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Full Length Research Paper

Effect of salidroside on learning and memory ability of vascular dementia rats

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The effect of salidroside (SD) on learning and memory ability of vascular dementia (VaD) rats were studied. The Wistar rats were randomly divided into six groups: Sham-operation group, VaD group, positive control (PC) group, SD high dose (SD-HD) treatment group, SD moderate dose (SD-MD) treatment group and SD low dose (SD-LD) treatment group. The bilateral common carotid arteries of Wistar rats were permanently ligated to established VaD models. The special study and memory were observed by Y-maze test. At postoperative 4, 8 and 12 weeks, error number (EN) and total reaction time (TRT) in the VaD, PC, SD-HD, SD-MD and SD-LD group were significantly more than those in the sham-operation group (*P*< 0.05 or 0.01), while EN and TRT in the PC, SD-HD, SD-MD and SD-LD groups were significantly less than those in the VaD group (*P*<0.05 or 0.01). Compared with PC group, EN and TRT in SD-MD and SD-LD groups were also higher (*P*<0.05 or 0.01). However, there were no significant differences in EN and TRT between PC and SD-HD groups (*P*>0.05). These results suggested that SD could improve the learning and memory ability of VaD rats by different dosages.

Key words: Salidroside, vascular dementia, rats.

INTRODUCTION

In the western world, vascular dementia (VaD) is the second most common form of adult-onset dementia after Alzheimer's disease (AD). It has an overall prevalence of 1.2 to 4.2% in people aged 65 years or older, and accounts for 10 to 50% of dementia cases, depending on the diagnostic criteria and study population (Knopman et al., 2003). In terms of symptomatology, VaD is characterized by progressive cognition decline, functional ability impairment and behavioral problems. VaD results mainly from ischemic injury or oligaemia to brain areas involved in cognition, memory and behaviour (Román, 2003).

Rhodiola crenulata (Crassulaceae, Hongjingtian in Chinese) has been known as a medicinal plant for a long time. This precious perennial herbaceous plant distributed at high altitudes in the Polar Arctic and Alpine regions throughout Europe and Asia (Qu et al., 2009). It was reported that *R. crenulata* has multiple pharmacological activities such as, antioxidant (Panossian et al., 2010), antihypoxia (Ming, 1986), antifatigue (Shevtsov et al., 2003), antiapoptosis (Jung et al., 2002), anticancer (Kwon et al., 2008) and enhancement in learning and memory (Petkov et al., 1986). Recently, it was reported that the extract of *R. crenulata* could improve memory and behaviour of VaD rats and some mechanisms were also investigated (Chen et al., 2008a, b, 2010; Wang and Yang, 2008; Yang et al., 2008). However, the exact neuroprotective constituents in the extract of *R. crenulata* are still unclear. Salidroside (SD, p-hydroxyphenethyl-b-D-glucoside, $C_{14}H_{20}O_7$, structure is shown in Figure 1) is one of the major active constituents in *R. crenulata*. It has been reported to possess various pharmacological properties including resisting anoxia, antiaging, anticancer, antiinflammation, antioxidative, antifatigue, antiviral, neuroprotective, hepatoprotective and cardioprotective effects (Diaz Lanza et al., 2001; Laremii and Grigor'eva, 2002; Kanupriya et al., 2005; Kelly, 2001; Kucinskaite et al., 2004; Ma et al., 2009; Nan et al., 2003; Wang et al., 2004, 2009; Zhang et al., 2007).

According to its neuroprotective activity and high

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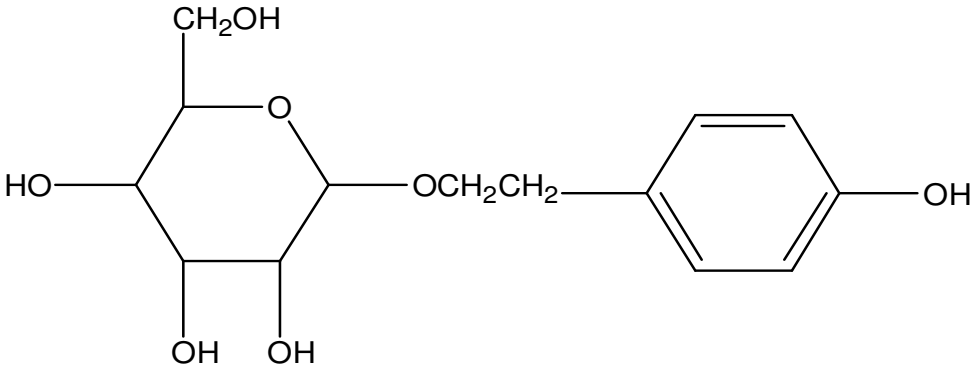

Figure 1. The chemical structure of salidroside.

content in *R. crenulata*, we assumed that SD might have some effects on learning and memory ability of VaD.

