Neurosci Bull June 1, 2014, 30(3): 417–424. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1375-2

·Original Article·

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

Nan Zhang[1,2], Mengya Xing[1,2], Yiyi Wang[3], Hao Liang[2], Zhuo Yang[4], Fudong Shi[1,2], Yan Cheng[1,2]

[1]*Department of Neurology, Tianjin Medical University General Hospital, Tianjin 300052, China*

[2]*Tianjin Neurological Institute; Key Laboratory of Post-traumatic Neuro-repair and Regeneration in the Central Nervous System, Ministry of Education; Tianjin Key Laboratory of Injuries, Variations and Regeneration of the Nervous System, Tianjin 300052, China*

[3]*Tianjin Haihe Hospital, Tianjin 300350, China*

[4]*Nankai University, Tianjin 300071, China*

Corresponding author: Yan Cheng. E-mail: drcylfl@gmail.com

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[1], while an increasing number of studies suggest an even higher percentage of elderly dementia caused by VaD[2]. 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, 15]. 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 intrapertoneal 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.

Evaluating LTP

Six weeks after model establishment, five rats were randomly selected from each group for LTP evaluation at CA3-CA1 synapses by applying the following procedures: (1) Intra-abdominal injection of 30% urethane (4 mL/kg) for anesthesia; (2) after anesthesia, rats were fixed onto the head stereotaxic apparatus; (3) at the top of the rat's head, a skin incision of 1.5 cm was made at the midline to expose the skull, then a round skull window (5 mm in diameter) was drilled at the projection positions of stimulation and recording electrodes, and the underneath dural was removed using surgical tweezers; (4) using the anterior fontanelle as the reference point, the stimulation electrode was inserted 4.2 mm behind the reference point and 3.5 mm left to the anterior fontanelle, and the recording electrode was 3.5 mm behind and 2.5 mm left to the anterior fontanelle; (5) the electrodes were gently inserted into the left hippocampus, with a depth at approxiately 2.5 mm beneath the pia mater for the stimulation electrode and 2 mm for the recording electrode; (6) after placing the stimulating and recording electrodes, the LTP signal was acquired with Neurolog NL 104, NL 125 and PowerLab; (7) field excitatory postsynaptic potentials (fEPSPs) were recorded, and by adjusting the electrodes, maximal fEPSPs were induced; (8) then, with single stimuli ranging from 0.1 mA to 1.0 mA, the fEPSPs were recorded, and the intensity that induced 1/3 of the maximum negative signal was selected as the recording current intensity. Then 3 stimuli/min were applied for 20 min and baseline fEPSPs were recorded; (9) high-frequency stimulation was applied (200-Hz trains, 10 pulses/train every 2 s, repeated 10 times) and then 3 stimuli/min were applied for 60 min while fEPSPs were recorded; and (10) by normalization to the baseline, the slope of the fEPSP at each time point after high-frequency stimulation was standardized for statistical analysis.

Analysis of VEGF and NR1 by Western Blot

The hippocampus was taken from the brain after decapitation of rat. After 30-min incubation on ice, 500 mg tissue was homogenized on ice with an ultrasonic homogenizer in 1 mL radioimmunoprecipitation assay (RIPA) and phenylmethylsulphonyl fluoride (PMSF) lysis buffer (Millipore, Billerica, MA) with RIPA:PMSF at 100:1. Then the homogenate was centrifuged at 13 000 rpm for 15 min at 4°C, 20 μL supernatant was used to determine the protein concentration, and the remaining lysate was boiled for 10 min after mixing with 2× SDS gel loading buffer at 1:1 (*v*:*v*).

The total amount of protein in the loaded sample was adjusted to 80–150 μg to ensure even loading across samples in the same electrophoresis. Bromophenol blue (Sigma-Aldrich, St. Louis, MO) was used as a tracking dye to monitor the vertical electrophoresis. The proteins were separated with a Mini-Protean 3 electrophoresis system (Bio-Rad, Hercules, CA) at 150 V until the dye front reached the lower gel. Separated proteins were then transferred onto polyvinylidene-fluoride membrane (Millipore) at 80 V for 80 min. Non-specific binding was blocked with 4 mL PBS containing 5% nonfat dry milk for 1 h at room temperature. Proteins were immunodetected with specific primary antibodies (Millipore; 1:500 and 1:1 000 in the above blocking solution). After 12-h incubation at 4°C, the membranes were washed and incubated with secondary antibodies (Millipore; 1:1 000 with TBST) for 1 h at room temperature. Immunoreactive proteins were visualized using a chemiluminescent assay kit (CGQ/D2, Syngene, Cambridge, UK), and images were captured.

