Original Paper

Sulforaphane Prevents Neuronal Apoptosis and Memory Impairment in Diabetic Rats

Gengyin Wang^a Hui Fang^{a,b} Yanfeng Zhen^b Gang Xu^c Jinli Tian^b Yazhong Zhang^b Dandan Zhang^b Guyue Zhang^b Jing Xu^b Zhiyue Zhang^d Mingyue Qiu^a Yijia Ma^d Hongrui Zhang^e Xinxin Zhang^a

^aDepartment of Internal Medicine, Hebei Medical University, Shijiazhuang, ^bSecond Department of Endocrinology, Tangshan Gongren Hospital, Tangshan, ^cDepartment of Burns and Orthopedics, Tangshan Gongren Hospital, Tangshan, ^dGraduate School, North China University of Science and Technology, Tangshan, ^eCenter of Health Examination, Tangshan People's Hospital, Tangshan, China

Key Words

Sulforaphane • Diabetes • Memory impairment • Apoptosis

Abstract

Background/Aims: To explore the effects of sulforaphane (SFN) on neuronal apoptosis in hippocampus and memory impairment in diabetic rats. **Methods:** Thirty male rats were randomly divided into normal control, diabetic model and SFN treatment groups (N = 10 in each group). Streptozotocin (STZ) was applied to establish diabetic model. Water Morris maze task was applied to test learning and memory. Tunel assaying was used to detect apoptosis in hippocampus. The expressions of Caspase-3 and myeloid cell leukemia 1(MCL-1) were detected by western blotting. Neurotrophic factor levels and AKT/GSK3 β pathway were also detected. **Results:** Compared with normal control, learning and memory were apparently impaired, with up-regulation of Caspase-3 and down-regulation of MCL-1 in diabetic rats. Apoptotic neurons were also found in CA1 region after diabetic modeling. By contrast, SFN treatment prevented the memory impairment, decreased the apoptosis of hippocampal neurons. SFN also attenuated the abnormal expression of Caspase-3 and MCL-1 in diabetic model. Mechanically, SFN treatment reversed diabetic modeling-induced decrease of p-Akt, p-GSK3 β , NGF and BDNF expressions. **Conclusion:** SFN could prevent the memory impairment and apoptosis of hippocampal neurons in diabetic rat. The possible mechanism was related to the regulation of neurotrophic factors and Akt/GSK3 β pathway.

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Introduction

Diabetes mellitus (DM) was a metabolism disease characterized by insulin resistance and related decrease of insulin release [1]. Many complications were found in diabetic patients, including brain abnormality. Clinically, the ability of learning and memory, the

Hui Fang

Department of Internal Medicine, Hebei Medical University, Shijiazhuang, Hebei 050017, (China); Tel. +86-13831581838, E-Mail fanghui@medmail.com.cn

space navigation obviously decreased in diabetic patients [2, 3]. Dementia was also found in some severe diabetic cases. Although there was no consistent definition of diabetic and brain disease, electrophysiological and image abnormalities could be used to confirm the chronic mild cognition impairment in diabetic patients [4]. Epidemiologically, the diabetic patients with mild cognition impairment have a high rate to progress into dementia [5]. Nevertheless, the exact pathological mechanisms for cognitive impairment in diabetic patients were still elusive.

Accumulating evidence suggested that neurological diseases involved in diabetes were related to apoptosis of neurons [6]. Loss of neurons contributes to neuronal and circuit dysfunction. Possible mechanisms were related to metabolism dysfunction, vascular factors, and deficient of nerve growth factors. In addition, the blockage of cell survival signaling pathways was also related to neurological diseases regulated by diabetes [7-9].

Sulforaphane (SFN) is a compound within the isothiocyanate group of organosulfur compounds, which widely distributed in cruciferous vegetables such as broccoli, Brussels sprouts or cabbages. SFN has a mount of pharmacological activities, including antioxidative [10], anticancer [11], antibacterial [12] and anti-inflammation [13], etc. Recent studies also suggested that SFN could be a potential compound to enhance memory in disease models [14]. However, their mechanisms were not disclosed. In this study, we aimed to explore whether SFN could prevent memory deficit in diabetic model and its underlying mechanisms.

Materials and Methods

Experimental animals

30 sprague-Dawley (SD) rats (male, 12 weeks) in clean grade were obtained from Animal Center of Hebei Medical University (1511040) and raised in the temperature of $25 \pm 2^\circ\text{C}$ with free access to food and water. All the experiments were performed with the approval of Ethics Committee of Hebei Medical University. After one week adaption, 30 rats were randomly divided into three groups: normal control, diabetes mellitus group and SFN treatment groups. 50 mg/kg streptozotocin (STZ, Sigma) was administrated through intraperitoneal injection to induce diabetic model. Diabetic models were regarded as successful if blood glucose levels were higher than 16.7 mmol/L. 11 weeks after successfully modeling, the rats in SFN group were treated with SFN (25 mg/kg) orally once daily for consecutive 14 days. After treatment, behavioral tests and biochemical experiments were carried out.

