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Full Length Research Paper

Effect of Xingnaojing on somatostain and arginine vasopressin in rats with vascular dementia

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To explore the effect of Xingnaojing on the somatostain and arginine vasopressin in a rat vascular dementia model, a total of 24 male Sprague-Dawley rats were randomly divided into three groups: sham group (S), vascular dementia group (VD) and Xingnaojing group (XNJ). The memory was measured by Morris water maze test before operation and before and after Xingnaojing treatment. Treatment was performed for 15 days, and then rats were sacrificed. The contents of somatostain (SS) and arginine vasopressin (AVP) in different brain regions (frontal cortex, temporal lobe, hippocampus, cerebral ganglion and corpora striatum) were determined by radioimmunoassay. When compared with S and XNJ groups, the escape latency was significantly prolonged and the platform crossing dramatically decreased in the VD group ($P < 0.01$); and the SS content was predominantly decreased in frontal area cortex, temporal lobe, hippocampus, cerebral ganglion and corpora striatum ($P < 0.01$). The AVP content was reduced, but significant difference was only found in the temporal lobe and corpora striatum ($P < 0.05$). The abnormal learning and memory in VD animals may be attributed to the decrease of SS and AVP after multiple cerebral infractions. Xingnaojing can predominantly raise the contents of SS and AVP in the brain of VD rats, and consequently improve the spontaneous activity and the ability of learning and memory. Xingnaojing injection should be sealed from light, in ampoule bottles, 10 ml each, and this product is an aromatic drug, used immediately after opening, to prevent evaporation.

Key words: Vascular dementia, rat, memory, somatostain, arginine vasopression.

INTRODUCTION

Vascular dementia (VD) is a syndrome characterized by cognitive impairment and caused by some cerebral vascular diseases. VD is characterized by the disturbance of central nervous system, particularly cognitive impairment, including abnormal learning, memory and thinking and neuropsychological symptoms or signs (aphasia, apraxia, stolen identification, etc). The pathogenesis of VD has not yet been fully elucidated to date. In the brain, a lot of neuropeptides, including somatostatin (SS) and arginine vasopressin (AVP) are found to be closely related to the learning and memory (Lu et al., 2006). They not only possess the hormone like properties in the central nervous system, but are involve in the knowledge records, and the formation and maintenance of conditioned reflex (Golubeva, 2006; Garcia-Horsman et al., 2007). Xingnaojing injection, a new type of Chinese medicine, is scientifically crafted from musk, borneol, gardenia, turmeric and other traditional Chinese medicine. It has the properties of clearing away heat and toxic materials and activating collateral circulation.

So far, no studies have reported the effect of Xingnaojing on the SS and AVP in the brain of patients with VD, and the relationship between therapeutic efficacy of Xingnaojing on VD and the SS and AVP is largely unclear. The present study aimed to investigate the effects of Xingnaojing on the SS and AVP in the brain of VD rats and to further explore the pathogenesis of VD. Our results may provide a reliable evidence for the clinical use of Chinese medicine for the treatment of VD.

MATERIALS AND METHODS

Animals, reagents and instrument

A total of 24 male Sprague-Dawley rats weighing 300 to 350 g and aged about 10 to 12 months were purchased from the Animal Center

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Table 1. Escape latency and platform crossing in different groups ($\bar{x} \pm s$).

Group (n)	Escape latency	Platform crossing
S (8)	14.24 ± 1.81	7.88 ± 1.07
XNJ (8)	15.28 ± 1.79	7.23 ± 0.98
VD (8)	97.97 ± 7.39#	1.62 ± 0.34#
*F*1	13.25	15.99
P	0.00	0.00

#P < 0.01 versus XNJ group.

of School of Medicine of Zhejiang University. The SS and AVP immunohistochemistry kits were from Wuhan Boster Health Engineering Co., Ltd, and the mice water maze from Anhui Huaibei Zhenghua Biological Equipment Co., Ltd. Animal care was in accordance with the guidelines of institutional committees and this study was approved by the local ethics authorities.

Grouping

Rats were randomly divided into sham group (S, n = 8), vascular dementia group (VD, n = 8) and Xingnaojing treatment group (XNJ, n = 8). Rats in the XNJ group were intraperitoneally treated with Xingnaojing injection at 2 ml/kg and animals in the S group and VD group received intraperitoneal injection of normal saline of equal volume. Treatment continued for 15 days.

