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The effect of isoliquiritigenin on learning and memory impairments induced by high-fat diet via inhibiting TNF-α/JNK/IRS signaling

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ABSTRACT

Isoliquiritigenin (ILG), a chalcone from *Glycyrrhiza uralensis,* has various biological properties. ILG markedly inhibited inflammation, but the effects on the brain inflammation and insulin resistance induced by high-fat diet (HFD) are still unknown, so our study intended to investigate its effect on cognitive dysfunction induced by HFD and the relevant mechanisms. ICR mice were treated with HFD diet for 8 weeks to induce peripheral insulin resistance prior to being intervened with rosiglitazone, ILG (30, 60 mg/kg). 4 weeks later, Morris Water Maze (MWM) was used to assess the learning and memory, the insulin resistance index was measured, and the brain inflammation cytokines (IL-1β, TNF-α) were assessed. Meanwhile, the p-JNK, p-IRS Ser^{307} protein expressions in the hippocampus were also detected using the western blot to explore the corresponding mechanisms. Our results suggested that ILG could significantly alleviate the cognitive impairments in the MWM test and attenuate peripheral insulin resistance. The IL-1β, TNF-α levels declined with the administration of ILG, meanwhile the p-IRS Ser^{307} expression decreased with the inhibition of p-JNK. In conclusion, ILG could improve the spatial learning and memory lesions induced by HFD via the inhibition of TNF-α/JNK/IRS pathway.

1. Introduction

Nowadays, Type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) bring a serious threat to health care system, gaining much more attention. AD is the most common neurodegenerative disease worldwide [1], characterized by progressive learning and memory deficits. T2DM, is characterized by hyper-insulinemia, hyper-glycemia and insulin resistance (IR) [2]. Historically, T2DM and AD were considered irrelevant diseases [3], but in regard to epidemiology, the aspect of IR in T2DM is an independent risk factor for the incidence of AD [4]. Meanwhile, clinical studies suggest that people with diabetes mellitus risk occurred in AD were 65% higher than those without it [5]. Thus, in the past decades, many studies have focused on the correlation between T2DM and AD. It has been now widely recognized that T2DM and AD share several kinds of abnormalities, including the increased oxidative stress, impaired glucose metabolism and IR [2]. The close relationship between T2DM and AD is considered to be mainly derived from the establishment of IR and the relevant changes of insulin on the central normal functions [3]. Thus it is meaningful to know more about IR for treating T2DM and AD.

The functions of insulin in the brain are worth to being noted. As known that insulin is secreted by pancreatic beta cells, acting largely as a metabolic regulator in the periphery [6], which has relative different functions in the brain. Insulin regulates glucose uptake of peripheral tissues by binding to the cell surface protein–Insulin Receptors (IR_S) [7]. Yet IR_S are distributed throughout the brain. In rodents, the highest density of IR_S is found in the olfactory bulb, hypothalamus, cerebral cortex, hippocampus and thalamus [8]. In the hypothalamus, the insulin attributes to the anorexigenic effect which leads to the reduction of food intake; in addition, the action of insulin may involve in the reduction in body weight due to its regulation in energy expenditure and locomotor activity [3,9]. Above all, function of insulin in the brain involves energy homeostasis as well as learning and memory. Meanwhile, insulin can directly modulate neurotransmitter release, neuronal survival and synaptic plasticity [10]. Insulin is transported across the blood–brain barrier (BBB) by a saturated transport system mediated by the insulin receptor protein [6]. Brain insulin is mainly obtained from peripheral via insulin receptor mediated transport.

Insulin exerts its function through binding to the IRs and leads to activated tyrosine kinase activity of the IRs which phosphorylation

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of insulin receptor substrate (IRS) at tyrosine level. Then the activated IRS initiates downstream signaling cascades, one of which is the phosphatidylinositol 3-kinase (PI3K) cascade, regulating almost all of the metabolic actions of insulin and in turn leading to the activation of Akt and Glycogen Synthase Kinase 3β (GSK3β) [3,7,11].

When the normal functions of insulin are destroyed, problems arise. The most common condition is called the insulin resistance syndrome, which is characterized by continuously high level of insulin in the periphery, and reduction of insulin to perform various functions [12]. It has been reported that long-term hyperinsulinemia can damage BBB function and the insulin activity [13], thus the peripheral hyperinsulinemia is related to the lack of insulin action in the brain. Most recent studies have focused on the role of IR which might be an important pathology on the AD-like change. Date from epidemiological, clinical and animal studies have shown that excessive energy intake could adversely affect the brain [14]. Furthermore animal studies have shown that high-calories diets damage the structure and functions of the hippocampus [15], the specific mechanism is unclear. Although the effect of chronic hyperinsulinemia on the brain is not fully understood, some studies proposed that the disturbances in brain insulin signaling pathways may contribute to the biochemical, molecular, and histopathological lesions in AD [16]. The deactivation of IRs can be triggered by downstream signaling molecules such as insulin receptor substrates-1 (IRS-1) and some factors including inflammatory factors TNF-α, free fatty acids (FFAs) and their downstream effector the c-Jun amino-terminal kinases (JNKs) have been identified as potent mediators for obesity-related insulin resistance [17] through influences on phosphorylation of IRS-1 on serine level [18]. Moreover, peripheral insulin sensitization agent-rosiglitazone, has been shown to reverse the cognitive deficits induced by high-fat diet via correcting peripheral insulin resistance [19], indicating that improved insulin activity has beneficial effect on the memory performance.

