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Paeoniflorin ameliorates cognitive dysfunction via regulating SOCS2/IRS-1 pathway in diabetic rats

Xiaoxu Sun [a,1], Shanshan Li [a,1], Lixing Xu [a], Hao Wang [a], Zhanqiang Ma [a], Qiang Fu [a], Rong Qu [b], Shiping Ma [a,*]

[a] Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, 639, Longmian Road, Nanjing 211198, China
[b] Department of Pharmacology of Traditional Chinese Medical Formulae, Nanjing University of Traditional Chinese Medicine, 138, Xianlin Road, Nanjing 210029, China

HIGHLIGHTS

- Paeoniflorin ameliorated diabetes-associated cognitive deficits in rats.
- Paeoniflorin prevented tau hyperphosphorylation in the hippocampus of diabetic rats.
- Paeoniflorin restored SOCS2/IRS-1 pathway by suppressing inflammatory reaction in the hippocampus.

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ABSTRACT

Paeoniflorin is a natural monoterpene glycoside in *Paeonia lactiflora pall* with various biological properties including promising anti-inflammatory activity. Current evidences support that inflammatory reaction, oxidative stress, as well as abnormal insulin signaling in the hippocampus are potential causes of tau hyperphosphorylation and finally induce cognitive dysfunction. The present study aims to explore the effects of paeoniflorin on the cognitive deficits and investigate the underlying mechanisms in diabetic rats induced by a high-sucrose, high-fat diet and low dose of streptozotocin (STZ). Paeoniflorin treatment effectively improved the performance of diabetic rats in the Morris water maze test via decreasing escape latency and increasing the spent time in the target quadrant. Immunohistochemistry staining also had shown that tau hyperphosphorylation in the hippocampus was prevented after paeoniflorin administration. This function was correlated with its abilities of reducing the brain inflammatory cytokines (IL-1β and TNF-α), decreasing suppressor of cytokine signaling 2 (SOCS2) expressions and promoting insulin receptor substrate-1 (IRS-1) activity. Additionally, we also found paeoniflorin administration significantly promoted the phosphorylation levels of protein kinase B (Akt) and glycogen synthase kinase-3β (GSK-3β). Together, these results showed that paeoniflorin had beneficial effects on relieving diabetes-associated cognitive deficits via regulating SOCS2/IRS-1 pathway and might provide a feasible method for the treatment of diabetes-associated cognitive dysfunction.

1. Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases characterized with hyperglycemia. Patients with diabetes have suffered secondary complications followed by multiple system dysfunction. The impairment of diabetes in the central nervous system has received more and more attention, except for vascular diseases, retinopathy and nephropathy [1,2]. Cognitive disorder induced by diabetes, known as diabetic encephalopathy (DE), is characterized with neurochemical and neurostructural dysfunction [3]. A growing number of epidemiological investigations suggested there was a 1.2–1.5 fold greater cognitive dysfunction in individuals who were diagnosed with diabetes compared with healthy subjects [4,5]. As known, neural fibrillary tangles formed by hyperphosphorylated tau are closely related to cognitive disorder and act as an obvious histopathological marker. Accumulating evidences suggest that tau not only participates in regulating microtubule stability, synaptic activity and cognitive processes, but also involves in cellular processes including inflammatory response, oxidative stress and neuron survival [6,7]. Elevated inflammatory response in the hippocampal tissue leads to a promotion in tau hyperphosphorylation, and

Abbreviations: Akt, protein kinase B; DE, diabetic encephalopathy; DM, diabetes mellitus; GSK-3β, glycogen synthase kinase-3β; IL-1β, interleukin-1β; IRS-1, insulin receptor substrate-1; JAK/STAT, Janus kinase/signal transducer and activator of transcription; PI3K, phosphoinositide 3-kinase; SOCS2, suppressor of cytokine signaling 2; STZ, streptozotocin; TNF-α, tumor necrosis factor-α.

* Corresponding author.
E-mail address: spma@cpu.edu.cn (S. Ma).

[1] Sun Xiaoxu and Li Shanshan contributed equally to this work.

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then reduces the memory capacity in diabetic rats [8]. Hence, the high levels of inflammatory factor are gradually identified as an initiation factor of diabetic encephalopathy. Furthermore, previous studies revealed that the productions of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were accelerated in neural tissues of diabetic rats [9]. The previous study also showed the association between increased SOCS2 expressions and inflammation is considered as a potential target in central neuropathy [10,11]. Therefore, the function of SOCS2 in the hippocampus is worth to be emphasized. As an inhibitor of IRS-1, SOCS2 suppresses insulin pathway, which plays an important role in synaptic function, cognitive process and memory ability [12]. Overexpression of SOCS2 inhibits the activity of IRS-1, blocks the phosphorylation of Akt and GSK-3β, and eventually causes tau hyperphosphorylation [13–15]. These events above suggested that SOCS2 might be the pivotal link between inflammatory response and insulin signaling pathway, thereby relieving hyperglycemia-induced inflammatory response and restoring SOCS2/IRS-1 activation might be a feasible therapeutic strategy for preventing tau phosphorylation and alleviating cognitive impairment.