Animal modeling and drug intervention

Rats were fasted 12 h given *ad libitum* access to water before surgery. After being anesthetized with 10% chloral hydrate aldehyde (0.3 ml/100 g body weight), they were fixed in an operation table in a prone position. A midline incision was made at the back of the neck. Then, the transverse processes of the first cervical vertebra were exposed, and two vertebral arteries were permanently occluded by electric coagulation. Then, convulsions, slow deep breathing and increase of heartbeat were observed in these rats. These animals were fixed in supine position and a midline incision was made at the neck. The bilateral carotid arteries were separated, and then occluded using clamps for 5 min thrice, and the interval was 1 h. When the clamps were removed, followed by reperfusion, the rats breathed freely and had stable heartbeat. The wound was closed and intramuscular injection of penicillin (400,000 U) was carried out to prevent infection. In the S group, the vertebral arteries and carotid arteries were not occluded. Rats in the XNJ group received the aforementioned treatment and then were intraperitoneally treated with Xingnaojing. During the surgery, the rectal temperature was maintained at 37 to 38°C.

Animals non-responding to external stimuli, having increased breathing rate and being absent of righting reflex were diagnosed with VD and used in the following experiments.

Water maze test

The Morris water maze is a round pool with 90 to 180 cm in diameter, 50 to 60 cm in height and 30 cm in depth. A round platform with 8 to 10 cm in diameter and 29 cm in height was put in the pool and the water temperature was maintained at 24 ± 1°C. The camera was placed at 2 m away from the central top of the pool and could capture the whole experimental area. The system was sampled only within the region. The hardware system, includes computer and the video capture card connected with the black and white charge-coupled devices (CCD) camera. The video signal was converted by the video capture card through a specific software system analysis. Before the experiment, the rats were placed in the pool (without Platform) for 2 min to familiarize with the environment. During the experiment, the pool wall was divided into four quadrants: A, B, C and D. Each rat was put into the water gently at four quadrants. The escape latency was recorded as the time from the rat being put in the water to it climing on the platform. If the rats find the hidden platform, they were allowed to stay there for 15 s, and then put in the cage for rest. If the rats can not find the platform in 2 min, they were guided to the platform, allowed to stay about 15 s and then put in the cage. In each test, rat in the water swam from one point to another point is to be marked as 1 circle, each round for four circles, and 15 min rest between each circle. Experiment was performed twice daily and continued for 3 consecutive days (a total of 6 times). Then, the escape latency and platform-crossing were compared.

Sample preparation and determination of neuropeptides

All animals were sacrificed at 15 days after treatment. The whole brain was obtained and kept in the boiling saline for 5 min. Then, after removal of water, the frontal lobe, temporal lobe, hippocampus, thalamus and striatum were separated, collected and weighed. Then, these samples were homogenized in 1 mol/L NaCl at room temperature. Then, 1 ml of 1 mol/L NaOH was added followed by centrifugation at 4°C, 3000 rpm/min for 20 min. The supernatant was kept at -70°C for use. Radioimmunoassay was used to detect the contents of SS and AVP in different brain regions.

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS) version 11.0. Data were presented as mean ± standard deviation ($\bar{x} \pm s$). Comparisons of means among groups were done with one-way analysis of variance (ANOVA) and those between two groups were carried out with Student-Newman-Keul (SNK) test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Learning ability

The escape latency in the VD group was significantly prolonged accompanied by a significant reduction in the platform crossing (P<0.01), when compared with the XNJ and S groups (Table 1 and Figure 1).

SS content in different brain regions

When compared with the S group, the SS content in the bilateral frontal lobe, temporal lobe, hippocampus, thalamus and striatum was significantly lowered in the VD group (all P < 0.01, Table 2 and Figure 2); when compared with the VD group, SS content in the bilateral frontal, temporal lobe, hippocampus, thalamus and striatum was markedly increased in the XNJ group (all P

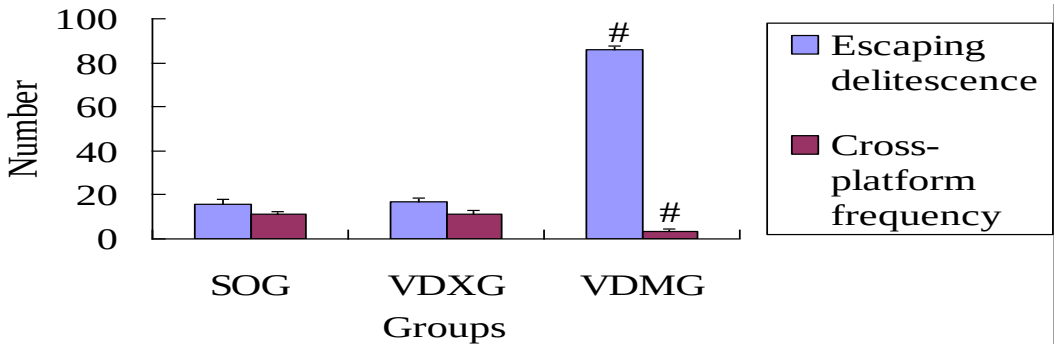

Figure 1. Escape latency and platform crossing in different groups.