Statistical Analyses

Statistical analyses were conducted with SPSS (ver. 13.0). Data are presented as mean ± standard deviation (SD). The one-way ANOVA was used to assess differences among groups, and the LSD method was used for multiple comparisons. P <0.05 was considered to be significantly different.

RESULTS

Neurobehavioral Assessment with Water Maze Tasks

Dementia was successfully developed in all three groups undergoing the 2-VO procedure, and there was no significant difference in the escape latency among groups C, H and S.

During the 5-day place navigation task, there was a trend for shorter escape latencies in all the groups, groups X and Y being the most evident, and groups C and S the least. From day 1, group C had longer escape latencies than groups X (P = 0.009 on day 1, P = 0.000 on days 2–5 respectively) and Y (P = 0.013 on day 1, P = 0.000 on days

Table 1. Escape latencies (s) during place navigation tasks (mean ± SD)

Group	*n*	Day 1	Day 2	Day 3	Day 4	Day 5
Sham	6	78.65 ± 20.91	45.52 ± 10.36	26.19 ± 8.39	24.21 ± 9.56	17.79 ± 7.02
VaD model	6	107.38 ± 8.40*	99.12 ± 10.97*	98.73 ± 20.12*	90.41 ± 17.86*	87.69 ± 11.91*
VaD+saline	6	111.86 ± 8.85*	97.71 ± 8.37*	94.18 ± 7.21*	87.03 ± 4.38*	80.51 ± 8.62*
VaD+HSYA	6	95.12 ± 23.29	79.38 ± 28.68*	61.81 ± 29.73*#	49.35 ± 33.24*#	40.14 ± 29.99*#
F		4.698	13.338	19.252	15.737	22.768
P		0.012	0.000	0.000	0.000	0.000

*P <0.05 compared with sham operation group; #P <0.05 compared with VaD model group.

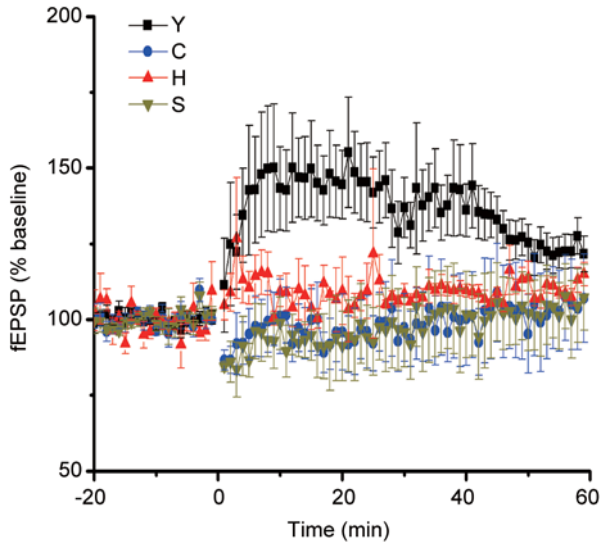

Fig. 1. fEPSPs after high-frequency stimulation. LTP at Schaffer collateral-CA1 synapses elicited by high-frequency stimulation in groups Y, C, H and S. Slopes of fEPSPs are normalized to baseline (20 min) before high-frequency stimulation and plotted against time. Each point represents the mean ± SEM of three consecutive evoked responses. Group Y had the greatest fEPSP slope, and in group H it was increased compared with groups C and S. (ANOVA, F = 396.027, P = 0.000, 5 mice each group; LSD comparison, P = 0.000, group H *versus* groups C and S). C, VaD model group; H, VaD plus HSYA group; S, VaD plus saline group; Y, SHAM operation group.

2–5), but group H showed a shorter escape latency than group C after day 3 (P = 0.220, 0.055, 0.003, 0.002, and 0.000 on days 1–5 respectively) (Table 1).