Water Morris maze

Animals were tested by water Morris maze 2 weeks after SFN treatments. At the beginning of place navigation task, rats were placed in a circle pool, allowing them to swim for 5 min. Tests were carried out at a fixed time every day for consecutive 4 days. Video camera tracking system recorded the time it took for rats to find platforms (escape latency) and their swimming routes. During the 4 training sections, rats were placed into the pool at 4 different starting points (different quadrants). If rats found platforms or found different platforms within 120 sec during Morris water maze test (latency is recorded as 120 sec), they were taken onto platforms, allowing 15 sec's rest before next training section. The average of latency during 4 training sections every day was recorded as the study result of that day. At day 5 of spatial probe, platform was removed, and rats were placed into water at any starting point. All rats were placed at the same starting point, and the time of rats staying in the quadrant where platform was placed was recorded.

Western blot

After the behavioral tests, the rats were decapitated and brains were obtained. The brain tissue lysates were separated by SDS-PAGE, transferred to PVDF, and immunoblotted using specific antibodies against Caspase-3 (1:1000; Abcam), myeloid cell leukemia 1 (MCL-1) (1:1000, Abcam), NGF (1:1000; Abcam), BDNF (1:1000, Abcam), p-Akt (1:1000, Abcam), Akt (1:1000, Abcam), GSK3 β (1:1000, Abcam) and p-GSK3 β (1:1000, Abcam). The bound antibodies were detected using horseradish peroxidase-conjugated secondary antibodies and an enhanced chemiluminescence detection system.

Tunel Assay

The apoptosis of hippocampal neurons were detected by Tunel assay. The brain tissue was fixed in 4% PFA and embedded into paraffin wax. In Situ Cell Death Detection Kit (Roche, Switzerland) was applied to detect the apoptosis according to the manufacturer's instructions. Apoptotic changes were measured via fluorescence microscopy (Olympus, Tokyo, Japan). The images were taken using a light microscope (Olympus, Japan). We referred to the previous publication to analyze the apoptotic rate [15]. The positive rate of 0-1% was defined as score 0, 1-10% as score 1, 10-50% as 2, 50-80% as 3 and 80-100% as 4. The expressions were divided into negative (score 0), weak positive (score 1), positive (score 2) and strong positive expressions (score 3), respectively.

Statistical Analysis

All data were presented as mean $\pm$ SED. Differences among three or more groups were compared by one way analysis of variance (ANOVA), followed by Bonferroni post hoc testing for multiple comparisons. *p* values of 0.05 or less were regarded as significant.

Results

SFN prevented the impairments of spatial learning and memory in diabetic model

We next tested the learning and memory in different groups. As shown in Fig. 1A, the latency to find the platform was significantly higher in model group than that in normal control group. By contrast, SFN treatment significantly decreased the latency to find the platform compared with diabetic model group. After four-day training, the platform was removed. Under this condition, the time spent in target quadrant was significantly lower in model group than that in control group (Fig. 1B). By contrast, the time in SFN treatment group was significantly increased compared with model group.

SFN prevented apoptosis of hippocampal neurons in CA1 region

As shown in Fig. 2, apoptotic neurons were significantly increased in the CA1 region in model group. However, SFN could significantly decrease the numbers of apoptotic neurons in CA1 region.

SFN prevented the abnormalities of Caspase-3 and MCL-1 expressions in diabetic model

We also detected caspase-3 and MCL-1 expressions (Fig. 3). Compared with normal control group, hippocampal Caspase-3 was increased in model group, while it was down-regulated by SFN treatment. MCL-1 expression was increased in model group, however decreased by SFN treatment.

Fig. 1. SFN enhanced learning and memory in diabetic rat model. (A) The latency to find the platform during the 4-day training. (B) The time spent in target quadrant after removal of the platform. * *p* < 0.05 compared with control group. # *p* < 0.05 compared with model group.

Fig. 2. SFN mitigated the neuronal apoptosis in diabetic rats. (A) A few apoptotic neurons were found in control group. (B) Significant increase of apoptotic neurons were found in diabetic model. (C) The apoptotic neurons were eliminated in SFN treatment group. (D) Quantification data of the apoptotic cells. * $p < 0.05$ compared with control group. # $p < 0.05$ compared with model group.

Fig. 3. SFN decreased Caspase-3 and increased MCL-1 expressions in diabetic rats. (A) Representative blots of Caspase-3. (B) Quantified data of Caspase-3. (C) Representative blots of MCL-1. (D) Quantified data of MCL-1. * $p < 0.05$ compared with control group. # $p < 0.05$ compared with model group.

Fig. 4. SFN reversed neurotrophic factors level induced by diabetic modeling. (A) NGF expression; (B) BDNF expression. * $p < 0.05$ compared with control group. # $p < 0.05$ compared with model group.

