Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Protective effect of sakuranetin in brain cells of dementia model rats

Chen Li*, Chunting Hu, Ruili Wang, Hui Wang, Qiaoya Ma, Songsheng Chen, Ya He

Department of Geriatrics Neurology, the Second Affiliated Hospital of Xi'an Jiaotong University, 710004, Xi'an, Shaanxi, China

*Correspondence to: nfhdw8@163.com

Received April 1, 2019; Accepted June 19, 2019; Published June 30, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.5.9

Abstract: Alzheimer's disease (AD) is a high-incidence neurodegenerative disease with complex and diverse pathogenesis. With aging of the population and continuous improvement of living standards, the incidence of AD is on the increase. Therefore, there is need to develop more effective AD drugs in order to improve the quality of life of the elderly. Sakuranetin (SAK) is a dihydroflavonoid compound extracted from plants. It has many physiological properties. In this study, the effect of SAK on spatial discrimination in a rat model of cognitive dysfunction exposed to D-galactose was investigated with respect to its effect on malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels, and on the expressions of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and nuclear factor-κB inhibitory factor-α (IκBα) in hippocampus of rats. The results obtained suggest that SAK may exert protective effects on brain cells through anti-oxidation mechanism. Moreover, the improvement in learning and memory impairment by SAK may also be related to the inhibition of inflammatory mediators in brain tissue. These findings provide scientific evidence that can be exploited for more effective treatment of Alzheimer's disease.

Key words: Sakuranetin; Dementia model; Brain cells; D-galactose.

Introduction

Alzheimer's disease (AD) is a frequently-occurring degenerative disease of the central nervous system in the elderly (1). This disease which often starts insidiously, manifests as typical clinical symptom of progressive deterioration of cognitive dysfunction which can lead to complete loss of social functionality in patients (2). The pathogeny and pathogenesis of AD are very complex, and the existing hypotheses on its pathogenesis have not adequately revealed the etiology of AD and its pathophysiological mechanisms (3-5). The number of dementia patients is on the increase worldwide, and it is expected to reach 1.15 billion by 2050. The problem of aging population is very serious in China, and AD has become a serious social public health problem (1). Therefore, there is a dire need to evolve more effective drugs for treating AD. Traditional Chinese Medicine (TCM) has a wide range of sources and targets. In recent years, many scholars in China and abroad have carried out extensive studies on natural drugs for the treatment of AD, starting from TCM and its effective components (6, 7).

Sakuranetin (SAK, Figure 1) is a dihydroflavonoid present in the bark of *Populus tomentosa*, *Populus aspen* (8), *Betula ermanii* (9), and *Dodonaea viscose* leaves (10). Sakuranetin has anti-inflammatory activity (11). It interferes with calcium metabolism in smooth muscle cells, and relaxes smooth muscles (12). It effectively regulates the expression of p-glycoprotein or MRP1 in mice drug-resistant tumor cells (13). A study has shown that SAK exerts therapeutic effect against asthma (14). Due its high antioxidant activity, it effectively resists the deposition of melanin, thereby reducing skin darkness, and it whitens and tenderizes the skin. These properties make SAK very popular in the cosmetics market. However, its influence on cognitive function has not been studied. In this study, the effect of SAK on spatial discrimination in a rat model of cognitive dysfunction exposed to D-galactose was investigated, as well as its possible mechanism of action.

Materials and Methods

Drugs and main reagents

Sakuranetin was provided by the Institute of Traditional Chinese medicine, Chinese Academy of Traditional Chinese Medicine. Piracetam tablets were purchased from Northeast Pharmaceutical Group Shenyang First Pharmaceutical Co., Ltd. D-Galactose (D-gal) was bought from Beijing Dingguo Changsheng Biotechnology Co., Ltd, while IL-6 kit and TNF-α assay kits were purchased from RD Company (US). Coomassie bright blue kit, and assay kits for superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-PX) were products of Nanjing Institute of Biology. The kit for nuclear factor κB inhibitor α was purchased from Sigma Company (US).