Paeoniflorin (Fig. 1), mainly extracted from the root of *Paeonia lactiflora pall*, exerts many pharmacological effects such as anti-inflammatory, anti-oxidative and anti-hyperglycemic properties [16]. Recently, the neuroprotective effects of paeoniflorin attracted considerable attention. Our previous experiments showed that paeoniflorin attenuated amyloid-beta peptide-induced neuron impairment and enhanced the ability of learning and memory in Alzheimer's disease rats [17]. Although the neuroprotective effects of paeoniflorin have been well documented, whether paeoniflorin can improve diabetes-associated cognitive decline is less well-known and the relevant mechanism is unclear. SOCS2/IRS-1 signaling plays an important role in sustaining neuronal function. Additionally, it is demonstrated that the overexpression of SOCS2 suppresses insulin pathway, and then accelerates the hyperphosphorylation of tau [18–20]. For this purpose, we investigated the beneficial effects of paeoniflorin on cognitive dysfunction in diabetic rats. Therefore, we examined behavioral changes, blood glucose levels and hippocampal inflammatory cytokine contents in diabetic rats. At the meantime, in order to investigate the possible mechanisms of paeoniflorin in diabetes-associated cognitive dysfunction, we also measured the expressions of SOCS2, p-IRS-1 Ser307, p-Akt Ser437, p-GSK-3β Ser9 and p-tau Ser396 in the hippocampus.

2. Materials and methods

2.1. Drugs and reagents

Paeoniflorin (purity ≥ 98%) and rosiglitazone were purchased from Nanjing Zelang Pharmaceutical Technology Co., Ltd. (Nanjing, China) and Chengdu Hengrui Pharmaceutical Co. Ltd. (Chengdu, China). Streptozotocin was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Glucose kit was provided by Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-1β and TNF-α were purchased from Dizhao biological technology Co. Ltd. (Nanjing, China).

2.2. Animals and treatment schedule

Male Sprague-Dawley (SD) rats weighting 200–220 g were provided by Experimental Animal Center in Jiangsu Province. All the animals were housed in standard cages for 7 days to adapt to the new environment before experiments. Rats were located in 12 h light-dark cycle experimental condition at temperature 22 ± 1 °C with food and water available. All the laboratory procedures and animals care were performed according to the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation. The protocols were approved by the Science and Technology Department of Jiangsu Province.

Rats were divided randomly into the following groups: control diet group (n = 11) and the remaining for DM group (n = 44). DM group rats were given a high-sucrose, high-fat diet containing 15% sucrose, 10% lard, 5% egg yolk powder, 1% cholesterol, 1% sodium chloride and 68% standard rat feed for 8 weeks. Diabetes was induced by intraperitoneal injection of 35 mg/kg STZ prepared in citrate buffer (pH = 4.4, 0.1 M). Rats with fasting blood glucose level equal or above 11.1 mM after 7 days were administered with paeoniflorin (15 or 30 mg/kg), rosiglitazone (4 mg/kg) or vehicle (10 ml/kg distilled water instead) by the way of lavage once a day at 9:00–10:00 a.m. for 4 weeks (Fig. 2).

2.3. Morris water maze test

Spatial learning and memory ability of rats was assessed by Morris water maze test 1 h after the last drug administration, which was performed as previously described [21,22]. The test included two sections: (1) The training trials: The Morris water maze test was conducted in a black circular water pool (180 cm in diameter and 60 cm in height) with a platform (10 cm in diameter) placed at the middle of a virtual quadrant. The water temperature was controlled at 22 ± 1 °C. Each rat was put into the tank from one of the four virtual quadrants to find the platform, which was hidden 1 cm under the water surface. The rats needed to reach the platform within 90 s and stay on it for 15 s, the escape latency was recorded by a video-tracking program for analysis. If the rats failed to find the platform within the required time, they were guided to the target and also allowed to stay on it for 15 s, and the escape latency was recorded as 90 s. The training trials were carried out for four consecutive days with two trials daily. (2) The probe trials: On the fifth day, rats were allowed to swim freely 90 s with the platform

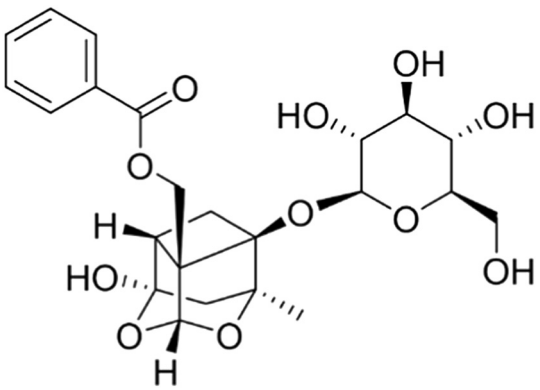

Fig. 1. Structural formula of paeoniflorin.

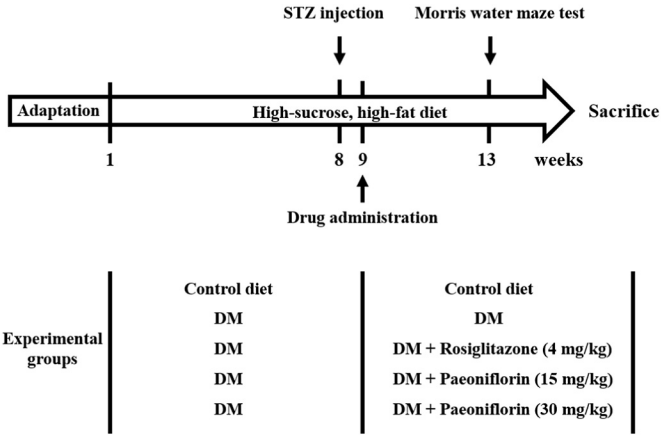

Fig. 2. Schematic illustration for the experimental procedures.