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Naringin ameliorates cognitive deficits in streptozotocin-induced diabetic rats

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ABSTRACT

Objective(s): Previous research demonstrated that diabetes is one of the leading causes of learning and memory deficits. Naringin, a bioflavonoid isolated from grapefruits and oranges, has potent protective effects on streptozotocin (STZ)-induced diabetic rats. Recently, the effects of naringin on learning and memory performances were monitored in many animal models of cognitive impairment. However, to date, no studies have investigated the ameliorative effects of naringin on diabetes-associated cognitive decline (DACD). In this study, we investigated the effects of naringin, using a STZ-injected rat model and explored its potential mechanism.

Materials and Methods: Diabetic rats were treated with naringin (100 mg/kg/d) for 7 days. The learning and memory function were assessed by Morris water maze test. The oxidative stress indicators [superoxide dismutase (SOD) and malondialdehyde (MDA)] and inflammatory cytokines (TNF-a, IL-1β, and IL-6) were measured in hippocampus using corresponding commercial kits. The mRNA and protein levels of PPARγ were evaluated by real time (RT)-PCR and Western blot analysis.

Results: The results showed that supplementation of naringin improved learning and memory performances compared with the STZ group. Moreover, naringin supplement dramatically increased SOD levels, reduced MDA levels, and alleviated TNF-α, IL-1β, and IL-6 compared with the STZ group in the hippocampus. The pretreatment with naringin also significantly increased PPARγ expression.

Conclusion: Our results showed that naringin may be a promising therapeutic agent for improving cognitive decline in DACD.

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Introduction

Diabetes mellitus results from defects in insulin secretion and/or insulin function and causes glucose metabolic disorders with some secondary complications affecting eyes, kidneys, heart, and brain (1). Patients and animals' brains with diabetes mellitus show widespread angiopathy and degenerative abnormalities. It was previously reported that diabetes mellitus have adverse effects on nervous systems, resulting in cognitive impairment (2). In 2006, a new concept "diabetes-associated cognitive decline (DACD)" has been proposed on the importance of strengthening the learning and memory deficits in both clinical and experimental settings (3).

The pathogenesis of cognitive dysfunction seems to be triggered by many factors in diabetic patients (4). Hyperglycemia leads to oxidative stress and inflammatory response, which are the risk factors of Alzheimer's disease (AD) in diabetes mellitus (5-7). The oxidative stress increased in diabetes mellitus, causes morphological and functional alterations in the hippocampus, due to the excessive production of malanoldehyde (MDA) and decreased efficiency of superoxide dismutase (SOD). Inflammation related to DACD, manifested as dysregulated production of TNF-a, IL-1β, and IL-6 plays a crucial role in insulin resistance (8-9). Peroxisome proliferator-activated receptor (PPAR) γ, a ligand-dependent transcription factor, is involved in type 2 insulin-resistant diabetes. Activation of the PPARγ, with anti-inflammatory and antioxidant effects, can increase insulin sensitization and modulate insulin-mediated glucose (10-12).

Naringin, a naturally flavanone glycoside in grapefruits and oranges, has been widely used in traditional medicine. It has been reported to possess antiapoptotic, antiosteoporosis, antiulcer, antioxidant, antiinflammatory, and anticarcinogenic properties (13-14). Emerging data indicate that naringin is involved in amelioration of hyperglycemia. Naringin possesses lipid-lowering and insulin-like properties to decrease insulin resistance,

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Table 1. Primer sequences used in RT-PCR for GAPDH and PPARγ

Gene	Primer sequences	Produce size	TM(℃)	Cycles
GAPDH	Forward：5' AAGTTCAACGGCACAGTCAA 3'	466 bp	58	32
	Reverse：5' ATGCAGGGATGATGTTCTGG 3'			
PPARγ	Forward：5' CCAAAGTGCGATCAAAGTAG 3'	411 bp	60	32
	Reverse：5' AGATCAGCAGACTCTGGGTT 3'			

hyperglycemia, and dyslipidaemia. Recent findings in epidemiological and dietary interventional studies suggest the possible role of naringin in the treatment of learning and memory (15). Naringin administration prevents and delays neurodegeneration in aluminum chloride-induced cognitive deficits (16). Moreover, naringin reverses a glucose uptake defect and improves cognitive function in the APPswe/PSΔE9 transgenic mouse model of Alzheimer's disease (17). More importantly, neuroprotective effect of naringin is a result of its antioxidant and anti-inflammatory activity to protect against kainic acid-induced status epilepticus in rats (18). However, there is little information on the effect of naringin on DACD. Hence, the purpose of this study was to assess the effect of naringin treatment on cognitive, in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Animals

Male Wistar rats (210–230 g) were purchased from Hunan SJA Laboratory Animal Co, Ltd (Changsha, China). They were kept under specific pathogen free (SPF) environment with free access to water and rodent chow. The experiments were executed in accordance with the Experimental Animal Care and Use Committee of Hunan University of Chinese Medicine (Changsha, China).

