Tropical Journal of Pharmaceutical Research January 2022; 21 (1): 13-18
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i1.3

Original Research Article

Asiaticoside ameliorates neuronal apoptosis and oxidative stress in vascular dementia rats via cAMP-PKA axis activation

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Sent for review: 9 September 2021 *Revised accepted: 15 December 2021*

Abstract

Purpose: *To investigate the effect of asiaticoside (AS) on vascular dementia (VD) development and elucidate its mechanism of action.*
Methods: *A VD rat model was established via bilateral common carotid artery occlusion. The cognitive function of VD rats was evaluated using Morris water maze test. The contents of malondialdehyde (MAD) and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) were determined by enzyme-linked immunosorbent assay (ELISA) while Western blot was used to determine protein levels.*
Results: *Compared to model group, AS significantly improved the cognitive dysfunction of VD rats ($p < 0.01$). In addition, AS significantly inhibited oxidative stress of VD rats compared with model group ($p < 0.01$). AS also alleviated the apoptosis of brain tissue and activated cAMP-PKA axis in VD rats, relative to model group ($p < 0.01$).*
Conclusion: *AS attenuates cognitive dysfunction, oxidative stress, and neuronal apoptosis in VD via activation of cAMP-PKA axis. Thus, AS is a potential drug for the treatment of VD, but further investigation is required to ascertain this.*

Keywords: *Asiaticoside, Vascular dementia, Cognitive dysfunction, Oxidative stress, Apoptosis*

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INTRODUCTION

Vascular dementia (VD) is the second leading cause of dementia after Alzheimer's disease, accounting for approximately 15 % of dementia cases [1]. Vascular dementia is a syndrome of cerebral circulation dysfunction and brain injury caused by cerebrovascular factors [2]. The pathogenesis of VD remains unknown. Studies have revealed that increased apoptosis in VD leads to cognitive dysfunction [3]. Furthermore, oxidative stress and neuroinflammation also play essential roles in the development of VD [4]. Despite some advances in experimental and clinical neuroscience, no drug has been approved in the world to date for the treatment of VD [3]. Hence, it is important to identify an effective medicine for treating VD.

Asiaticoside (AS) is a triterpenoid bioactive compound extracted from *Centella asiatica* [5]. Increasing evidence demonstrate that AS exhibits various effects in many diseases, such as anti-tumor, wound healing, anti-inflammatory, and antioxidant action [6,7]. For example, AS inhibits proliferation of colorectal cancer by suppressing the NF-κB signaling pathway [6]. Moreover, Luo, *et al*. reported that AS alleviate spinal cord injury via antioxidant and anti-inflammatory effects and suppressing the p38-MAPK pathway [7]. Similarly, AS exerts protective effects on central nervous system (CNS)-related diseases [8,9]. For instance, Yin *et al* showed that AS relieves diabetes-induced cognition deficits through modulation of the PI3K/Akt/NF-κB pathway [8]. Fan *et al* found that AS suppressed neuronal apoptosis and promoted functional recovery in rats with spinal cord injury [9].

Nevertheless, the effect of AS on VD remains unclear at present. A previous study reported that AS activates the cAMP-induced PKA/pCREB/BDNF pathway and alleviate chronic unpredictable mild stress-induced depressive symptoms [10]. Interestingly, Han *et al*. demonstrated that the cAMP/PKA/CREB signaling pathway is involved in regulating cognitive dysfunction in VD [11]. Therefore, AS might be involved in the regulation of VD by modulating the cAMP-PKA axis. In this study, the effect of AS on VD development and whether the cAMP-PKA axis is involved in the mechanism of AS on VD were investigated.

EXPERIMENTAL

Animal experiments

Thirty male Sprague-Dawley rats aged 4 – 6 weeks were purchased from the Beijing Laboratory Animal Research Center (Beijing, China). The rats were assigned to five groups (n = 6): sham, VD, VD + 10 mg/kg AS, VD + 20 mg/kg AS, and VD + 40 mg/kg AS. The rats in the VD, VD + 10 mg/kg AS, VD + 20 mg/kg AS, and VD + 40 mg/kg AS groups underwent bilateral common carotid artery occlusion to establish a VD model according to the method described previously [12].

