^{1)}$	20.3±5.4$^{1)}$	21.3±7.4$^{1)}$	24.3±3.2$^{1)}$	26.8±3.1$^{1)}$	29.3±3.4$^{1)}$
Model	-	26.3±6.4	28.5±5.2	61.3±6. 8	35.2±6. 6	38.7±6.3	41.2±6.7

Table 4: Detection value of TNF-α of rats in every group ($x\pm s$, n=10)

Group	Dosage /g·kg^{-1}·d^{-1}	Serum/ng·L^{-1}			Brain tissue/μg·L^{-1}		
		2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
Normal	-	13.0±5.3$^{2)}$	13.1±3.8$^{2)}$	13.3±3.2$^{2)}$	15.4±4.1$^{2)}$	15.1±3.3$^{2)}$	15.3±6.3$^{2)}$
Sham	-	14.7±5.6$^{2)}$	14.5±5.7$^{2)}$	14.3±5.5$^{2)}$	16.9±5.4$^{2)}$	15.1±3.3$^{2)}$	18.6±6.6$^{2)}$
Bushen Xingnao decoction	15.0	22.6±5.5$^{1)}$	23.5±5.6$^{1)}$	24.3±5.4$^{1)}$	25.3±5.6$^{1)}$	26.5±5.2$^{1)}$	28.6±5.4$^{1)}$
Model	-	30.3±3.3	31.6±3.4	33.4 ±5.1	34.2±2.7	34.7 ±4.3	36.4±4.8

augment was shown at the 4th and 6th week (Compared with model group and sham operation group, P<0.01 was shown in three groups); TNF-α value of phase point at each time in Bushen Xingnao decoction treatment group was distinctly smaller than that of phase point at the same time of model group (Comparison in the 2nd, 4th and 6th week showed that P<0.05) (table 4).

DISCUSSION

Traditional Chinese medicine considers that the pathogenesis of vascular dementia was associated with the deficiency of vital energy, the deficiency of the kidney, phlegm and blood stasis. The pathogeny includes deficiency of kidney qi, intermin-gled phlegm and blood stasis which block the brain collaterals, thus inducing the imbalance of yin and yang and impair and apraxia of spiritual mechanism and becoming dementia (Jia 2003). The deficiency of the kidney and blood stasis and phlegm stagnation are the main pathological changes of vascular dementia (Zhou and Li 2001). Therefore, Bushen Huayu Qutan recipe is consistent of the dementia pathogenesis. Bushen Xingnao decoction, on the basis of repeated clinical observation, study and drug screening, is the developed Chinese herbal medicine compound preparation to treat vascular dementia, which is component with ginseng, prepared rehmannia root, red peony root, ligusticum chuanxiong hort, polygala tenuifolia, acorus tatarinowii and gastrodia elata. This recipe has the function of nourishing kidney and qi, eliminating stagnation and dissolving phlegm and activating brain and regaining consciousness. Nourishing

kidney and qi is conducive to treat the primary while eliminating stagnation and dissolving phlegm help to treatment the secondary symptoms. Relieing both primary and secondary symptoms has a significant efficacy for vascular dementia.

Inflammation response is the main pathomechanism for ischemic brain tissue injury. The cytokines which appear in the early inflammation response of cerebral ischemic injury are TNF-α and IL-1β. Suppressing on inflammation is the major strategy on treating cerebrovascular diseases. VEGF is a growth factor discoved in recent years, having the effect on promoting the division of endothelial cells. What's more, it is able to promote the growth of vacular and the establishment of collateral circulation. The animal experiment of focal cerebral ischemia has verified that the 3rd day after infarction is the most active vascular proliferative stage, on which significant VEGF expression can be visualized (Roberts and Palade 1995 and Rafael 2006). VEGF, as the vascular repair factor of cerebral ischemia injury, having a cerebral protection effect on cerebral ischemia diseases has been confirmed (Wang *et a1.,* 2007).

This study, through the behavior observation of vascular dementia rats, found that Bushen Xingnao decoction had the ability to approve the learning-memory ability of vascular dementia rats. From the second modeling week, AAR acquisition rate in model group was significantly smaller than that in control group and further reduce was shown at the 4th and 6th week (Compared with sham operation group, $P<0.01$ was shown in three groups); After treatment of Bushen Xingnao decoction, AAR acquisition rate of phase point at each time was distinctly larger than that of phase point at the same time of model group (Comparison in the 2nd week showed that $P<0.05$ and that in 4th and 6th week showed that $P<0.01$). The synptoms in treatment group was significantly improved compared those in model group, which directly indicated that Bushen Xingnao decoction had the cerebral protection effect. This experiment, with the detection on VEGF, IL-1β and TNF-α, presented that the VEGF, IL-1β and TNF-α content in the tissues and serum of chronic cerebral ischemia rats was increased. Compared with model group at the same time point, VEGF activity in treatment group increased at varying degrees while the content of IL-1β and TNF-α decreased at varying degrees, which indicated that Bushen Xingnao decoction was able to effectively promote the bioactivity of VEGF, increase the IL-1β and TNF-α contents in tissues and serum of chronic cerebral ischemia rats. Therefore, it suggested that Bushen Xingnao decoction was able to suppress the inflammation response of vascular dementia rats, augment the vascular repair function and play the cerebral protective role.

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