Isoliquiritigenin (ILG), a component of roots of *Glycyrrhiza uralensis* plants, is a flavonoid with a chalcone structure. It has multiple biological activities, including anti-inflammation [20], anti-tumor activity [21]. ILG treatment also potently attenuated the LPS-induced transcription activity of nuclear factor-kappa B (NF-κB) and the inflammasome activation [22], mediator of cellular inflammation and oxidative stress which lead to neuronal stress and apoptosis. However, few reports are available that are aimed to investigate the protective effect of ILG against cognitive impairments induced by a high-fat diet (HFD) in mice. Previous findings of the anti-inflammatory mechanisms of ILG may provide a favorable basis for its role on the AD-like change in the brain induced by HFD. The purpose of our present study is designed to attempt to indicate the issues above, to investigate the protective effects of ILG on cognitive impairments and IR induced by HFD. The mechanisms were discussed below.

2. Materials and methods

2.1. Reagents

Glucose test kit (Rongsheng bio-pharmaceutical Co. Ltd. Shanghai, P.R China). Enzyme-linked immunosorbent assay (ELISA) for mice (Dizhao biological technology Co. Ltd. Nanjing, P.R. China). BCA Protein Assay Kit (Biyuntian biotechnology research institute). ILG (Xian Kailai Biotechnology Co., Ltd. Shanxi, P.R. China). Rosiglitazone (Hengrui Pharmaceutical Co. Ltd. Chengdu, P.R. China). Equipment Morris water maze (Shanghai Jiliang software co., Ltd. Pierce).

2.2. Animal treatment and diets

ICR male mice weighted 18–22 g were purchased from Animal Breeder of Mount Qinglong in Nanjing, China. All animals were housed in standard cages at China Pharmaceutical University and received food and water ad libitum prior to the experiment. Mice were maintained in 12 h dark–light cycle under the constant temperature (22 ± 1 °C). All experiment procedures and the animal care were handled according to the Provision and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Jiangsu Province.

At the end of acclimation of the animals for 1 week with standard laboratory pellet food and water freely available prior to assignment to HFD or control diet conditions, for continuously feeding of 8 weeks the blood glucose and insulin levels were monitored at any time of each group. After oral glucose tolerance test, the mice were divided randomly into 5 groups: control diet (Normal), HFD (Model), HFD + Rosiglitazone (Rosi), HFD + ILG (ILG-L, ILG 30 mg/kg), HFD + ILG (ILG-H, ILG 60 mg/kg). The HFD consisted of the following: sucrose (15%), lard (10%), yolk powder (5%), cholate (0.5%), cholesterol (0.5%), basic feed (69%). All animals were given pure water. All the drugs were suspended in 0.3% carboxymethylcellulose solution by gavage once daily for 4 consecutive weeks. Then Followed by Morris Water Maze (MWM) test, the peripheral insulin resistance was evaluated. At the end of the trail, the mice were euthanized, blood were collected, and the plasma separated for use. Brains were rapidly removed and the hippocampus and cortex was isolated. The samples were stored at −80 °C until use.

2.3. Behavioral testing

The MWM test is widely used to test spatial learning and memory of rodents [23]. The experimental facilities consisted of a circular water pool (100 cm diameter, 50 cm in height), containing water to a depth of 15.5 cm. The water was controlled at 22 ± 1 °C and was rendered opaque by addition of milk powder. The pool was virtually divided into four quadrants, i.e., NE, SE, SW, and NW, a transparent platform (10 cm in diameter) was hidden 1.0 cm below the surface of water and placed at the midpoint of one quadrant. According to MWM test, the mice received four consecutive daily training trials during the following five days, with each trial lasted until either the mice had found the platform or for a maximum of 90 s. Once the mice found the platform within 90s, they were allowed to stay on it for 10s and the escape latency was recorded, but if they failed, they were guided to the platform and stayed on it for 15s and the escape latency was recorded 90s.The time needed for the mice to find the platform was escape latency. A probe trial was performed on the fifth day when the extent of memory consolidation was assessed. In the probe trial, the platform was removed, then the mice were placed and released opposite the site where the platform had been located. The single-probe trial consisted of a 90 s free swim in the pool without the platform. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning, which was shown in the results.

2.4. Insulin resistance test

For fasting plasma insulin and glucose, the mice were fasted for 12 h. The blood was collected from orbital venous plexus, then extracted 3000r for 15 min in a centrifuge, the supernatants were separated to measure the glucose an insulin levels respectively using commercial kits and ELISA according to the manufacturer's

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