The results of the spatial probe task on day 6 showed that in group C, the time spent in the platform quadrant and the swimming distance within that quadrant were both significantly shorter than those in group Y (both P = 0.000). However, group H spent more time (P = 0.014) and had longer swimming distances (P = 0.045) in the platform quadrant than group C (Table 2).

LTP at CA3-CA1 Synapses in Hippocampus

After high-frequency stimulation, the slopes of fEPSP were standardized to the baselines, and the results showed that groups X and Y had the highest values while groups C and S had the lowest. Besides, the values in group H were higher than in groups C and S (P <0.05) (Fig. 1).

Assessment of Protein Expression by Western Blot

VEGF was found in the hippocampus in all groups at 44 kD (its molecular weight) (Fig. 2). Groups C and S had slightly higher VEGF expression than group Y, while group H had significantly higher VEGF expression than groups C and S (Fig. 2).

NR1 expression was found in the hippocampus of each group at 120 kD (Fig. 3). Group C showed decreased NR1 expression while in group H it was increased (Fig. 3).

DISCUSSION

The pathogenesis of VaD involves a series of molecular and structural changes that include inflammatory reactions, oxidative stress, apoptosis, excitotoxicity and impaired synaptic transmission. Due to the complex etiological and pathological processes of VaD, its clinical treatment is challenging. As in most cases VaD progresses gradually, the continuous development of the vascular lesions and persistent cerebral ischemia further complicate

Table 2. Results of spatial probe tasks (mean ± SD)

Group	*n*	Time spent in platform quadrant (s)	Swimming distance in platform quadrant (%)
Sham	6	13.50 ± 3.54	46.85 ± 12.54
VaD model	6	5.50 ± 0.83*	19.78 ± 3.54*
VaD+saline	6	5.57 ± 0.87*	19.98 ± 3.35*
VaD+HSYA	6	9.20 ± 1.23*#	30.49 ± 2.40*#
F		22.254	20.855
P		0.000	0.000

**P* <0.05 compared with sham operation group; #*P* <0.05 compared with VaD model group.

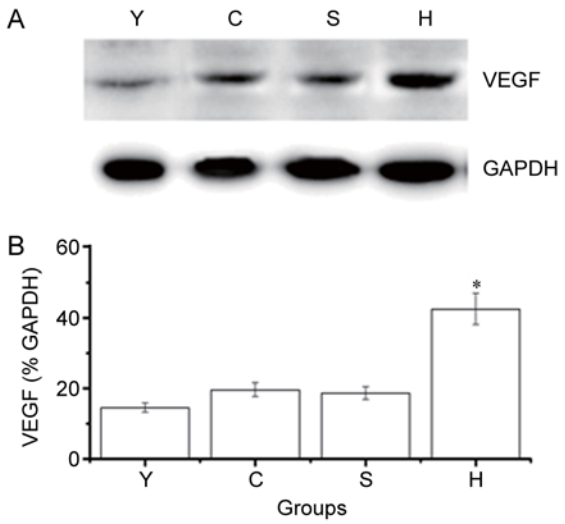

Fig. 2. VEGF expression in the hippocampus. A: Western blots of VEGF expression in groups Y, C, S and H. B: VEGF expression in group H was higher than in groups C and S (ANOVA, *F* = 39.271, *P* = 0.000, *n* = 6; LSD comparison, *P* = 0.000, group H *versus* groups C and S). C, VaD model group; H, VaD plus HSYA group; S, VaD plus saline group; Y, sham operation group.

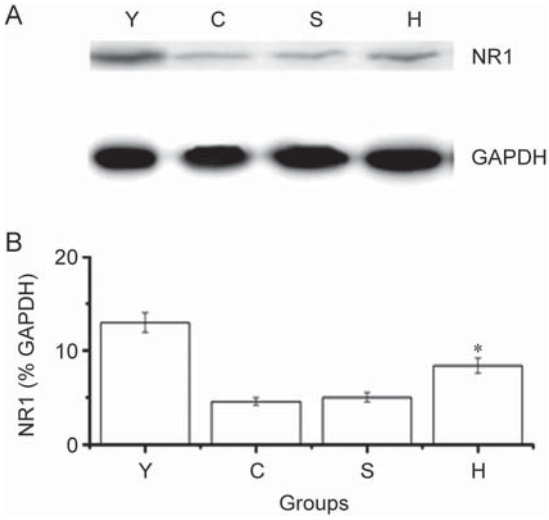

Fig. 3. NR1 expression in the hippocampus. A: Western blots of NR1 expression in groups Y, C, S and H. B: NR1 expression in group H was higher than in groups C and S (ANOVA, *F* = 32.465, *P* = 0.000, *n* = 6; LSD comparison, *P* = 0.000, group H *versus* groups C and S). C, VaD model group; H, VaD plus HSYA group; S, VaD plus saline group; Y, sham operation group.

the treatment. Therefore, our study explored a feasible treatment for VaD that could repair the damaged neural tissue and improve synaptic plasticity, as well as improving angiogenesis and blood perfusion.