Briefly, the rats were anesthetized via intraperitoneal injection of 10 % chloral hydrate (0.3 ml/100 g). Skin from the neck was cut to expose the bilateral common carotid artery. The tissues around the carotid arteries were then separated, and the bilateral common carotid arteries were occluded using silk sutures. The skin was stitched and sterilized [12]. The rats in the sham group underwent the same procedures except for occlusion of the carotid arteries. After establishing the VD model, rats in the AS administration groups were received 10, 20, and 40 mg/kg AS, respectively, for 4 weeks via gastric perfusion. All procedures were carried out following “Principles of Laboratory Animal Care” [13]. The execution of this study was permitted by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (approval no. IACUC-20180423-11).

Morris water maze test

The Morris water maze test was conducted as described previously [14]. The water maze utilized in this study was 100 cm in diameter and 30 cm in depth. The bottom of the water maze was black. The water maze was filled with opaque water, and the water temperature was kept at 22 ± 1 °C. The water maze was divided into four quadrants, and a circular platform (1.5 cm beneath the water surface) was placed in one quadrant of the pool. Before starting the formal test, all rats were pretrained three times a day with an interval of 2 h each time for 2 days. During training, the rats were placed randomly in different quadrants facing the wall and were guided to search the platform in a straight line and stay on the platform for 30 sec. To conduct the formal Morris water maze test, the rats were placed in different quadrants randomly facing the wall to find the platform. During the experimental trial, the times spent finding the platform (escape latency) and times spent on the platform were recorded. All rats were given 60 s to explore the platform. If the rats could not find the platform, they were placed on the platform for 10 sec, and escape latency was recorded as 60 sec. The test was performed four times a day for 5 days.

Enzyme-linked immunosorbent assay (ELISA)

After the rats were euthanized, brain tissues were collected and homogenized. After centrifugation, the supernatant was harvested to determine the contents of cyclic adenosine monophosphate (cAMP) and malondialdehyde (MAD), as well as the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), using the corresponding commercial ELISA assay kits according to the manufacturer’s procedures.

Western blot

Brain tissues were lysed using RIPA buffer, and the protein concentration of the lysates was quantified using a BCA Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). Equivalent

amounts of lysate were subjected to SDS-PAGE and electro-transferred onto PVDF membranes. After blocking with 5 % non-fat milk, the membranes were incubated with primary antibodies against cleaved caspase-3 (1:1000; Cell Signaling Technology), cleaved Caspase-9 (1:1000; Cell Signaling Technology), Bax (1:1000; Abcam), BCL-2 (1:1000; Abcam), PKA (1:1000; Cell Signaling Technology), p-CREB (1:1000; Cell Signaling Technology), CREB (1:1000; Cell Signaling Technology), BDNF (1:1000; Abcam), and β-actin (1:2000; Abcam) at 4°C overnight. The membranes were then incubated with HRP-conjugated IgG H&L secondary antibody (Abcam, Cambridge, UK) for 2 h. Then, the bands were visualized utilizing ECL Plus Western Blotting Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

All data are shown as mean ± standard deviation (SD). One-way ANOVA plus LSD *post hoc* test were employed to determine differences using SPSS 22.0 software (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

AS improves cognitive dysfunction in rats with VD

To investigate the effects of AS on VD, a rat VD model was established and then treated the animals with 10, 20 or 40 mg/kg AS for 4 weeks. The chemical formula of AS is presented in Figure 1 A. After administering AS, the cognitive function of VD rats was evaluated utilizing the Morris water maze test. The escape latency of VD rats was significantly increased compared to sham rats ($p < 0.01$) but was decreased by AS administration in a dose-dependent manner ($p < 0.01$, Figure 1 B). In contrast, the time spent in the target quadrant by VD rats was less than that of sham rats ($p < 0.01$), but AS administration increased this in a dose-dependent manner ($p < 0.01$, Figure 1 C). The movement routes in the Morris water maze test of rats in the different groups are shown in Figure 1 D. These results indicate that AS improved cognitive dysfunction in rats with VD.

Asiaticoside attenuates oxidative stress in rats with VD

To further investigate the effect of AS on VD, oxidative stress was assessed in VD rats after treatment with AS. The results show that the activities of GSH, SOD, and CAT were decreased in brain tissues of VD rats ($p < 0.01$), but AS administration increased these activities ($p < 0.01$, Figure 2). Furthermore, the content of MDA was elevated in brain tissues of VD rats ($p < 0.01$). However, AS administration decreased the MDA content ($p < 0.01$, Figure 2). Thus, these findings revealed that AS attenuated oxidative stress in rats with VD.