Chemicals and drugs

STZ and naringin were purchased from Sigma Chemicals. Level of specific markers for oxidative stress including MDA and SOD in hippocampus tissues was analyzed by commercial kits (Cat. No: A003-1 for MDA, A001-1 for SOD, Nanjing Jiancheng Bioengineering Institute, China). The activities of TNF-α, IL-1β, and IL-6 were evaluated by the commercial immunoassay kits (Cat. No: RTA00 for TNF-α, SRLB00 for IL-1β, and R6000B for IL-6, R&D Systems, USA).

Induction and assessment of diabetes

Diabetic model was induced by IP injection of a single dose of 65 mg/kg STZ. Seven days after STZ injection, fasting blood glucose level was determined. The control animals were injected with citrate buffer. Animals were considered diabetic if plasma glucose level exceeded 7.8 mmol/L (19). Animals in each experiment were randomly assigned to four groups: (1) Control group (Con) (n= 20); normal rats treated with saline, IP (physiological saline 0.1 ml/100 g), (2) Vehicle group (DM) (n=20); diabetic rats treated with saline, IP (physiological saline 0.1 ml/100 g), (3) DM+Naringin group (n=20); diabetic rats treated with naringin (100 mg/kg/d), for 7 days.

Morris water maze test

After naringin treatment for 7 days, the Morris water maze test was conducted to assess the learning and memory performance. The escape latency and path length were evaluated in each trial and averaged over three trials for each rat. The frequency the rat reached the platform, as well as the time spent in the target quadrant were detected within 60 sec (20).

RNA extraction and real time-PCR analysis

The total RNA was extracted from hippocampus samples using the trizol method, as directed by the manufacturer's protocol. Real time (RT)-PCR was used to determine the levels of PPARγ expressions. Primer sequences were synthesized by Shanghai Sangon Biotech Engineering Co, Ltd. (Shanghai, China) and summarized in Table 1. The RT-PCR consisted of 32 cycles (at 95 °C for 30 sec, 58/60 °C for 40 sec, and 72 °C for 8 min) after an initial denaturing step (at 95 °C for 5 min). The software used for bands analysis was designed by Shanghai Tanon Technology Co, Ltd. (Shanghai, China). The results were represented as a ratio by normalizing to GAPDH mRNA levels.

Western blot analysis

The soluble proteins were separated on a 12% acrylamide gel and transferred onto a nitrocellulose membrane. Samples were incubated with primary antibody specific for PPARγ (Proteintech, 16643-1-AP, 1:1000, at 4 ℃, overnight). Primary antibodies were visualized with anti-rabbit HRP-conjugated secondary antibody using a chemiluminescent detection system (CoWin Biotech, Beijing, China). Variations in sample loading were corrected by normalizing to β-actin levels.

Statistics

All results were represented as mean±SD. All statistical analyses were analyzed by SPSS 16.0 software. The data of trials in MWM were analyzed by repeated measurement ANOVA, while the others were evaluated using one-way ANOVA. $P < 0.05$ was deemed statistically significant.

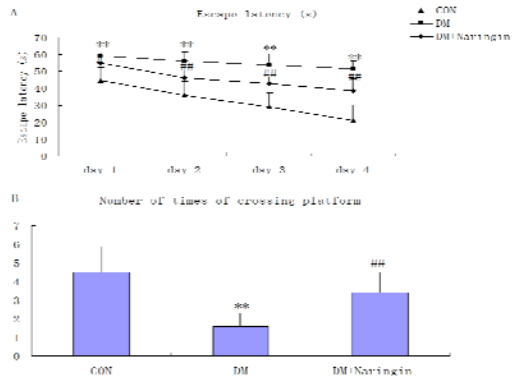

Figure 1. Effects of naringin on the escape latency (**A**) and the number of times of crossing the platform (**B**), in control and diabetic rats. $^{**}P<0.01$ compared with the Con group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with the DM group. Con, control; DM, diabetic rats; DM + naringin, diabetic rats treated with naringin (100 mg/kg/d)

