

Original Article

Neuroprotective and anti-dementia role of Tongqiao Huoxue decoction in rats with vascular dementia

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Abstract: Objective: Tongqiaohuoxue decoction (TQHDX) as a traditional Chinese medicine has the role of promoting blood circulation, and vascular dementia (VD) is a cognitive dysfunction syndrome caused by cerebral embolism. This study investigated the protective effects of TQHDX on memory impairment and brain damage in rat. Methods: Morphological changes in ischemic brains were performed for hematoxylin-eosin (HE) staining. The number of apoptotic neurons was detected by TUNEL staining. Administration with TQHDX for 4 weeks significantly improved the behavioral performance of rats with vascular dementia, as showed in the Morris water maze test. Superoxide dismutase (SOD), Glutathione peroxidase (GSH-PX), malondialdehyde (MDA) were determined by spectrophotometry using commercial kits. Results: TQHDX enhanced activities of SOD and GSH-PX but it decreased their MDA content. Conclusion: These results demonstrated TQHDX possesses neuroprotective and antidementia properties and suggested that TQHDX might be developed as an effective drug for the prevention of VD.

Keywords: Vascular, Tongqiaohuoxue decoction, neuroprotective effects, oxidative stress, morris water maze

Introduction

Vascular dementia (VD) is the second most common form of dementia in the elderly, after Alzheimer's disease, with frequency of 15% of all dementias. Vascular dementia accounts for approximately 30% of dementia cases in Asian countries and 10% of dementia cases in western countries [1]. Accumulating evidence showed vascular dementia disease in aged populations is a problem of enormous importance. VD is characterized by infarctions and subclinical vascular brain injury and severe cognitive impairment. Currently, VD refers to any type of dementia resulting from cerebral blood vessel disease, as well as hypoperfusive ischemic cerebral injury [2, 3]. As we know, cerebral blood vessel injury is a multifactor, multi-mechanism, malignant cascade reaction [4]. As the overall process of cerebral blood vessel injury is extremely complex, the protective effects of Chinese medicinal herbs are receiving more attention in the effort to find agents for the treatment of vascular dementia.

Tongqiaohuoxue decoction (TQHDX) is a classical prescription in traditional Chinese medicine, established by Wang Qingren-the distinguished doctor of the Qing Dynasty. TQHDX is made of moschus, Carthamus tinctorius, Rhizoma chuanxiong, Semen pruni persicae, and Radix Paeoniae Rubra. TQHDX promotes blood circulation by removing blood stasis, and induces resuscitation by dredging the channels. TQHDX has marked efficacy in the treatment of stagnation of blood in the head and face channel, has preventive and therapeutic effects on stroke, and can ameliorate symptoms of the apoplectic [5]. We have previously shown that TQHDX has beneficial effects in cerebral ischemia [6].

This study was aimed to investigate in more detail the mechanisms of this neuroprotective effect. We want to determine whether TQHDX could protect neuronal cells from cerebral blood vessel injury, improving the ability of learning and memory from vascular dementia, as well as its potential mechanism.