In the current work, we established rat models of VaD by permanent ligation of bilateral common carotid artery and attempted to investigate the effects of SD on learning and memory ability of VaD rats by Y-maze test.

MATERIALS AND METHODS

Reagents and apparatus

SD was purchased from China pharmaceutical and biological products inspection (Lot: 110818-201005). Nimodipine tablets were obtained from Beijing Bayer pharmaceutical and medicinal company (Lot: 100091211) and Y-maze (type MG-3) was obtained from Zhangjiagang Biomedical Instrument Factory.

Laboratory animals

Ninety (90) Wistar rats, aged greater than 16 months, weighing (300 ± 41) g, of either gender, were provided by the Experimental Animal Center of the Zhengzhou University of China between July, 2008 and November, 2011. The rats were housed under controlled conditions (room temperature, 22 ± 2°C).

Establishment of VaD rats models

Following the rats were intraperitoneally anesthetized by chloral hydrate, a median incision was made at the cervical part. Muscle was carefully bluntly dissected to expose bilateral common carotid artery. A No.7 surgical suture was embedded under common carotid artery. Vagus nerve was avoided to be stimulated and bilateral common carotid artery was ligated carefully and incision was sutured.

Grouping and intervention

Ninety (90) Wistar rats were randomized into six groups: sham-operation group (sham, n=15): bilateral common carotid artery was dissected, but ligation was not conducted following surgical suture embedding, then surgical suture was drawn out and incision was sutured; VaD model group (VaD, n=15): rat models of VaD were developed as previous described methods; Positive control group (PC, n=15): rat mdels of VaD were developed and intraperitoneally injected with nimodipine at a dose of 20 mg/(Kg·d), once a day. SD high dose treatment group (SD-HD, n=15): rat models of VaD were developed and intraperitoneally injected with SD at a dose of 30 mg/(Kg·d), once a day. SD moderate dose treatment group (SD-MD, n=15): rat models of VaD were developed and intraperitoneally injected with SD at a dose of 20 mg/(Kg·d), once a day; SD low dose treatment group (SD-LD, n=15): rat models of VaD were developed and intraperitoneally injected with SD at a dose of 10 mg/(Kg·d), once a day. The experimental temperature was kept at room temperature (22 ± 2°C). Rats in the sham-operation group and VaD group were intraperitoneally injected with the same volume of normal saline.

Behavioral evaluation

The changes in action and behavior of rats were observed with type MG - 3 Y-maze before and 4, 8 and 12 weeks after operation. The parameters of Y-maze were set as voltage 70 V and delay time 3 s. Following random rest method (Wu et al., 2007), safety area was changed in irregular order. The arm which rat stood on was used as the starting point of test. After rats escaped to safety area, signal lamp still lighted on for 15 s. Test began following another 1 s. Test was conducted repeatedly to train rats to form the conditioned reflex of light-dark discrimination, and there was 30 s time interval between two tests. The rats were daily trained 20 times for 3 days successively. Error number (EN), which referred to the times of errors in everyday training, in the last day ≤ 2 and total reaction time (TRT) ≤ 120 s indicated that the rats had formed the conditional reflex (Chen et al., 2006); EN ≥ 8 was used as the criterion of cognitive dysfunction.

Criteria of correct and wrong reaction

Rats escape to safety area in about after pelma was electrified for 10 s, which indicated correct reaction, otherwise indicated wrong reaction. Rats escaped to starting area that was also considered as wrong reaction. Generally, rats were daily trained 20 times, which reflected the degree of correct reaction of rats. Reaction time (latency) referred to time period from signal lamp was lighted to rats firstly escaped to light area. TRT referred to the time needed by all the reactions (including correct reaction and wrong reaction) in a whole experimental day. TRT reflected the reaction time of rats. Both EN and TRT could be used together to evaluate the learning and memory ability of rats. All the rats were daily re-trained 20 times for 3 days successively with Y-maze. EN and TRT in the last day were recorded.

Table 1. EN of rats at different time points among different groups.