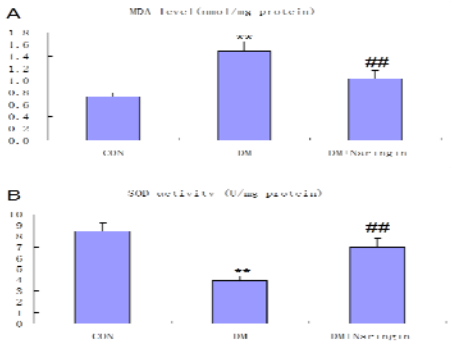

Figure 2. Effects of naringin on concentration of MDA and the activities of SOD in hippocampus of control and diabetic rats. $^{**}P < 0.01$ compared with the Con group; $^{\#\#}P<0.01$ compared with the DM group. Con, control; DM, diabetic rats; DM+naringin, diabetic rats treated with naringin (100 mg/kg/d)

Results

Effects of naringin on diabetes-induced cognitive deficit

After 7 days of naringin treatment, the Morris water maze task was used to assess the cognitive performance in different groups. Diabetic rats exhibited longer escape latency ($P<0.01$) than the control group, while naringin remarkably decreased the mean escapes latency ($P<0.01$), as illustrated in Figure 1A. Likewise, there was obvious reduction of number of times the diabetic rats crossed the former platform location ($P<0.01$), in comparison with control group. However, administration with naringin statistically increased number of times during the probe trial, compared with the STZ-treated group ($P<0.01$).

Effects of naringin on diabetes-induced changes in oxidative stress

As shown in Figure 2A, the oxidative production of MDA was markedly increased in STZ-induced diabetic rats ($P < 0.01$). Nevertheless, treatment with naringin statistically inhibited the content of MDA ($P<0.01$). In contrast, Figure 2B shows that antioxidant enzymes production including SOD was significantly reduced in the hippocampus of the STZ-treated rats. Administration of naringin significantly increased the SOD activity in the hippocampus, compared with the STZ-treated group ($P<0.01$).

Effects of naringin on inflammatory cytokines in the hippocampus of STZ-treated rats

To investigate the effect of naringin on the inflammation induced by diabetes in the hippocampus, major inflammatory factors including TNF-α, IL-1β, and IL-6 were detected by the commercial immunoassay kits. The levels of TNF-α, IL-1β, and IL-6 were all significantly augmented in the hippocampus of STZ-injected rats ($P<0.01$), compared with those of the control group; while, naringin treatment differentially regulated STZ-induced TNF-α, IL-1β, and IL-6 levels ($P<0.01$), as displayed in Figure 3. Together, these data indicate that the protective effects of naringin on STZ-induced inflammation could at least partially be the consequence of TNF-α, IL-1β, and IL-6 release in the hippocampus.

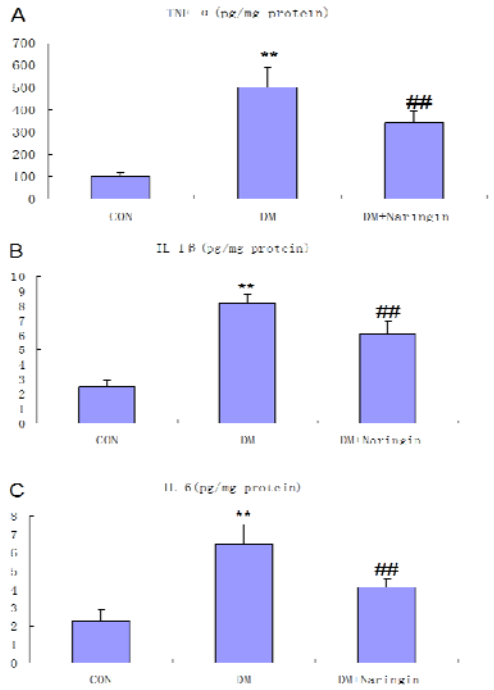

Figure 3. Effects of naringin on TNF-α (A), IL-1β (B), and IL-6 (C) levels in hippocampus of the control and the diabetic rats. $^{**}P<0.01$ compared with the Con group; ## $P<0.01$ compared with the DM group. Con, control; DM, diabetic rats; DM + naringin, diabetic rats treated with naringin (100 mg/kg/d)

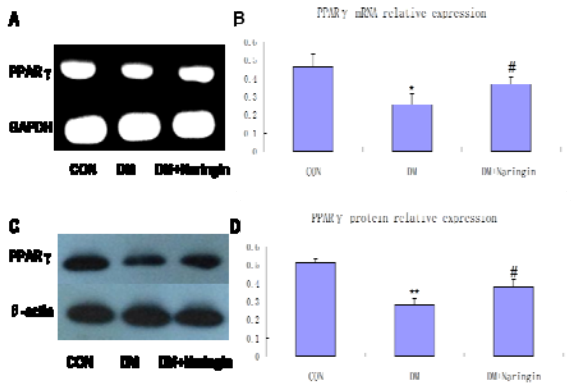

Figure 4. Effects of naringin on the mRNA (A and B) and protein (C and D) levels of PPARγ in hippocampus of control and diabetic rats. **P*<0.05, ***P*<0.01 compared with the Con group; #*P*<0.05, ##*P*<0.01 compared with the DM group. Con, control; DM, diabetic rats; DM + naringin, diabetic rats treated with naringin (100 mg/kg/d)