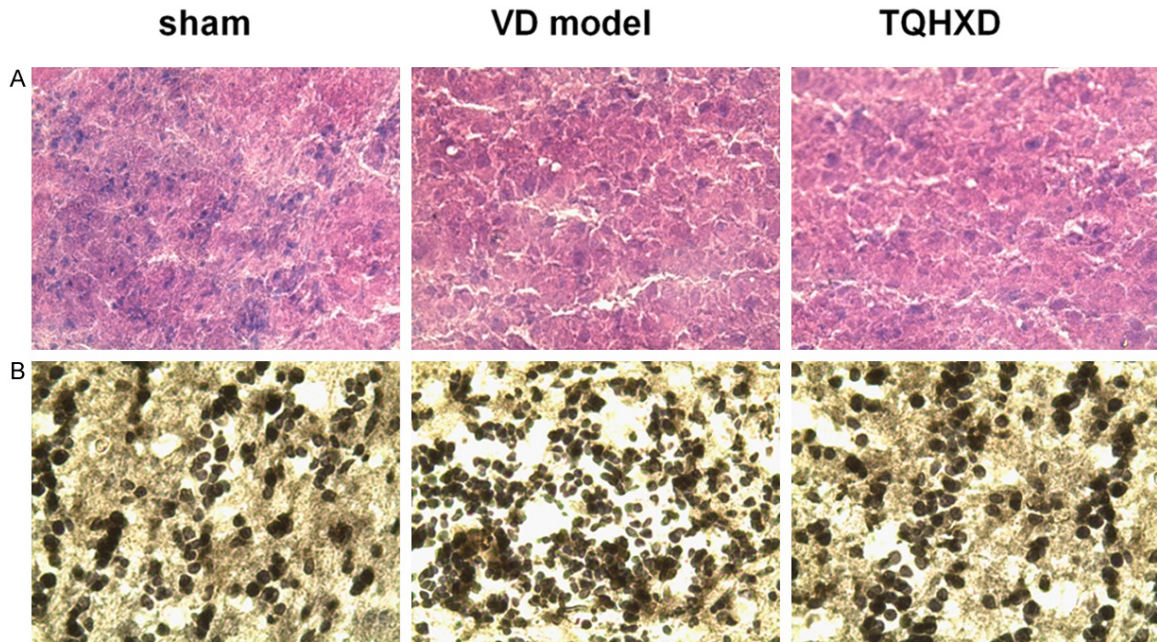


Figure 1. Effect of TQHXD treatment on the pathohistological changes of rats after cerebral injury. Haematoxylin-eosin staining and TUNEL of apoptosis in different groups of rats. A shows haematoxylin and eosin staining, original magnification 200 \times . B shows TUNEL staining, original magnification 400 \times .

Methods

Plant materials and plant extracts

TQHXD consists of *Radix paeoniae rubra* (3 g), *Rhizoma ligustici wallichii* (3 g), *Semen pruni persicae* (9 g), *Fructus Jujubae* (7 pills), *Carthamus tinctorius* (9 g), *Allium fistulosum* (3 root), *Tender ginger* (9 g), *moschus* (0.15 g), *Yellow rice wine* (250 ml). Each botanic medicine was bought from pharmaceutical company and was identified as eligible and pure medicinal material. These herbs were immersed in water for 20 min whose volume is 5 times than that of medicine compounds. It was decocted at boiling temperature for half an hour, then the decocted liquid was taken out. The compounds was continued to decoct twice in the water whose volume is 3 times than that of the compounds. Finally, the decoction was totally collected. It was placed in the sterile bottle and stored in the refrigerator.

Animals

All experimental procedures were conducted in accordance with the recommendations of the International Association for the Study of Pain and the National Institute of Health Guide for the Care and Use of Laboratory Animals. All experimental protocol was duly reviewed and

approved by the institutional animal ethics committee. Adult male Sprague-Dawley rats weighing 200 to 250 g [procured from the Experimental Animal Center of Guangxi Medical University] were used for this study. Animals were housed in groups of 5 rats per cage, with food and water available, and maintained in a climate controlled environment on a 12 h light/dark cycle at a temperature of $25 \pm 3^{\circ}\text{C}$ and at the relative humidity of $55 \pm 5\%$. The experiments were carried out in accordance with the current guidelines for the care of laboratory animals in Guangxi Medical University.

The rats were randomly divided into different groups: sham-operated group, VD model group, TQHXD group. The administered doses of TQHXD were 1 ml/100 g, once a day for 4 weeks. Rats in sham-operated and VD model group were administered with normal saline in the same volume. One hour after the last administration, the operation was performed.

Preparation of VD model

The animals were fasted overnight but allowed free access to water. After being anesthetized with 10% chloral hydrate, the skin of the rat was incised along the midline of the cervical region to expose the bilateral common carotid

