Hydroxysafflor yellow A improves learning and memory in a rat model of vascular dementia by increasing VEGF and NR1 in the hippocampus

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ABSTRACT

Hydroxysafflor yellow A (HSYA) has angiogenesis-regulating and neuro-protective effects, but its effects on vascular dementia (VaD) are unknown. In this study, 30 adult Sprague-Dawley rats were randomly allocated to five groups: normal, sham-operation, VaD alone (bilateral carotid artery occlusion), VaD plus saline (control), and VaD plus HSYA. One week after operation, the HSYA group received one daily tail-vein injection of 0.6 mg/100 g HSYA for two weeks. Five weeks after operation, the spatial memory of all five groups was evaluated by the water maze task, and synaptic plasticity in the hippocampus was assessed by the long-term potentiation (LTP) method. Vascular endothelial growth factor (VEGF) and N-methyl-D-aspartic acid receptor 1 (NR1) expression in the hippocampus was detected via Western blot. We found that, compared with the group with VaD alone, the group with HSYA had a reduced escape latency in the water maze (\(P < 0.05\)), and the LTP at CA3-CA1 synapses in the hippocampus was enhanced (\(P < 0.05\)). Western blot in the late-phase VaD group showed slight up-regulation of VEGF and down-regulation of NR1 in the hippocampus, while HSYA significantly up-regulated both VEGF and NR1. These results suggested that HSYA promotes angiogenesis and increases synaptic plasticity, thus improving spatial learning and memory in the rat model of VaD.

Keywords: vascular dementia; hydroxysafflor yellow A; long-term potentiation; NMDA receptor; vascular endothelial growth factor

INTRODUCTION

Vascular dementia (VaD) is a syndrome characterized by acquired mental dysfunctions resulting from brain damage of cerebrovascular origin. It is the second most common cause of dementia in the elderly after Alzheimer’s disease, causing 20–30\% of all elderly dementia cases\textsuperscript{11}, while an increasing number of studies suggest an even higher percentage of elderly dementia caused by VaD\textsuperscript{13}. However, so far, no drug has been approved by the FDA for VaD treatment.

Cognitive functions such as learning and memory are correlated with synapse number and function in the central nervous system, so cerebral ischemia-induced neuronal apoptosis and synapse reduction are considered to be the major causes of the symptoms of VaD. Since learning and memory are based on the long-lasting enhancement of synaptic efficacy, long-term potentiation (LTP) in the...
hippocampus is considered to be essential for cognition, and it is also an indicator of synaptic plasticity at the cellular level\[3\]. The N-methyl-D-aspartic acid receptor (NMDAR) is the main regulator of synaptic plasticity and LTP, and it is closely associated with learning and memory\[4, 5\]. NMDARs are composed of at least seven subunits: one NR1 subunit, four NR2 subunits (NR2A, NR2B, NR2C and NR2D) and two NR3 subunits (NR3A and NR3B). The NR1, NR2A and NR2B subunits are essential for the regulation of synaptic plasticity, however, the interactions between these subunits in LTP as well as their influence on learning and memory are yet unclear\[6-9\]. Vascular endothelial growth factor (VEGF) stimulates endothelial cell proliferation and promotes neovascularization\[10\]. Studies have reported that VEGF induces neurogenesis not only in the subependymal zone but also in the hippocampus, thus enhancing learning and memory, separately from increasing angiogenesis in the hippocampus\[11, 12\].

Safflower yellow is a natural pigment of the safflower, Carthamus tinctorius L, and it contains a mixture of water-soluble chalcones. Hydroxysafflor yellow A (HSYA, C_{27}H_{32}O_{16}, molecular weight 612) is the major component, and was first isolated by Meselhy et al. in 1993\[13\]. Its effects on angiogenesis and neuroprotective action in cerebrovascular and neurodegenerative diseases have become hot topics in recent years\[14-19\]. In addition to anti-inflammatory and antioxidant effects, its protection against apoptosis and effects on NMDARs have been found important for neuroprotection\[14-19\].

In this study, we evaluated the effects of HSYA on spatial memory, synaptic plasticity and VEGF, and explored the molecular mechanism for the improvement of spatial memory in a rat model of VaD.

**METHODS**

**Animals**

Thirty healthy adult male Sprague-Dawley rats weighing 280–300 g were provided by the Laboratory Animal Center of the Chinese Academy of Military Medical Science. The animals were group-housed at a stable temperature of ~20°C, with food and water *ad libitum* under a 12-h light/dark cycle (lights on at 07:00). This study was approved by Tianjin Medical University Animal Care and Use Committee.

Rats were randomly allocated to five groups (*n* = 6 rats/group): normal control (X), sham operation (Y), VaD alone (C), VaD + HSYA (H) and VaD + saline control (S).

**Rat Model of VaD**

Permanent bilateral common carotid occlusion (2-VO) was used to establish the VaD model, and the experimenters were blind to the grouping (C, H or S) before conducting operations. Prior to 2-VO, the animals were fasted for 12 h and water-deprived for 4 h. After weighing, the rats were anesthetized by intraperitoneal injection of 3.5 mg/kg chloral hydrate and then fixed supine on a heated pad; then both carotid arteries were gently exposed and permanent artery occlusion was implemented by double ligation.

**Groups and Treatments**

One week after model establishment, the VaD rats in group H received HSYA (Zhejiang Yongning Pharmaceutical Co., Ltd, Taizhou, China) *via* tail vein at 0.6 mg/100 g body weight dissolved in 1 mL saline. The same procedure was followed in group S but with 1 mL saline only. The treatment was continued for two weeks with one injection per day.

**Morris Water Maze**

The place navigation task in the water maze was used to assess learning and memory, and lasted for five days. From day 1, the rat was released into the water facing the pool wall at each landmark (the four quadrants each had a landmark on the wall) in a specific sequence. If the rat found the hidden platform in quadrant III and stayed on the platform for >2 s, it was deemed successful, and the time to find the platform was recorded as the escape latency. If the rat did not find the platform within 120 s, it was manually guided there, allowed to remain for 15 s, and its escape latency was recorded as 120 s. The swimming path and speed were also recorded. The rat was placed in the water from all 4 landmarks each day, with an interval of 30 min for resting; this procedure was continued for five days.

On day 6, a spatial probe task was conducted to evaluate spatial memory. The hidden platform was removed and rats were released into quadrant I, then within 30 s, the time spent in quadrant III (platform quadrant) was recorded. By multiplying the swimming speed by the time spent in quadrant III, the swimming distance in that quadrant was calculated. Then the distance covered in the platform quadrant was divided by the total swimming distance during the 30 s to provide an index of the spatial memory of that animal.