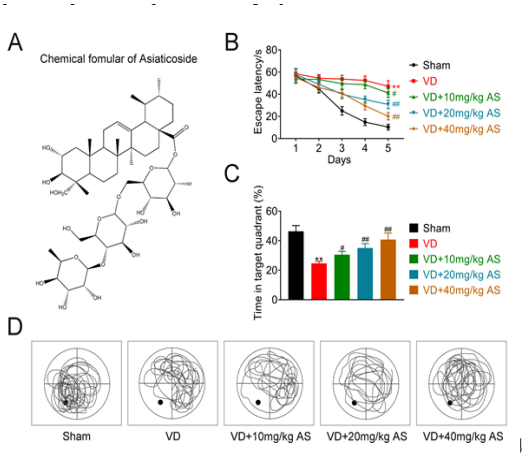

Figure 1: AS improves cognitive function in rats with VD. A. The chemical formula of AS was shown. **B.** The escape latency of VD rats with 10, 20 or 40 mg/kg AS administration was assessed using the Morris water maze test for 5 days. **C.** The time spent in the target quadrant of VD rats with 10, 20 or 40 mg/kg AS administration was investigated by the Morris water maze test. **D.** The movement routes in the Morris water maze test of VD rats after with 10, 20 or 40 mg/kg AS administration were shown. $^{**}p < 0.01$ compared to the sham group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ compared to the VD group

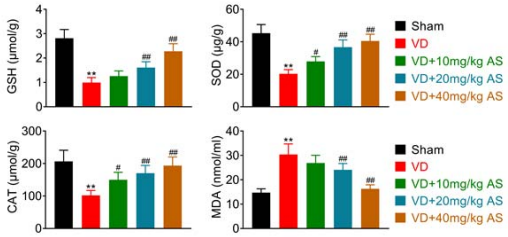

Figure 2: AS attenuates oxidative stress in rats with VD**.** GSH, SOD, and CAT activities, as well as MDA content in brain tissues of VD rats, after administrated with 10, 20 and 40 mg/kg AS were measured using ELISA; $^{**}p < 0.01$ compared to the sham group. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ compared to the VD group

AS alleviates apoptosis of brain tissue in rats with VD

To study the influence of AS on brain tissue apoptosis in VD, the expressions of cleaved caspase-3, cleaved caspase-9, Bax, and Bcl-2 in AS-treated VD rats were determined using

Western blot. The data demonstrate that the expression levels of cleaved caspase-3, cleaved caspase-9, and Bax were significantly increased in VD rats ($p < 0.01$) and the Bcl-2 level was decreased in brain tissues of VD rats ($p < 0.01$, Figure 3). However, AS treatment significantly reduced the levels of cleaved caspase-3, cleaved caspase-9, and Bax in VD rats and increased the level of Bcl-2 ($p < 0.01$, Figure 3). Hence, AS alleviated the apoptosis of brain tissue in rats with VD.

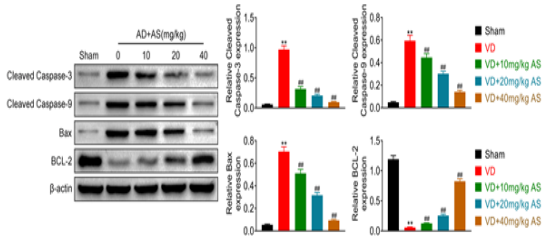

Figure 3: AS alleviates apoptosis of brain tissue in rats with VD**.** The expression levels of cleaved caspase-3, cleaved caspase-9, Bax, and Bcl-2 in brain tissues of VD rats after administrated with 10, 20 and 40 mg/kg AS were determined using Western blot; $^{**}p < 0.01$ compared to the sham group. $^{\#\#}p < 0.01$ compared to the VD group