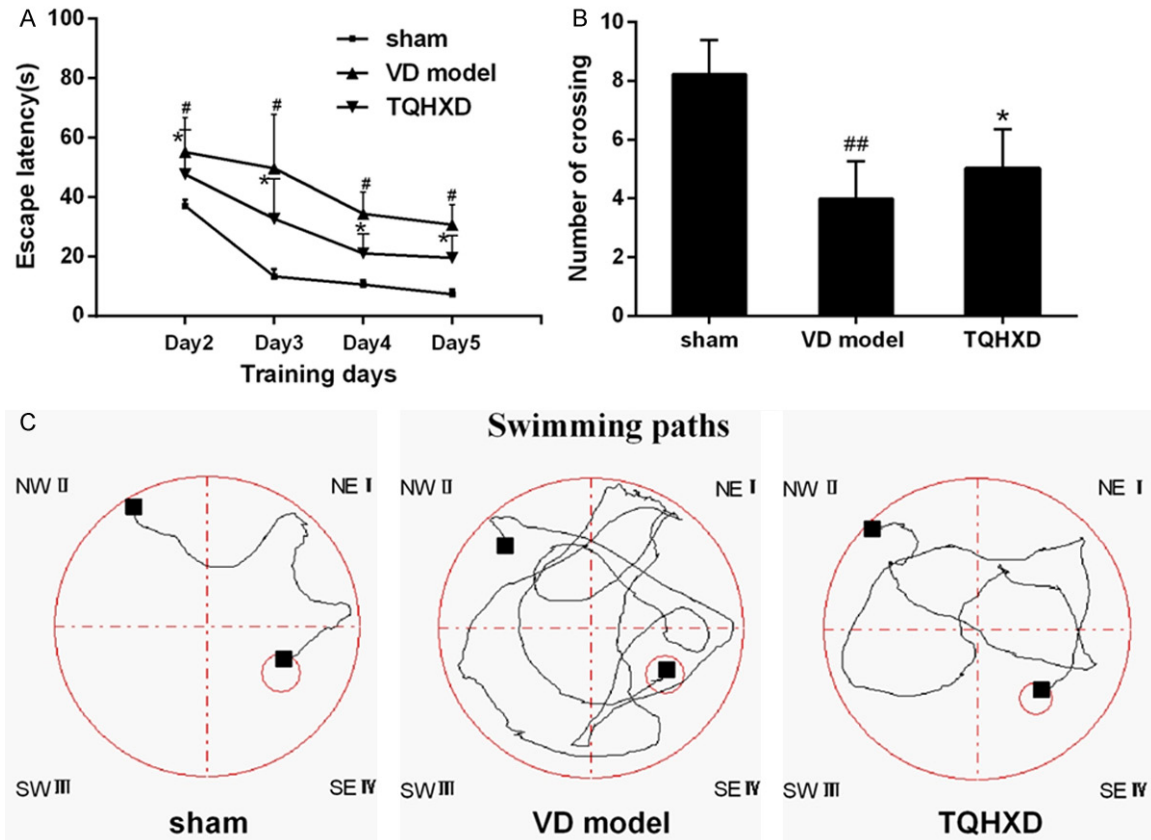


Figure 2. Performances of rats in Morris water maze tests. A. The escapes latency of rats in training trials. B. Number of crossings over the exact former location of the platform in the probe trial. C. Representative pathways in the last day of training trials. The smart cycle is the platform region. Data are presented as means \pm SD ($n = 10$). $^{***}P < 0.01$, $^{*}P < 0.05$, compared with the sham operation group. $^{*}P < 0.05$, compared with the model group.

arteries without nerve damage; they were carefully separated from the surrounding tissues, approximately 1 cm inferior to the origin of the external carotid artery [7]. Both common carotid arteries were tied twice with silk sutures. The skin incision was sutured and the animals were allowed to recover from the anesthesia. As a control, sham-operated rats underwent identical surgery without occlusion of arteries. Animals that exhibited an abnormal post-operative condition were excluded from the study.

Morris water maze test

Rats were tested for spatial learning and memory in the Morris water maze (MWM) [8]. The MWM consisted of a circular tank measuring 1.5 m in diameter with water temperature maintained at approximately 21°C and made opaque by non-toxic white paint powder (Reeves & Poole, Toronto) on the surface. Four points around the edge of the pool were arbitrarily

designated as north (N), south (S), east (E) and west (W), allowing the apparatus to be divided into 4 corresponding quadrants (NE, SE, NW and SW). A clear plexiglass escape platform was submerged approximately 2 cm below the water surface and placed in the NE quadrant of the maze. Extra-maze cues consisted of laboratory furniture and lights (held constant throughout the experiment). A video camera was mounted above the center of the pool and all performance was recorded for subsequent analyses. On the first day, rats were allowed a 120 s habituation session in the pool with out the platform. The following 4 days, rats were given 4 trials/day for each of 4 test days (120 s trial, 120 s inter-trial interval during which time the rat remained on the escape platform). If the rat did not find the escape platform within the allotted time, it was guided to the finish by the experimenter. Escape latencies and intertribal behaviour were recorded by observers blind to experimental treatment. A 60 s probe trial was