Originally developed by Morris in the 1980s, the water maze is used to assess spatial learning in rodents[20]. In this study, we used the reliable and efficient experimental procedure introduced by Vorhees[21], which detects changes in spatial learning and memory due to lesions or medications. The results showed that the VaD plus HSYA group had improved spatial learning and memory compared with the VaD model group and saline control group.

In 1973, Bliss *et al.* reported a significant response of granule cells to high-frequency stimulation of the perforant path in domestic rabbits[22]: after the initial stimulation there was a long-lasting potentiation or an increase in current flow, which was thus named LTP. Our study revealed that HSYA enhanced LTP at CA3-CA1 synapses in VaD rats; LTP in the hippocampus has been reported to induce long-lasting enhancement of synaptic efficacy and plasticity, and

consequently strengthens learning and memory[23-26].

The activation of NMDARs requires the combination of glutamate and a co-agonist, either glycine or *D*-serine, and this co-agonist combines with two NR1 subunits of the NMDAR tetramer[27]. With NR1 activated, LTP is established and the negative effects of NMDARs on spatial learning and memory are thus inhibited and reduced[28-30]. Yang studied cortical neurons from rats pre-treated with NMDA *in vitro*, and revealed that HSYA reduces Bax expression, restores the balance between pro- and anti-apoptotic proteins, and reverses the up-regulation of NR2B mediated by NMDA, while NR2A is not affected in this process[19]. In our study, Western blot showed that HSYA increased the expression of NR1 in the hippocampus of VaD rats, which facilitated the enhancement of LTP.

VEGF was initially found to be a vascular permeability factor, and was considered as an endothelium-specific mitogen for a long time[31]. The VEGF family contains six members: from VEGF-A to VEGF-E and placental growth factor. These factors are important for angiogenesis and the regulation of endothelial cell functions. VEGF-A is essential for angiogenesis during both development and adulthood, and its signal transduction is mediated by the activation of two tyrosine kinase receptors: VEGFR-1 (Flt21) and VEGFR-2 (KDR)[32]. Specifically inactivating VEFG-A in the developing brain impairs angiogenesis and retards brain and skull development with significant neuronal apoptosis[33, 34]. Other studies of anti-angiogenic treatment showed that VEGF-A not only influences vascular endothelial cells but also affects neurons. Early in brain development, VEGF-A is expressed in the neuroectoderm of the ventricular zone, while VEGFR-1 and VEGFR-2 exist mainly in migrating endothelial cells[35, 36]. *In vitro* studies revealed that VEGF-A and VEGFR-2 are expressed in neural stem cells of the subventricular zone of the lateral ventricles in rodents[12, 37, 38]. VEGF-A stimulates neural stem cell proliferation in a dose-dependent manner and inhibits apoptosis, and intraventricular injection of reconstituted VEGF-A promotes neurogenesis in the subventricular zone and the dentate gyrus, and reduces neuronal apoptosis[12]. In this study, VEGF-A was detected in all groups, and the HSYA group had the highest expression.

The regulatory effects of HSYA on VEGF are not yet clear. *In vitro* studies of a human endothelial cell line confirmed that under hypoxic conditions, HSYA raises the protein concentration and mRNA expression of VEGF, and increases the accumulation and transcriptional activity of HIF-1α, which improves the hypoxic tolerance of vascular endothelial cells, and increases the cell proliferation rate[39]. However, when the effects of HSYA on human umbilical vein endothelial cells under normal and tumor conditions were studied using the MTT method, EVC304 growth was promoted by a high concentration of HSYA, while a low concentration inhibited growth, and this inhibitory effect gradually increased as the concentration of HSYA decreased[40]. Therefore, the effects of HSYA on VEGF and vascular endothelial cell growth are dose-dependent: low concentrations inhibit cell growth while high concentrations show significant stimulatory effects. In this study, a standard dose of HSYA (6 mg/kg/day) was administered to VaD rats for 2 weeks. Western blots showed that this dose significantly increased the VEGF-A expression in the hippocampus of VaD rats, and this effect was still evident 3 weeks after the last administration of HSYA in groups H and Y compared with groups X, C and S. Therefore, this dose induced a long-lasting increase in VEGF-A expression, suggesting it might promote angiogenesis in rat hippocampus.