Effects of naringin on mRNA and protein Levels of PPARγ in the hippocampus of STZ-treated rats

To explore whether naringin could influence the activities of PPARγ in diabetes, hippocampus was separated from brain, and mRNA and protein expression levels of PPARγ were evaluated by RT-PCR and Western blot analysis. As shown in Figure 4, diabetic rats exhibited a lower mRNA and protein levels of PPARγ (*P*<0.01), compared with the controls. In contrast, naringin treatment statistically enhanced the mRNA and protein expression levels of PPARγ in the hippocampus of diabetic rats brain (*P*<0.01).

Discussion

Naringin, a bioflavonoid isolated from grapefruits and oranges, has antidiabetic properties to ameliorate learning and memory performances in STZ-induced diabetic rats. Naringin is involved in ameliorating hyperglycemia. Previous study has clarified that naringin can significantly decrease insulin resistance and hyperglycaemia (21-22). Moreover, naringin has potent neuroprotective effects. Diabetic neuropathy is characterized by slowly sensory loss, elevated pain, slowing of nerve conduction velocity, and nerve fiber degeneration. So, naringin can inhibit diabetes induced neuropathic pain by modulation of endogenous biomarkers (23). Lastly, Naringin protects memory impairment against STZ-induced cognitive deficits. Cognitive impairment disorder is the most common form of secondary complications of diabetes mellitus, which induces longterm complications in the brain and is involved in Alzheimer's disease (AD) pathogenesis. In this study, we characterized rat model of STZ-induced diabetes, and the findings from Morris water maze showed that supplementation of naringin improves learning and memory performances.

Diabetes mellitus is associated with abnormal regulation of glucose and impaired carbohydrate utilization. Hyperglycemia associated biochemical changes lead to hypoglycaemic brain injury which is involved in the occurrence of oxidative stresses and inflammation. Oxidative stress causes morphological and functional alterations in the brain and is associated with learning and memory deficits (24). Our results illustrated that naringin significantly inhibited the oxidative stresses (decreased SOD and increased MDA content) to improve cognition in diabetic rats. Additionally, in human diabetes, inflammatory cytokines could cause serious neuronal damages in the hippocampus, be involved in the occurrence of neurodegenerative diseases, and be negatively correlated with cognitive deficits (25-26). In the current research, the activities of major inflammatory cytokines including TNF-α, IL-1β, and IL-6 were all augmented in the diabetic rat hippocampus, and naringin treatment significantly inhibited the proinflammatory response, to improve the cognitive decline in DACD.

PPARγ agonists are used for their therapeutic potential in diabetes mellitus. Pioglitazone is a highly selective PPARγ agonist that improves insulin-mediated glucose, and is widely used for patients with type 2 diabetes mellitus (T2DM) (27). It can increase insulin sensitivity, reduce blood glucose, and lower blood pressure in insulin-resistant rheus monkey (28), and has a protective effect against oxidative stress in liver and kidney of diabetic rabbits (29). Recent findings in experimental models and in the clinical settings suggest that PPARγ agonists improve hippocampus-dependent cognitive deficits in brain disorders (27, 30). PPARγ agonists can be used for treating the cognitive dysfunction by reducing brain β-amyloid through PPARγ activation (31). Naringin has a partial agonistic effect on PPARγ, and increases PPARγ to ameliorate insulin resistance, dyslipidaemia, β-cell dysfunction, kidney damage, and hepatic steatosis in type 2 diabetic rats (22). In this study, we also speculate that the downregulation of PPARγ may be a leading cause of cognitive deficits in streptozotocin-induced diabetic rats and the pretreatment with naringin significantly increases PPARγ expression. We assessed the effects of naringin on DACD using a STZ-injected rat model, and the results showed that naringin significantly increased PPARγ expression. In all, naringin exerts an antidiabetic effect, possess antioxidant, anti-inflammatory, and a partial agonistic effects on PPARγ.

Conclusion

The results of the current study showed that learning and memory functions are decreased by diabetes, and naringin may be a suitable medicine to

improve learning and memory performances in DACD. Its neuroprotective, antioxidant, and anti-inflammatory effects could ameliorate DACD via modulation of PPARγ.

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