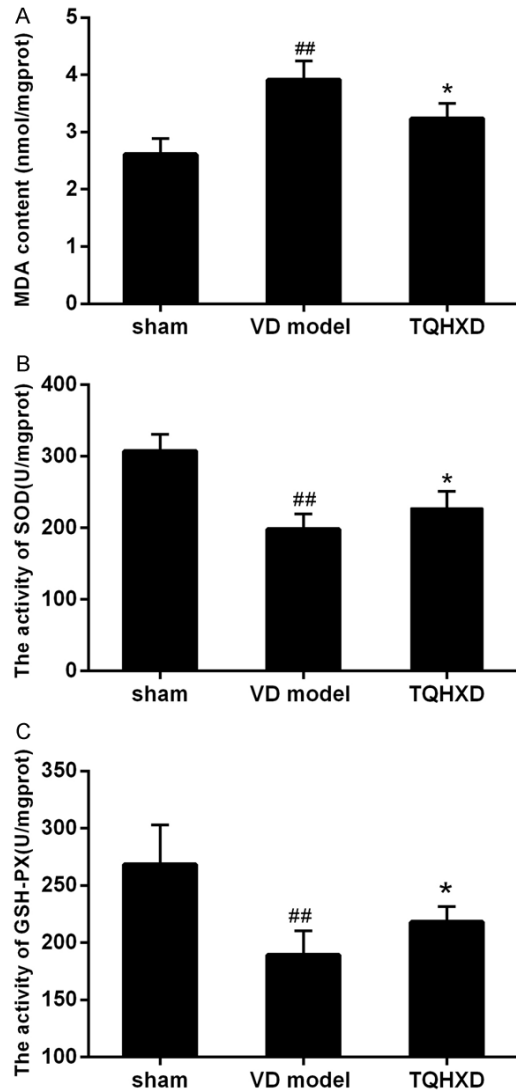


Figure 3. Effect of TQHXD on oxidative stress in VD rats. Oxidative stress was evaluated by examining MDA content (A), SOD activity (B), and GSH content (C). Values are expressed as mean \pm SD ($n = 8$). ^{##} $P < 0.01$, compared with the sham operation group. ^{*} $P < 0.05$, compared with the model group.

administered 24 h following the last test day, time and distance spent in the target quadrant was recorded.

Histopathology (H&E) and transferase dUTP nick-end labeling (TUNEL) staining

Rats were perfused with 100 ml of saline and 200 ml of 4% paraformaldehyde in phosphate buffered saline (pH 7.4). Subsequently, the brains were collected and placed in the fixative overnight. Coronal sections of the brain were cut from 4 mm in coronal sections, and stained

with H&E. Apoptotic cells in the hippocampus were detected by TUNEL staining using an In Situ Cell Death Detection Kit (Roche, Germany), according to the manufacturer's instructions. Sections were rinsed and visualized with DAB, and then mounted with cover slips. The number of TUNEL-positive cells was counted under the microscope and compared in each group.

Measurement of cellular SOD, GSH-PX, MDA levels

Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) assay kits were purchased from Jiancheng Institute of Biological Engineering (Nanjing, China). MDA, SOD and GSH assays were conducted by colorimetric kits according to the manufacturers' instructions. The levels of MDA and GSH and activity of SOD were expressed as per gram tissue.

Statistical analysis

Data were expressed as mean \pm S.D., student's t-test and analysis of variance (ANOVA) were used to determine statistical significance. A value of P less than 0.05 was considered significant.

Result

Injury assessment by haematoxylin and eosin (H&E) and TUNEL staining

The pathohistological alterations were assessed by H&E staining in different group rats. As **Figure 1A** showing, the number of hippocampal neurons, reduced obviously in the model group than sham-operated group, and the vascular structure was relatively unnormal, most cells looked nucleus pycnosis, vacuolar and edema. There were less pathological changes in TQHXD treating group of rats. In the TUNEL staining (**Figure 1B**), the TUNEL-positive cells were darkly stained and showed the morphologic signs of apoptosis. The number of cells exhibiting TUNEL-positive cells was maximal in the model group, but was less in sham-operated group and TQHXD treating group.