We evaluated LTP in the hippocampus along with performance in the water maze tasks and VEGF-A and NR1 levels in the hippocampus, and found that the HSYA-induced improvement in the spatial memory of VaD rats may occur in three ways: first, HSYA increases VEGF-A expression which may promote angiogenesis and facilitate the recovery of cerebral circulation and the rebuilding of neuronal networks; second, *via* VEGF-related mechanisms, HSYA protects neurons against hypoxia and may prevent the cascade of reactions such as oxidative stress which are essential in the pathological process of vascular dementia, and inhibits disease progression; last, HSYA increases NR1 expression in post-ischemic neurons, which promotes LTP and increases synaptic plasticity.

ACKNOWLEDGEMENTS

This work was supported by the Science and Technology Development Project of Colleges and Universities of Tianjin Municipality, China (20110116).

Received date: 2013-05-09; Accepted date: 2013-06-30

REFERENCES

[1] Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K*, et al.* Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. Lancet Neurol 2008, 7: 812–826.

[2] Ronnemaa E, Zethelius B, Lannfelt L, Kilander L. Vascular risk factors and dementia: 40-year follow-up of a population-based cohort. Dement Geriatr Cogn Disord 2011, 31: 460–466.

[3] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993, 361: 31–39.

[4] Dupret D, O'Neill J, Pleydell-Bouverie B, Csicsvari J. The reorganization and reactivation of hippocampal maps predict spatial memory performance. Nat Neurosci 2010, 13: 995–1002.

[5] Micu I, Jiang Q, Coderre E, Ridsdale A, Zhang L, Woulfe J*, et al.* NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature 2006, 439: 988–992.

[6] Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A*, et al*. Paraneoplastic anti-N-methyl-*D*-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007, 61: 25–36.

[7] Gielen M, Siegler Retchless B, Mony L, Johnson JW, Paoletti P. Mechanism of differential control of NMDA receptor activity by NR2 subunits. Nature 2009, 459: 703–707.

[8] Liu Z, Zhao W, Xu T, Pei D, Peng Y. Alterations of NMDA receptor subunits NR1, NR2A and NR2B mRNA expression and their relationship to apoptosis following transient forebrain ischemia. Brain Res 2010, 1361: 133–139.

[9] Vacotto M, Rapacioli M, Flores V, de Plazas SF. Acute hypoxia differentially affects the NMDA receptor NR1, NR2A and NR2B subunit mRNA levels in the developing chick optic tectum: stage-dependent plasticity in the 2B-2A ratio. Neurochem Res 2010, 35: 1609–1619.

[10] Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004, 25: 581–611.

[11] Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D*, et al.* VEGF links hippocampal activity with neurogenesis, learning and memory. Nat Genet 2004, 36: 827–835.

[12] Schanzer A, Wachs FP, Wilhelm D, Acker T, Cooper-Kuhn C, Beck H*, et al.* Direct stimulation of adult neural stem cells in vitro and neurogenesis *in vivo* by vascular endothelial growth factor. Brain Pathol 2004, 14: 237–248.

[13] Meselhy MR, Kadota S, Momose Y, Hatakeyama N, Kusai A, Hattori M*, et al.* Two new quinochalcone yellow pigments from Carthamus tinctorius and Ca^{2+} antagonistic activity of tinctormine. Chem Pharm Bull (Tokyo) 1993, 41: 1796–1802.

[14] Han B, Zhao H. Effects of hydroxysafflor yellow A in the attenuation of MPTP neurotoxicity in mice. Neurochem Res 2010, 35: 107–113.

[15] Ye SY, Gao WY. Hydroxysafflor yellow A protects neuron against hypoxia injury and suppresses inflammatory responses following focal ischemia reperfusion in rats. Arch Pharm Res 2008, 31: 1010–1015.