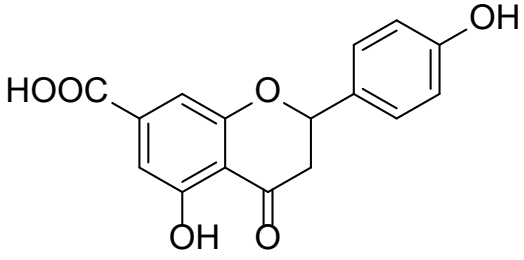

Figure 1. The structure of sakuranetin.

Experimental animals

Healthy Wistar male rats (pure breed) weighing 200 ± 20 g were purchased from Changchun Yisi Experimental Animal Technology Co., Ltd. (animal production license: SCXK (Ji) 2011-0004). Prior to commencement of the experiment, the rats were subjected to one week of adaptive training at a temperature of 20 ℃ and humidity of 70%).The Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University approved this study.

Animal modeling and grouping

After an initial 3-day adaptation to the laboratory environment, the animals were randomly divided into 6 groups: normal control group, model control group, 10 mg/kg piracetam (Pir) positive group, and 3 SAK groups (10, 20 and 40 mg/kg), with 10 rats in each group. In addition to the normal control group, rats in the other five experimental groups were subcutaneously injected with D-galactose at a dose of 50 mg/kg body weight daily, at the back of the neck, and fed with semi-high fat diet for 6 weeks. At the same time, the corresponding drugs were gavaged as suspension once a day. Rats in the model group were daily gavaged an equivalent volume of water in place of drug, while the normal control group received equivalent volume of normal saline through subcutaneous injection at the back of the neck, and were given normal feed. The experiment lasted for six weeks (15).

Behavioral observation

After 6 weeks of SAK treatment, water maze test (15) was performed to determine the spatial learning and memory ability of rats in each group. The wall of the water maze pool was 50 cm high, the water depth was 40 cm, and the water temperature was maintained at 20 to 24 ℃. The diameter of the pool was 150 cm. At the positive center of the target quadrant of the water maze, an underwater hidden platform of diameter 10cm was placed, and the surface of the platform was located 1 cm beneath the water. During the first 5 days, positioning navigation test was carried out, 4 times a day, and rats were put in from different water entry points each time. Each rat was placed in the pool facing the wall of the pool, and the escape latency, that is, the time taken for rats to search and locate the platform, was recorded. If any rat did not reach the underwater platform within 2 min, it was carefully directed to the platform and allowed to stay for 20 sec, and 120 sec was recorded as the escape latency. On the 6th day, the underwater platform was removed and the rats were placed facing the pool wall into the pool. The rats were evaluated with respect to the time taken to reach the first passage, and the number of times of crossing the original platform position within 3 min.

Tissue sample processing

After the behavioral test, the rats were fasted, and after 12 h, intraperitoneal anesthesia (10 % chloral hydrate at a dose of 0.3 mL/100 g) was administered. The rats were then sacrificed by decapitation, and the hippocampal tissues were quickly separated on the operating table. One gram (1 g) of hippocampal tissue sample was taken, and normal saline pre-cooled at 4 ℃ was added. It was then centrifuged 4 times at 13500 rpm, with low temperature homogenization time of 10 sec, at 30 sec intervals. A 10 % tissue homogenate was prepared and centrifuged at low temperature and low speed (4 ℃, 3000 rpm) for 15 min. The supernatant was collected and kept at -40 ℃ prior to use in determination of levels of expressions of inflammatory factors, and oxidative stress markers.

Assay of biochemical indices in brain tissue

The contents of TNF-α and IL-6 were determined using enzyme linked immunosorbent assay (ELISA) in line with instructions on the corresponding kit manuals. The contents of SOD, MDA and GSH-PX were determined using immuno-turbidimetry. Protein quantification was done with the Coomassie brilliant blue method in accordance with the kit protocol.