TSD ameliorates learning and memory impairments induced by MCAO

The effect of TQHXD on spatial memory in animal model of VD was shown in **Figure 2**. **Figure 2A** showed that the escape latency of all groups presenting a gradually decreasing in a day-

dependent manner during the former 4 days' trials. The sham-operated group rapidly learned the location of the platform and quickly reached the escape platform. In contrast, the VD model group exhibited a swimming behavior in which animals "wasted" time exploring the margin of the pool during the testing period. On the fourth day, TQHX treatment groups and significantly shortened the escape latency by comparison with model group. These results showed TQHXD can ameliorate the impairment of learning and memory of VD rats. **Figure 2B** shows that in the probe trial, rats in the VD model group had completed fewer platform crossings than rats in the sham-operated group. Significant improvement of these indexes was observed in the TQHXD group. The swimming paths of the last day were record as shown in **Figure 2C**.

Measurement of MDA and GSH levels and SOD

We tested the oxidative stress effects of TQHXD on SOD, GSH-PX and MDA activity in rats. **Figure 3** shows that MDA level was significantly increased in the model group, while GSH level and SOD activity were decreased. Treatment with TQHXD significantly reversed the oxidative stress in rats, compared with the model group.

Discussion

Vascular dementia disease has become one of the most devastating diseases, causing high morbidity, high disability rate and high mortality in aged persons [9-11]. The activity of anti-oxidant enzymes, such as superoxide dismutase (SOD) and heme oxygenase/biliverdin reductase, is decreased in patients with VD [12, 13]. A major contributing factor in the pathogenesis of VD is oxidative stress. Increased oxidative stress in the brain parenchyma, has proven to be passed by lipid peroxidation, protein oxidation, and DNA oxidative damage, is considered to be a main characteristic feature of VD [14].

Oxidative stress occurs as the results of a shift in balance that favors the generation of oxygen-derived free radicals or ROS over anti-oxidant defense mechanisms [15]. But the balance will be destroyed when a blood vessel in the brain is injury. Anti-oxidant enzymes such as SOD and GSH-PX activity decline, the O₂ cannot clean clearly in time, it will form toxic hydroxyl free radicals. Highly toxic hydroxyl free radicals would trigger the peroxide of arachidonic acid, and generate large amounts of lipid peroxides,

such as MDA, NO, etc. Excessive free radical and lipid peroxide can make the nerve cell membrane structure damage, exacerbating the brain injury.

We measured both oxidant and antioxidant parameters. TQHXD enhanced activities of SOD and GSH-PX in vascular dementia rats but it decreased their MDA content. These results indicated that the protective mechanisms of TQHXD on vascular dementia disease may be related with anti-oxidative stress.

Moreover, behavioral performance was tested by the Morris water maze. The MWM is a valuable approach to assess the ability of spatial learning and memory in animal models. It was found that the rats performed a significant prolongation of escape latency in the VD group compared to that in the Sham group in the place navigation phase, suggesting that the learning ability was significantly damaged in rats with VD. However, TQHXD significantly improved the ability of spatial learning of VD rats. Similarly, in experiment, our findings showed TQHXD could decrease the number of TUNEL-positive cells. It implies that TQHXD could take neuroprotective effect through preventing neural cells from necrosis and apoptosis caused by the cerebrovascular injury [16].

In conclusion, this study has indicated that treatment with TQHXD could markedly attenuate the cognitive impairment of rats with VD, and that the protective effects may be mediated through its anti-oxidant activities. Therefore, TQHXD may be a potential agent for the treatment of VD.

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Disclosure of conflict of interest

None.

Authors' contribution

Changjun Lu and Linglu Dun conceived and designed the experiments. Guocheng Liu,

Xiaocui Lei and Bingxin Wei performed the experiments. Hongwei An and Zheyi Zhou analyzed the data. Yufen Wu and Junlei Lu contributed reagents/materials/analysis tools. Jianyuan Zeng and Linglu Dun wrote the manuscript.

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