Group	Error number (EN)			
	Before operation	4 weeks after operation	8 weeks after operation	12 weeks after operation
Sham	1.12 ± 0.51 (n=15)	0.97 ± 0.57 (n=15)	0.98 ± 0.62 (n=15)	0.99 ± 0.69 (n=15)
VaD	1.14 ± 0.52 (n=15)	2.78 ± 0.83 (n=10) $^{\triangle\triangle}$	8.15 ± 1.72 (n=10) $^{\triangle\triangle}$	13.64 ± 1.92 (n=10) $^{\triangle\triangle}$
PC	1.18 ± 0.34(n=15)	1.99 ± 0.64 (n=11) $^{\triangle}$*	5.25 ± 1.02 (n=11) $^{\triangle\triangle}$**	9.73 ± 1.48 (n=11) $^{\triangle\triangle}$**
SD-HD	1.19 ± 0.52 (n=15)	2.04 ± 0.73 (n=11) $^{\triangle}$*	5.27 ± 1.05 (n=11) $^{\triangle\triangle}$**	9.78 ± 1.52 (n=11) $^{\triangle\triangle}$**
SD-MD	1.16 ± 0.49 (n=15)	2.26 ± 0.83 (n=11) $^{\triangle}$*$^{\#}$	6.32 ± 0.95 (n=11) $^{\triangle\triangle}$**$^{\#\#}$	10.78 ± 1.45 (n=11) $^{\triangle\triangle}$**$^{\#\#}$
SD-LD	1.12 ± 0.47 (n=15)	2.43 ± 0.68 (n=11) $^{\triangle}$*$^{\#}$	6.75 ± 1.02 (n=11) $^{\triangle\triangle}$**$^{\#\#}$	11.88 ± 1.62 (n=11) $^{\triangle\triangle}$**$^{\#\#}$

VS Sham, ($^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01); VS VaD, (*P<0.05, **P<0.01); VS PC, ($^{\#}P$<0.05, $^{\#\#}P$<0.01).

Table 2. TRT of rats at different time points among different groups.

Group	Total reaction time (TRT)			
	Before operation	4 weeks after operation	8 weeks after operation	12 weeks after operation
Sham	100.65 ± 2.52 (n=15)	100.43 ± 1.93 (n=15)	101.34 ± 3.05 (n=15)	107.25 ± 1.98 (n=15)
VaD	100.86 ± 2.55 (n=15)	173.54 ± 5.62 (n=10) $^{\triangle\triangle}$	212.38 ± 8.24 (n=10) $^{\triangle\triangle}$	275.25 ± 4.94 (n=10) $^{\triangle\triangle}$
PC	100.47 ± 2.51 (n=15)	128.39 ± 6.19 (n=11) $^{\triangle}$*	169.84 ± 4.72 (n=11) $^{\triangle\triangle}$**	216.80 ± 3.98 (n=11) $^{\triangle\triangle}$**
SD-HD	100.67 ± 2.32 (n=15)	131.41 ± 7.08 (n=11) $^{\triangle}$*	170.44 ± 4.96 (n=11) $^{\triangle\triangle}$**	215.76 ± 3.96 (n=11) $^{\triangle\triangle}$**
SD-MD	100.53 ± 2.44 (n=15)	140.23 ± 7.02 (n=11) $^{\triangle}$*$^{\#}$	179.94 ± 5.04 (n=11) $^{\triangle\triangle}$**$^{\#\#}$	236.80 ± 5.46 (n=11) $^{\triangle\triangle}$**$^{\#\#}$
SD-LD	100.82 ± 2.41 (n=15)	145.35 ± 7.06 (n=11) $^{\triangle}$*$^{\#}$	180.45 ± 5.12 (n=11) $^{\triangle\triangle}$**$^{\#\#}$	246.75 ± 5.67 (n=11) $^{\triangle\triangle}$**$^{\#\#}$

VS Sham, ($^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01); VS VaD, (*P<0.05, **P<0.01); VS PC, ($^{\#}P$<0.05, $^{\#\#}P$<0.01).

RESULTS

Quantitative analysis of the experimental animals

Ninety (90) rats were involved. At postoperative 1 day, 2 rats of VaD group, 2 of PC group, 2 of SD-HD group, 2 of SD-MD treatment group and 2 of SD-LD group died. At postoperative 4 weeks, 3 rats of VaD group, 2 of PC group, 2 of SD-HD group, 2 of SD-MD group and 2 of SD-LD group died. Rats of sham-operation group all survived. Finally, 69 rats were involved in the result analysis.