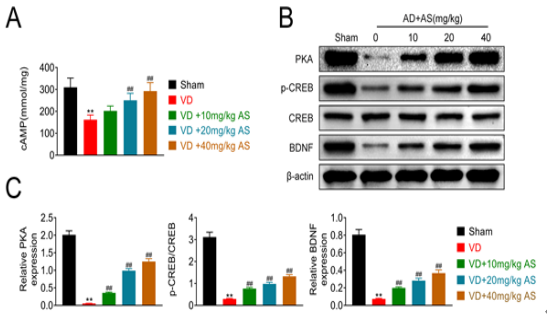

Figure 4: AS activates the cAMP-PKA axis in rats with VD. The expression levels of PKA, p-CREB, CREB, and BDNF in brain tissues of VD rats after treatment with 10, 20 or 40 mg/kg AS were determined using Western blot. The levels of cAMP in brain tissues of VD rats after administrated with 10, 20 and 40 mg/kg AS were determined using ELISA. $^{**}P < 0.01$ compared to the sham group, $^{\#\#}p < 0.01$ compared to the VD group

AS activates the cAMP-PKA axis in rats with VD

To determine the mechanism of AS on pathogenesis of VD, the activity of the cAMP-PKA axis was determined in AS-treated VD rats. Results show that the levels of cAMP, PKA, BDNF, and p-CREB were decreased in VD rats ($p < 0.01$) but AS administration increased these protein levels ($p < 0.01$, Figure 4). Collectively, our results demonstrate that AS activated the cAMP-PKA axis.

DISCUSSION

Vascular dementia is a syndrome of cerebrovascular disorders and brain injury caused by cerebrovascular factors and is a major cause of dementia. No drug has been approved in the world yet for the treatment of VD [3]. AS has been shown to exhibit various effects in many diseases such as anti-tumor, wound healing, anti-inflammatory, and antioxidant action [6]. However, the effect of AS on VD development remained unknown. Thus, this study investigated the role of AS in VD development and its underlying mechanism of action.

To investigate the effect of AS on pathogenesis of AD, the cognitive function of VD rats was evaluated after administration of AS. The Morris water maze test has been a widely used behavioral test in neuroscience studies to assess spatial learning and memory in rodents. The results indicate that AS improved cognitive function in rats with VD, which is consistent with previous findings. Zhang *et al* found that AS attenuated learning and memory deficits in rats with β-amyloid-induced Alzheimer's disease [15]. Oxidative stress has been shown to be involved in the pathogenesis of VD [16]. Du *et al* reported that ROS production was increased and SOD activity was decreased in VD rats [16]. Therefore, to better understand the beneficial effects of AS on pathogenesis of VD, the role of AS in oxidative stress in VD rats was explored. Results revealed that AS increased the VD-induced decrease of GSH, SOD, and CAT activities and decreased the VD-induced content of MDA. In other words, AS attenuated oxidative stress in rats with VD. Similarly, the protective effects of AS on alleviating oxidative stress have been reported in previous studies [7]. For example, Luo *et al* showed that AS attenuated spinal cord injury by exerting antioxidant effects [7].

Furthermore, apoptosis has been considered a critical event in VD pathogenesis. Zhang *et al* found that hydrogen sulfide alleviated neuronal injury induced by VD by inhibiting apoptosis in rats [17]. Thus, the effect of AS on brain tissue apoptosis was examined in this study. Our results demonstrate that AS alleviated apoptosis of brain tissue in rats with VD. The anti-apoptotic effects of AS have also been observed in other diseases. For instance, in rats with spinal cord injury, AS inhibited neuronal apoptosis and promoted functional recovery [9]. In β-amyloid-induced Alzheimer's disease, AS ameliorated

learning and memory deficits by suppressing apoptosis [15]. In contrast, AS has been demonstrated to induce apoptosis in tumor cells [6]. This evidence suggests that the response to AS regarding apoptosis may differ in different diseases.

To elucidate the mechanism of AS on pathogenesis of VD, activation of the cAMP-PKA axis was studied. The data show that AS increased the activation of cAMP, PKA, BDNF, and p-CREB. In other words, AS activated the cAMP-PKA axis in VD rats. Han *et al* revealed that the cAMP/PKA/CREB signaling pathway is involved in regulating cognitive dysfunction in VD [11]. Hence, AS might alleviate cognitive dysfunction, oxidative stress, and neuronal apoptosis in VD rats through the cAMP-PKA axis.

CONCLUSION

Asiaticoside attenuates cognitive dysfunction, oxidative stress, and neuronal apoptosis in VD by activating cAMP-PKA axis, suggesting that AS is a potential drug for the management of VD. However, further studies are required to ascertain this.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zhiwei Wang designed the study and supervised data collection. Hao Shen analyzed and interpreted the data. Yishu Hao and Qing Zhu prepared the manuscript for publication. All authors have read and approved the manuscript.

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