Determination of IκBα protein in the hippocampus

After the rat hippocampus was isolated on ice, the total protein was extracted and the protein concentration was determined with Coomassie brilliant blue method. Equal amount of protein in each sample was separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride (PVDF) membrane. Following sealing with 5 % BSA for 1 h at room temperature, IκBα-specific primary antibody was added and incubation was carried out at 4 ℃ overnight on a shaking table. Thereafter, the membrane was rinsed 3 times with TBST (pH 7.6), each time for 5 min, followed by the addition of secondary antibody labeled with horseradish peroxidase (HRP), and incubation at room temperature for 2 h. Grayscale analysis was carried out with Image Lab version 3.0 software. The expression level was reflected as gray value ratio of the target protein to β-actin. Each assay was repeated three times.

Statistical methods

Measurement data are expressed as mean ± standard deviation (x(—);x±s). One-way analysis of variance (ANOVA) was used for comparison between groups. Student-Newman-Keuls (SNK) was performed for post-HOC comparison. All statistical analyses were done with GraphpadPrism7.0 statistical software. Statistical significance was assumed at $p < 0.05$.

Results

Effect of SAK on the memory function and spatial learning in the model rats

In order to investigate the effect of SAK on spatial learning and memory function of model rats, Morris water maze test was used. Compared with the control group, the escape latency of rats in the model group was significantly prolonged; the number of platform crossings was markedly decreased, and the avoidance latency of the rats in the treatment group was significantly shortened (Figure 2A). However, SAK brought about significant and dose-dependent reductions in escape latency, and marked increases in the number of platform crossings, when compared to the model group ($p < 0.05$; Figure 2B).

Effect of SAK on the contents of TNF-α and IL-6 in hippocampus of model rats

In order to investigate the effect of SAK on the contents of TNF-α and IL-6 in hippocampus of the model rats, the production of TNF-α and IL-6 in the supernatant of hippocampus was determined with ELISA. As shown in Figure 3, compared with the model group, SAK significantly decreased the production of TNF-α and IL-6 in a dose-dependent fashion ($p < 0.05$).

Effect of SAK on the hippocampal expression of IκBα in rats

Degradation of IκBα protein increases the transcription activity of NF-κB, thereby increasing the release of inflammatory factors. As shown in Figure 4, western blot results indicated that the expression of IκBα was down-regulated in the model group, and the expression levels of IκBα protein in the hippocampus after SAK treatment at the three doses were significantly higher than that in the model control group ($p < 0.01$).

Effect of SAK on SOD, GSH-PX and MDA in the hippocampus of the model rats

Immuno-turbidimetry was used to determine the effect of SAK on the activities of SOD and GPx, and the content of MDA in hippocampus of model rats. The results showed that SAK increased the activities of SOD and GPx, and decrease MDA content in hippocampus, both dose-dependently ($p < 0.01$; Figure 5). These results suggest that SAK may exert protective effect on brain cells through antioxidation mechanism.

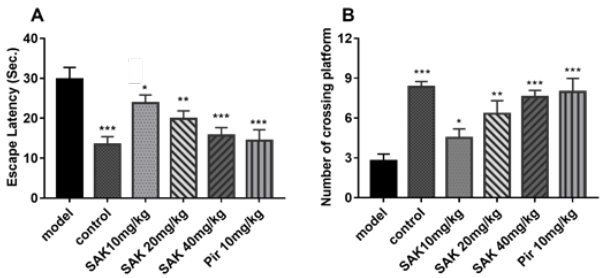

Figure 2. Effect of SAK on cognitive function in rats. (A) Escape latency of the six groups of rats; (B) number of platform crossings of the six groups of rats (n = 10). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with model rats.