Table 2. SS contents in different brain regions of three groups (ng/L).

Group	Frontal lobe	Temporal lobe	Hippocampus	Thalamus	Striatum
S (n = 8)	134.21 ± 6.67	305.86 ± 23.98	178.39 ± 11.02	194.23 ± 8.11	172.01 ± 9.72
VD (n = 8)	82.1 ± 12.12*	126.63 ± 17.27*	75.81 ± 22.54*	123.26 ± 31.25*	91.6 ± 14.36*
XNJ (n = 8)	121.1 ± 7.26$^{\#\#}$	237.38 ± 22.72$^{\#\#}$	93.01 ± 12.61$^{\#}$	146.63 ± 12.38$^{\#}$	157.62 ± 17.73$^{\#\#}$

*P<0.01 versus S group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 versus VD group.

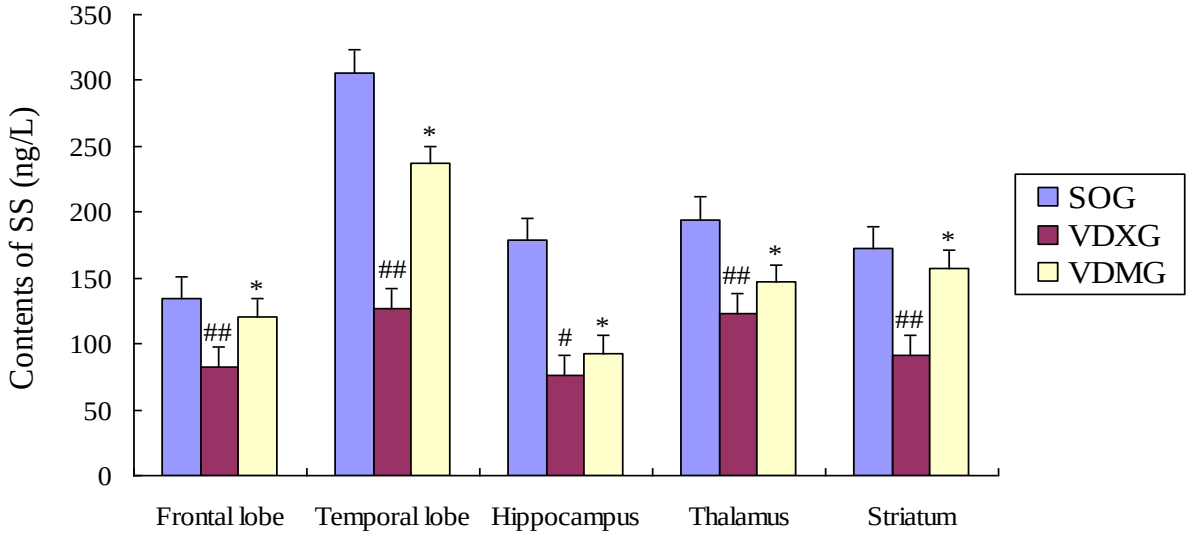

Figure 2. SS contents in different brain regions.

< 0.01), particularly in the hippocampus and thalamus (P <0.05, Table 2).

AVP in different brain regions

The AVP content in the different brain regions in the VD group was reduced as compared to the remaining two groups. When compared with the S group, significant difference in the AVP content was only found in the temporal lobe and striatum in the VD group (P < 0.05). When compared with VD group, the AVP content in the frontal lobe, temporal lobe, hippocampus, thalamus and striatum of XNJ group was increased, but significant difference was only noted in the temporal lobe and striatum (P < 0.05) (Table 3 and Figure 3).

DISCUSSION

VD is a chronic, progressive, persistent cognitive impairment syndrome resulting from cerebral vascular diseases,

Table 3. AVP contents in various brain regions of three groups ($\bar{x} \pm s$, ng/L).

Group	Frontal lobe	Temporal lobe	Hippocampus	Thalamus	Striatum
S (n = 8)	5.85 ± 0.71	11.37 ± 1.13	7.38 ± 0.62	9.03 ± 1.11	8.39 ± 0.85
VD (n = 8)	4.22 ± 0.50	7.20 ± 0.98*	5.85 ± 0.64	6.41 ± 0.61	5.37 ± 0.62**
XNJ (n = 8)	5.11 ± 0.67	10.39 ± 0.88 #	6.72 ± 0.59	8.41 ± 0.82	7.73 ± 0.84 #

*P < 0.05, **P < 0.01 versus S group; #P < 0.01 versus VD group.