Fig. 5. SFN reversed p-Akt and p-GSK3β expression induced by diabetic modeling. (A) NGF expression; (B) BDNF expression. * $p < 0.05$ compared with control group. # $p < 0.05$ compared with model group.

SFN reversed the decrease of neurotrophic factors

The neurotrophic factors, NGF and BDNF were also detected in different groups. Diabetic modeling decreased both NGF and BDNF expressions. By contrast, SFN treatment could reverse the decrease (Fig. 4).

SFN reversed the deficit of Akt/GSK3β pathway

Akt/GSK3β pathway was supposed as a cell survival pathway. Therefore, we also determined the Akt/GSK3β pathway. As shown in Fig. 5, Diabetic modeling decreased both p-Akt and p-GSK3β expressions. SFN treatment reversed the decrease of p-Akt and p-GSK3β expressions. By contrast, diabetic modeling or SFN treatment did not affect total Akt and GSK3β expressions.

Discussion

Previous study reported that dietary intake of sulforaphane-rich broccoli sprout extracts during juvenile and adolescence prevented phencyclidine-induced cognitive deficits [16]. That indicated the pharmacological activity of SFN in recovery of memory impairment. SFN is a natural dietary isothiocyanate and was reported to ameliorate memory impairment caused by okadaic acid [17] and scopolamine [18]. SFN also improved cognitive function in traumatic brain injury [19]. In addition, SFN was also reported to ameliorate memory impairment in AD model. In this study, we demonstrated the pharmacological activity of SFN in preventing the memory impairment in diabetic model. This study further evidenced the memory ameliorating effect of SFN in different disease models.

Hippocampus was thought to be an important brain region responsible for memory formation and consolidation [20]. The loss of pyramid neurons in neurological diseases caused deficit of neuronal circuit and impairment of hippocampal synaptic plasticity. As evidenced by previous studies, hippocampal neurons were severely damaged in STZ-induced diabetic models [21]. Moreover, apoptosis was apparently elicited after STZ injection [7]. How STZ caused hippocampal neuronal death was not clear. Intriguingly, there were a lot of

reports which might represent the mechanisms. On the one hand, neurotrophic factors were decreased after STZ injection. The decrease of neurotrophic factors have been confirmed to regulate the neuronal survival and might partially explain the pyramid neuronal loss [22]. On the other hand, cell survival signaling pathways were also found to be decreased after STZ injection. Typically, Akt and GSK-3 were dephosphorylated in diabetic model [7-9]. The abnormality of those signaling pathways might be potential reasons for the loss of neurons.

Apoptosis is an important type of important programmed cell death, which has been specifically clarified previously. A lot of signaling pathways or organelle damage will elicit apoptosis. Undoubtedly, apoptosis is an important way for pyramid neuron loss. BCL family and caspases are prominent for the apoptosis. MCL-1 is one gene in BCL family, which inhibits caspase-3 and prohibits apoptosis [23]. In our study, we verified that hippocampal MCL-1 was down-regulated, while Caspase-3 was increased after diabetic modeling. However, SFN could antagonize the changes of MCL-1 and caspase-3. These data further support that SFN ameliorates the memory impairment in diabetic model through inhibiting apoptosis of hippocampal neurons.

SFN is a kind of isocyanate, mainly from green Cruciferous vegetables like broccoli. SFN possesses a serial of pharmacological activities, such as antioxidant and antitumor. As a chemical preventive medicine, SFN has attracted extensive attention. The compound is known to be an important activator of Nrf2. SFN can induce Nrf2 activation and nucleus translocation, and combines with ARE to regulate the expression of phase II detoxification enzyme to clear ROS. Oxidative stress was thought to be one pathological factor for cell death. The antioxidants, such as grape seed extract and vitamin E were also reported to mitigate hippocampal cell loss in diabetic model [24]. In addition, SFN also possibly ameliorated the memory impairment in diabetic model through anti-inflammation [25]. Recent studies have demonstrated that Nrf2-ARE signaling is also involved in attenuating inflammation-associated pathogenesis [26]. However, these potential reasons still require further verifications.

Neurotrophic factors are important regulators, which not only modulate synaptic transmission or neuronal cell death [27, 28]. In this study, we found that after diabetic modeling, both of NGF and BDNF were down-regulated, while SFN treatment reversed the down-regulation. PI3K/Akt signaling pathway is a cell survival pathway [28]. In our study, we also found that diabetic modeling decreased the phosphorylation of Akt and GSK3 β , which were reversed by SFN treatment.

Conclusion

In this study, we reported that SFN could ameliorate the memory impairment in STZ-induced diabetic model. The potential mechanism underlying the protection was through inhibiting caspase-3 and MCL-1 dependent apoptosis of hippocampal neurons.

Disclosure Statement

None.

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