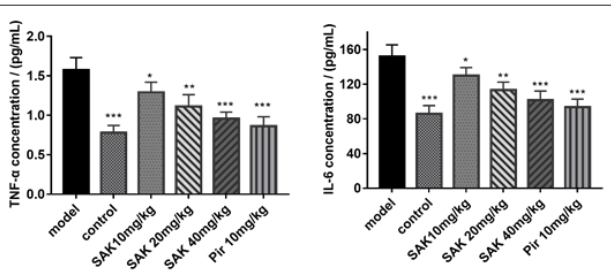

Figure 3. Effect of SAK on the expressions of TNF-α and IL-6 in hippocampus of rats (n=10). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with model rats.

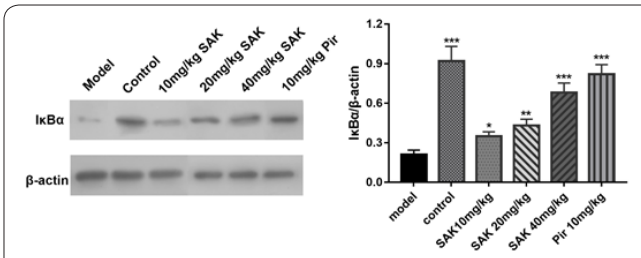

Figure 4. Effect of SAK on the protein expression of hippocampal IκBα in the different groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with model rats (n = 10).

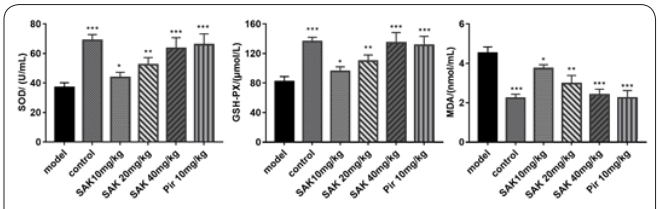

Figure 5. Effect of SAK on activities of SOD and GPx, and MDA content in rat hippocampus. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with model rats (n = 10).

Discussion

Alzheimer's disease (AD) is a frequently-occurring neurodegenerative disease in the elderly. The main clinical manifestations are decreases in motor ability, thinking ability, sensory ability, memory, and judgment ability. The pathogenesis of Alzheimer's disease is complicated and diverse. With the aging of populations and continuous improvement of living standards, the incidence of AD is on the increase. In terms of fatality, the disease has become fourth in ranking after tumor, cardiovascular disease and stroke. More worrying is the fact that there is still no specific method for the clinical treatment of AD.

Many plant extracts have obvious therapeutic properties due to their phytochemical compositions. The biological activities of these compounds against neurodegeneration is mainly due to their anti-oxidant and anti-amyloid deposition effects (17, 18). Sakuranetin (SAK) is found mainly in *Populus tomentosa*, *Populus aspen* and some other plants. It is a dihydroflavonoid with anti-inflammatory and antioxidant properties. In this study, a rat model of cognitive dysfunction exposed to D-galactose was used to investigate the protective effect of SAK on brain cells. Continuous injection of D-galactose led to production of large amounts of free radicals in the rats. This caused lipid peroxidation and interfered with cell function, resulting in a significant decline in learning and memory abilities (19). The Morris water maze test was used to assess learning and memory ability in the experimental rats. The results showed that the space exploration latency period of the model group was significantly prolonged at 6 weeks after hypodermic injection of 50 mg/kg D-galactose. The learning ability of D-galactose-dementia rats was also significantly reduced, which indicated that the model was successfully established. The results also showed that SAK improved the learning and memory abilities of model rats with D-galactose-induced cognitive dysfunction, shortened the latency period for spatial exploration, and increased the number of platform crossings in mice with D-galactose dementia (9).