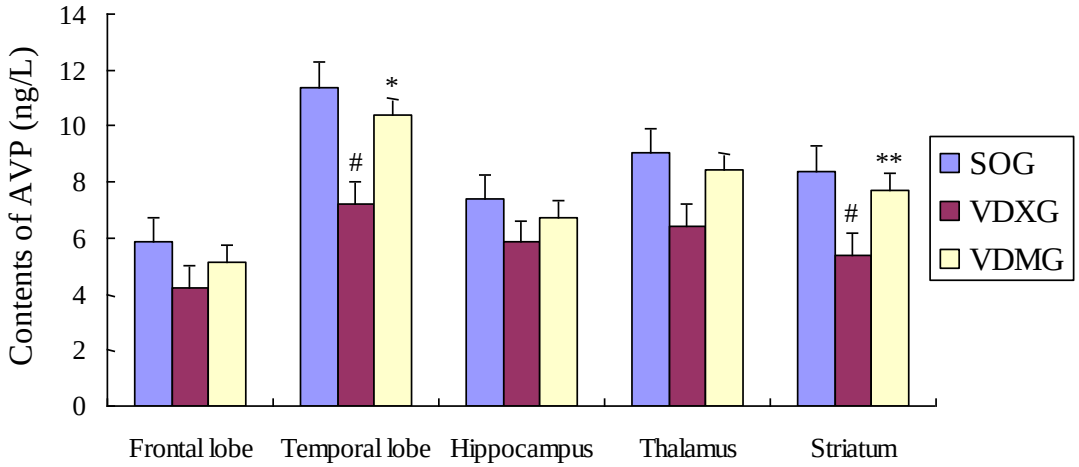

Figure 3. AVP contents in various brain regions of three groups.

including ischemia or hemorrhage. It has been the most common type of dementia. In Western countries, VD becomes the second leading cause of dementia after Alzheimer's disease, and in some countries of Asia, VD is the main type of senile dementia (Knopman et al., 2003). The annual prevalence of VD is 1 to 3/1000 (Knopman et al., 2002) which is increasing with the increase of cerebral vascular diseases. Currently, the pathogenesis of VD is still unclear. Thus, to establish an ideal animal, VD model is critical for the investigation of the pathogenesis of VD and the development of new drugs for the prevention and treatment of VD.

In the present study, four-vessel occlusion was introduced to induce global cerebral ischemia (4-VO) (Hou and Qin, 2005). The production process is that at first the bilateral vertebral artery were occluded, and then the bilateral common carotid artery (CCA) were repeatedly clipped. This animal model represents a repeated cerebral ischemia and reperfusion of the pathogenesis of which was close to that in VD. In the VD, the frontal lobe, temporal lobe, hippocampus, thalamus and caudate nucleus are severely damaged, and the physiological stability after ischemia was that there was no obvious limb movement disorder. In the present study, mortality was 27.27%, no obvious limb movement disorder was found, and all indicators were in normal ranges, which suggest the successful establishment of animal VD model.

Learning and memory are the important functions of the brain. Morris water maze test is a way in which animals learn to find hidden platform in the water. By analyzing the time spent on finding the platform and the path to the platform, we can determine the quality of memory. It has been a standard experiment to study the spatial learning and memory. Results from Morris water maze test can reflect the space cognitive function and this test is easy to perform and more sensitive to learning and memory function test (Ye and Zhang, 2004). Morris water maze is composed of a circular pool and automatic video recording and analysis system. In this test, the animals require 1 to 2 days of training and then the learning and memory are measured (Morris, 1984; Zhang, 2002). In the present study, when compared with S group, the escape latency in the VD group was prolonged, and the platform-crossing was significantly reduced, showing obvious learning and memory impairment. After treatment with Xingnaojing, the escape latency was shortened and the platform-crossing markedly increased when compared with VD group, but there were no significant differences between the S group and XNJ group.

In our study, the contents of SS and AVP in different brain regions of VD rats were also measured. In the VD group, the SS content in various brain region was significantly lower than that in rats of S group and the AVP content in different brain regions was also reduced to different extents, although significant difference was

only found in the temporal lobe and striatum ($P < 0.05$). However, in the XNJ group, the contents of SS and AVP in different brain regions increased significantly as compared to VD group ($P < 0.05$ or $P < 0.01$).