[16] Bie XD, Han J, Dai HB. Effects of hydroxysafflor yellow A on the experimental traumatic brain injury in rats. J Asian Nat Prod Res 2010, 12: 239–247.

[17] Ji DB, Zhang LY, Li CL, Ye J, Zhu HB. Effect of Hydroxysafflor yellow A on human umbilical vein endothelial cells under hypoxia. Vascul Pharmacol 2009, 50: 137–145.

[18] Shan LQ, Ma S, Qiu XC, Zhou Y, Zhang Y, Zheng LH*, et al.* Hydroxysafflor Yellow A protects spinal cords from ischemia/reperfusion injury in rabbits. BMC Neurosci 2010, 11: 98.

[19] Yang Q, Yang ZF, Liu SB, Zhang XN, Hou Y, Li XQ*, et al.* Neuroprotective effects of hydroxysafflor yellow A against excitotoxic neuronal death partially through down-regulation of NR2B-containing NMDA receptors. Neurochem Res 2010, 35: 1353–1360.

[20] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984, 11: 47–60.

[21] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006, 1: 848–858.

[22] Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973, 232: 331–356.

[23] Bannerman DM, Good MA, Butcher SP, Ramsay M, Morris RG. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature 1995, 378: 182–186.

[24] Jeffery KJ, Morris RG. Cumulative long-term potentiation in the rat dentate gyrus correlates with, but does not modify, performance in the water maze. Hippocampus 1993, 3: 133–140.

[25] Morris RG, Davis S, Butcher SP. Hippocampal synaptic plasticity and NMDA receptors: a role in information storage? Philos Trans R Soc Lond B Biol Sci 1990, 329: 187–204.

[26] Moser EI, Krobert KA, Moser MB, Morris RG. Impaired spatial learning after saturation of long-term potentiation. Science 1998, 281: 2038–2042.

[27] Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev 1999, 51: 7–61.

[28] Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature

1986, 319: 774–776.

[29] Sakimura K, Kutsuwada T, Ito I, Manabe T, Takayama C, Kushiya E*, et al.* Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor epsilon 1 subunit. Nature 1995, 373: 151–155.

[30] Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell 1996, 87: 1327–1338.

[31] Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983, 219: 983–985.

[32] Shen H, Clauss M, Ryan J, Schmidt AM, Tijburg P, Borden L*, et al.* Characterization of vascular permeability factor/vascular endothelial growth factor receptors on mononuclear phagocytes. Blood 1993, 81: 2767–2773.

[33] Haigh JJ, Morelli PI, Gerhardt H, Haigh K, Tsien J, Damert A*, et al.* Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. Dev Biol 2003, 262: 225–241.

[34] Raab S, Beck H, Gaumann A, Yuce A, Gerber HP, Plate K*, et al.* Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. Thromb Haemost 2004, 91: 595–605.

[35] Breier G, Albrecht U, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 1992, 114: 521–532.

[36] Breier G, Clauss M, Risau W. Coordinate expression of vascular endothelial growth factor receptor-1 (flt-1) and its ligand suggests a paracrine regulation of murine vascular development. Dev Dyn 1995, 204: 228–239.

[37] Maurer MH, Tripps WK, Feldmann RE Jr, Kuschinsky W. Expression of vascular endothelial growth factor and its receptors in rat neural stem cells. Neurosci Lett 2003, 344: 165–168.

[38] Meng H, Zhang Z, Zhang R, Liu X, Wang L, Robin AM*, et al.* Biphasic effects of exogenous VEGF on VEGF expression of adult neural progenitors. Neurosci Lett 2006, 393: 97–101.

[39] Ji DB, Zhu MC, Zhu B, Zhu YZ, Li CL, Ye J*, et al*. Hydroxysafflor yellow A enhances survival of vascular endothelial cells under hypoxia via upregulation of the HIF-1 alpha-VEGF pathway and regulation of Bcl-2/Bax. J Cardiovasc Pharmacol 2008, 52: 191–202.

[40] Zhang Q, Niu X, Yan Y, Jin M, Yang XZ, Li JR. Research on the mechanism of hydroxysafflor yellow A in inhibiting ngiogenesis. J Beijing Univ Tradit Chin Med 2004, 27: 25–29.