The hippocampus is involved in the important functions of brain information storage and memory, and plays an irreplaceable role in learning, memory and emotional stress (20). It has been found that TNF-α and IL-6 participate in neuroinflammatory reactions in hippocampus in neurodegenerative diseases, and that co-

gnitive function can be improved by blocking the release of TNF-α and IL-6 (21). Sakuranetin (SAK) inhibits the expression of cytokines, chemokines and adhesion molecules induced by inflammatory stimulation, and its mechanism is related to the inhibition of NF-κB activity. The degradation of IκBα is the central link to NF-κB release (22). In this study, SAK inhibited the increase of TNF-α and IL-6, the mechanism of which may be linked to inhibition of the degradation of IκBα protein. By attenuating the degradation of IκBα, the transcriptional activity of NF-κB was suppressed, and the release of inflammatory factors (TNF-α and IL-6) was inhibited, leading to alleviation of neuro-inflammatory reactions and improvement in cognitive function.

Superoxide dismutase (SODE), one of the important antioxidant enzymes, eliminates superoxide anion free radicals and reduces the production of lipid peroxides(23). Malondialdehyde (MDA) is a lipid peroxide which reflects the degree of lipid peroxidation. Glutathione peroxidase (GPx) blocks free radical secondary reactions induced by lipid peroxides, and protects the cell membrane from peroxidative damage (24). The results obtained in this study showed that after subcutaneous injection of D-galactose for 6 weeks, the activity of SOD in blood decreased, the content of MDA increased, and the activity of GPx decreased significantly. After 6 weeks of subcutaneous injection of D-galactose, superoxide anion free radicals increased, lipid peroxidation was exacerbated, and there was impairment of learning and memory ability, with evidence of dementia in the rats. However, SAK significantly increased the activity of SOD, decreased the level of MDA, and increased the activity of GSH-Px. These results suggest that the effect of SAK on the prevention and treatment of dementia induced by D-galactose is related to the increases in plasma activities of SOD and GPx, and the decrease in MDA level, the elimination of oxygen free radicals and the reduction in lipid peroxidation. However, the specific mechanisms involved in these effects of SAK need to be further studied.

In conclusion, SAK increased the activities of SOD and GPx, and decreased the content of MDA in hippocampus of the model rat brain tissue, suggesting that it may have protective effect on brain cells through antioxidation mechanism. Moreover, SAK inhibited the degradation of IκBα protein and decreased the contents of TNF-α and IL-6 in cerebral hippocampus tissue, suggesting that the improvement in learning and memory disorder by SAK may be related to the inhibition of inflammatory mediators in brain tissue. This study has established that SAK exerts protective and therapeutic effects against Alzheimer's disease. These findings provide more scientific information for the effective treatment of Alzheimer's disease.

Acknowledgements

This study was supported by the Social Development Key Science and Technology Program of Shaanxi Province (2016SF-164, 2017SF-019, 2018SF-143), Xi'an Science and Technology Project (201805099YX7SF33(2)), the Health Research Fund Project of Shaanxi Province (2018D060).

Conflict of Interest

There are no conflicts of interest in this study.

Author's contribution

All work was done by the authors named in this article and the authors accept all liability resulting from claims which relate to this article and its content. The study was conceived and designed by Chen Li; Chen Li, Chunting Hu, Ruili Wang, Hui Wang, Qiaoya Ma, Songsheng Chen and Ya He collected and analysed the data; Chen Li wrote the text and all authors have read and approved the text prior to publication.