SS in the central nervous system can affect the learning, particularly in the process of consolidating the memory and promotion recall; therefore, increasing studies concerning the role of SS in the occurrence of VD. Recent studies revealed that, in the multiple infarct dementia, such as Alzheimer's disease and Parkinson disease, the SS content in the cerebrospinal fluid and plasma was significantly lowered, and the more severe the dementia, the lower the SS content in the plasma. The decrease of SS is related to the impairment of cognition and has become one of the factors resulting in the progression of dementia in patients with cerebral infarction (Lu et al., 2009). In the present study, in the VD rats, the proportion of SS positive cells in the hippocampus and temporal cortex was significantly reduced, which confirmed that the sustained cerebral ischemia may lead to decrease of SS in the hippocampus and cortex. The decrease of SS in VD rats was closely related to the impairment of learning and memory (Song et al., 2009). It has been found that sustained cerebral ischemia may lead to decrease of SS in the hippocampus and cortex (Sun and He, 2008). However, others researchers propose that, SS is not isolated in the learning and memory, but can interact with a number of neurotransmitters (Yamazaki et al., 1996; Ishida et al., 1997) of which Ach is an important one. Zhang et al. (2004) have shown that, in VD, the levels of SS and Ach were remarkably decreased, and positive correlation between decrease of SS and Ach levels and VD was also noted, suggesting that both neurotransmitters participate in the pathogenesis of VD. Of course, SS can interact with more than Ach, and the interaction among different neurotransmitters is extremely complex and further investigations are required.

AVP can inhibit the presynaptic uptake of γ-aminobutyric acid (GABA), increases the GABA and promote the glutamate uptake. It is conducive to long-term potentiation dependent glutamate, and can significantly enhance the memory, reduce the forgetting and indirectly extend the effect of postsynaptic GABA on GABA receptors, participating in the regulation of fluid balance (Ohbuchi et al., 2008). When compared with healthy controls and cerebral infarction patients without dementia, the serum AVP level was lowered in VD patients, and the severity of dementia was associated with the AVP level: the more severe the dementia, the lower the AVP level. Moreover, the serum AVP level was positively correlated with the mini-mental state examination (MMSE) score: the low serum AVP level may directly reflect the impairment of cognitive function (Lu et al., 2009). In the cerebrospinal fluid of patients with VD, AVP and SS contents were significantly lower than VD patients after treatment, and both were positively correlated with the mental state examination (MMSE) score, Hasegawa Dementia Scale Smart Check (HDS) score and daily living scale (ADL) score. The low AVP and SS contents in the cerebrospinal fluid may interfere with the metabolism of a series of learning and memory-related neurotransmitters, leading to the occurrence and development of VD (Wang et al., 2010). Studies have showed that in the VD rats, the reduction of AVP in different brain regions is inconsistent, which may be attributed to the different AVP levels in various brain regions in normal state: high AVP level in the striatum and temporal lobe and low AVP level in the hippocampus and frontal lobe. When cerebral ischemia is present, the cholinergic system may be damaged and the response of neuroendocrine axis is also compromised, causing the decrease of AVP. The temporal lobe and striatum rich in AVP are thereby the firstly affected regions. The AVP in the brain of VD rats was proved to be abnormally distributed and interfere with the intellectual activity, which is an important cause of VD.

Xingnaojing injection is derived from cow-bezoar bolus for cerebral resuscitation. It has the properties of clearing away heat and toxic materials and activating collateral circulation. Studies have shown that Xingnaojing can improve the brain edema, reduce the infarct area, decrease the neuronal apoptosis, alleviate the pathological damage, and protect the nerves through inhibiting the ischemic-reperfusion induced apoptosis of neurons (Wu et al., 2007). In the present study, Xingnaojing was found to increase SS content in all brain regions and AVP in the frontal lobe and striatum, shorten the escape latency and increase platform-crossing significantly leading to improvement of learning and memory in VD rats.

The SS content in various brain regions was significantly lower than that in rats of control group, and the AVP content in different brain regions was also reduced to different extents, although significant difference was only found in the temporal lobe and striatum. After the use of XNJ, the contents of SS and AVP in different brain regions were significantly increased.

The learning and memory impairment in VD rats is relevant with the depletion of endogenous SS and AVP in the brain. In different brain regions, AVP positive neurons are differentially sensitive to ischemia. SS and AVP, with the uneven distribution in different areas of the brain, participate in the formation of a conditioned reflex and information storage, and play important roles in the learning and memory. Xingnaojing can improve the function of neurons, and increase the contents of SS and AVP, which then promotes the functional recovery of damaged neurons. Thus, we speculate that the neuroprotective effect of Xingnaojing on VD is related to the increase of AVP and SS content in different brain regions following the treatment. However, more studies with excellent design are required to confirm our findings.

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