General condition of rats

Before operation, rats of each group were flexible in action, sensitive in pain sensation and quick in reaction. When bilateral common carotid artery of rats in model group was completely blocked, transient convulsion was firstly presented, then body-righting reflex disappeared accompanied by hypothermy and slowing breath. During the early period after operation, rats were found with decreasing actions, "horoscope" of hindlimb while crawling, defective coordination and circling. At postoperative 5 to 7 days, crawling of each group was basically recovered and no obvious motor disturbances were found.

Evaluation of learning and memory ability of rats

There were no significant differences in EN and TRT before operation among groups (P>0.05). At postoperative 4, 8 and 12 weeks, EN and TRT in the VaD, PC, SD-HD, SD-MD and SD-LD groups were significantly more than those in the sham-operation group (P<0.05 or 0.01), while EN and TRT in the PC, SD-HD, SD-MD and SD-LD group were significantly less than those in the VaD group (P<0.05 or 0.01). Compared with PC group, EN and TRT in SD-MD and SD-LD groups were also higher (P<0.05 or 0.01). However, there were no significant differences in EN and TRT between PC and SD-HD groups (P>0.05). These results indicated that SD could improve the learning and memory ability of VaD rats by different dosages (Table 1 and 2).

DISCUSSION

Ischemic injury is an important mechanism of VaD. Therefore, we established the VaD model by permanent ligation of bilateral common carotid artery, which could successfully induce ischemic injury *in vivo*.

Y-maze is an instrument used to evaluate learning and memory ability. There are many testing methods about Y-maze test, including fixed exercising times random method, fixed exercising times sequence method, unfixed exercising times random method, unfixed exercising times sequence method (Wang et al., 2007). The sequence method is to change the direction safe area according to a fixed regulation, and it is easy for the rats to form inertia to differentiate spatial direction, thus it cannot completely reflect the memory ability. The random method requires changing the direction safe area at random, thus it is hard to form inertia. The test with unfixed times is to train the rats continuously by different periods in 1 day, but repeated training might make the rats tired and distractibility, even dysphoria, thus it is hard to objectively reflect the learning and memory abilities of rats. The test with fixed times is to regulate the exercising times every day, and completed in several days, it is hard for the rats to feel tired, thus it can objectively reflect the memory ability. Therefore, the fixed exercising times random method was used for Y-maze test in our study.

Nimodipine is a dihydropyridine calcium antagonist with multiple mechanisms of action. It was reported that nimodipine has an effect on age-related microangiopathy as vasoactive agent not interfering with the autoregulation of cerebra blood flow. Moreover, it may contribute to neuroprotection by blocking L-type calcium receptors. In addition, an exploratory randomized, double-blind, controlled trial of nimodipine focusing on subcortical VaD has recently been published. The results of this study favor the nimodipine-treated group that showed a better performance on verbal fluency and global cognitive function assessed with Mini-Mental State Examination (MMSE) compared with the placebo group. Furthermore, the high drop-out rates in the placebo group suggest that nimodipine might protect against cardio-vascular comorbidities (Pantoni et al., 2005). Therefore, nimodipine was chosen as the PC in this study. Based on models of 2-vascular occlusion, we used Y-maze to test the learning and memory ability of VaD rats before and 4, 8 and 12 weeks after operation. Results found that as compared with sham-operation group, rats of VaD presented increased EN and TRT of Y-maze task (at post operative 4, 8 and 12 week, $P<0.05$ or 0.01), and these symptoms are more and more obvious with the elongation of time to blockage. As compared with VaD group, EN decreased and TRT shortened in PC, SD-HD, SD-MD and SD-LD treatment groups ($P<0.05$ or 0.01). These results demonstrated that SD could improve the learning and memory ability of VaD rats. Up to now, there are some therapy protocols forVaD, such as cholinergic neurotransmission, cholinesterase inhibitors, muscarinic agonists, noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists and dihydropyridine calcium antagonists (Abel et al., 2007). The precise mechanisms involved in VaD remain unclear, but some studies have indicated that cholinergic deficiency and post-ischemic inflammation occurred in response to ischemic injury and contributes to delayed brain damage (Kuang et al., 2008; Kumaran et al., 2008). Moreover, inflammatory injury is another significant characteristic of VaD pathology. Inflammatory events following cerebral ischemia include upregulation of inflammatory mediators such as inter-cellular adhesion molecule 1, selectins, tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), nitric oxide (NO), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Wang et al., 2007). However, the mechanisms of the effects of SD in VaD improvement are still unknown. Lots of studies will be needed to further understand the mechanisms of the effect of SD in VaD improvement in *vitro* and *vivo*.

In the present work, we found that the learning and memory ability of rats with VaD were obviously improved following SD therapy, suggesting that SD could improve the cognitive function of VaD rats.

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