References

1. Hu X, Wang T, Jin F. Alzheimer's disease and gut microbiota. Sci Sin (Vitae) 2016; 59: 1006-1023.
2. Reitz C, Mayeux R. Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers. Biochem Pharmacol 2014; 88: 640-651.
3. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 2016; 8: 595-608.
4. del Ser T, Steinwachs KC, Gertz HJ, Andres MV, Gomez-Carrillo B, Medina M, et al. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. J Alzheimers Dis 2013; 33: 205-215.
5. Swerdlow RH, Burns JM, Khan SM. The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives. Biochim Biophys Acta 2014; 1842: 1219-1231.
6. Zhang L, Fang Y, Xu Y, Lian Y, Xie N, Wu T, et al. Curcumin improves amyloid β-peptide (1-42) induced spatial memory deficits through BDNF-ERK signaling pathway. PLoS One 2015; 10: 0131525.
7. Zhan PY, Peng CX, Zhang LH. Behavior: Berberine rescues D-galactose-induced synaptic/memory impairment by regulating the levels of Arc. Pharmacol Biochem Behav 2014; 117: 47-51.
8. Kodama O, Miyakawa J, Akatsuka T, Kiyosawa S. Sakuranetin, a flavanone phytoalexin from ultraviolet-irradiated rice leaves. Phytochem 1992; 31: 3807-3809.
9. Wollenweber E, Egger K. Flavonoid-Aglykone im Knospen-Exkret von Betula ermani. Z Pflanzenphysiol 1971; 12: 1.
10. Mata R, Contreras JL, Crisanto D, Pereda-Miranda R, Castañeda P, Del Rio F. Chemical studies on Mexican plants used in traditional medicine, XVIII. New secondary metabolites from Dodonaea viscosa. J Nat Prod 1991; 54: 913-917.
11. Sakoda CPP, de Toledo AC, Perini A, Pinheiro NM, Hiyane MI, dos Santos Grecco S, et al. Sakuranetin reverses vascular peribronchial and lung parenchyma remodeling in a murine model of chronic allergic pulmonary inflammation. Acta Histochem 2016; 118: 615-624.
12. Rojas A, Cruz S, Ponce-Monter H, Mata R. Smooth muscle relaxing compounds from Dodonaea viscosa5. Planta Med 1996; 62: 154-159.
13. Molnár J, Engi H, Hohmann J, Molnár P, Deli J, Wesolowska O, et al. Reversal of multidrug resistance by natural substances from plants. Curr Top Med Chem 2010; 10: 1757-1768.
14. Toledo A, Sakoda C, Perini A, Pinheiro N, Magalhaes R, Grecco S, et al. Flavonone treatment reverses airway inflammation and remodelling in an asthma murine model. Br J Pharmacol 2013; 168: 1736-1749.
15. Bae ON, Serfozo K, Baek SH, Lee KY, Dorrance A, Rumbeiha W, et al. Safety and efficacy evaluation of carnosine, an endogenous neuroprotective agent for ischemic stroke. Stroke 2013; 44: 205-212.
16. Andrieu S, Coley N, Lovestone S, Aisen PS, Vellas B. Preven-

tion of sporadic Alzheimer's disease: lessons learned from clinical trials and future directions. Lancet Neurol 2015; 14: 926-944.
17. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxid Med Cell Longev 2015; 2015: 610813.
18. You SP, Zhao J, Ma L, Tudimat M, Zhang SL, Liu T. Preventive effects of phenylethanol glycosides from Cistanche tubulosa on bovine serum albumin-induced hepatic fibrosis in rats. Daru 2015; 23: 52.
19. Murrow JR, Sher S, Ali S, Uphoff I, Patel R, Porkert M, et al. The differential effect of statins on oxidative stress and endothelial function: atorvastatin versus pravastatin. J Clin Lipidol 2012; 6: 42-49.
20. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 2015; 85: 296-302.
21. Su D, Zhao Y, Wang B, Xu H, Li W, Chen J, et al. Isoflurane-induced spatial memory impairment in mice is prevented by the acetylcholinesterase inhibitor donepezil. PLoS One 2011; 6: 27632.
22. Wu J, Wang A, Min Z, Xiong Y, Yan Q, Zhang J, et al. Lipoxin A4 inhibits the production of proinflammatory cytokines induced by β-amyloid in vitro and in vivo. Biochem Biophys Res Commun 2011; 408: 382-387.
23. Roberts BR, Ryan TM, Bush AI, Masters CL, Duce J. The role of metallobiology and amyloid-β peptides in Alzheimer's disease. J Neurochem 2012; 120: 149-166.
24. Olanow C. A radical hypothesis for neurodegeneration. Trends Neurosci 1993; 16: 439-444.