Brain, Behavior, and Immunity 25 (2011) 1658–1667

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IκB kinase β/nuclear factor-κB-mediated inflammatory pathways in mice

Jun Lu [a,1], Dong-mei Wu [a,b,1], Yuan-lin Zheng [a,*], Bin Hu [a], Wei Cheng [b], Zi-feng Zhang [a], Qun Shan [a]

[a] Key Laboratory for Biotechnology on Medicinal Plants of Jiangsu Province, School of Life Science, Xuzhou Normal University, Xuzhou 221116, Jiangsu Province, PR China
[b] School of Environment and Spatial Informatics, China University of Mining and Technology, Xuzhou 221008, Jiangsu Province, PR China

ARTICLE INFO

Article history:
Received 30 March 2011
Received in revised form 10 May 2011
Accepted 13 June 2011
Available online 25 June 2011

Keywords:
Ursolic acid
High-fat diet
Endoplasmic reticulum stress
IκB kinase β/nuclear factor-κB-mediated inflammation
Insulin signaling
Cognitive impairments

ABSTRACT

Evidence suggests that obesity-induced cognitive impairments are driven by in brain inflammatory responses and inflammation-mediated brain insulin resistance. Ursolic acid (UA), a triterpenoid compound, has many important biological functions, including antioxidant and anti-inflammatory activities. Here, we evaluated the effect of UA on cognitive impairment induced by a high-fat diet (HFD), and we explored the potential mechanisms mediating this effect.

Results showed that UA administration significantly improved the behavioral performance of C57/BL6J mice fed a HFD in both the step-through test and the Morris water maze task. These results were associated with the inhibition of endoplasmic reticulum stress and IκB kinase β/nuclear factor-κB-mediated inflammatory signaling and the restoration of insulin signaling and phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. UA administration also increased memory-related protein expression in the hippocampus of mice given a HFD. However, the neuroprotective effects of UA were blocked by an intracerebroventricular (i.c.v.) injection of PI-103, a specific PI3K 110α inhibitor.

These results suggest that UA may be a potent candidate for the prevention and treatment of cognitive deficits caused by type 2 diabetes.

1. Introduction

Obesity is defined as abnormal or excessive fat accumulation and has become one of the most serious threats to human health with limited treatment options. A substantial amount of evidence indicates that obesity can contribute to the development of many chronic diseases, including type 2 diabetes, hypertension, cardiovascular diseases and cancer (Olufadi and Byrne, 2008; Özcan et al., 2004; Park et al., 2010; Poirier et al., 2006). Additionally, recent studies have reported that obesity also induces neuronal insulin resistance, impairs neural signaling pathways and results in cognitive function deficits (Banno et al., 2010; Lu et al., 2011; Neumann et al., 2008; Stranahan et al., 2008). The underlying mechanisms of these side-effects have been associated with the activation of cellular stress signaling and inflammatory pathways (Boden, 2009; Cai, 2009; Nakamura et al., 2010; Özcan et al., 2004; Zhang et al., 2008).

Ursolic acid (UA: 3β-hydroxy-urs-12-en-28-oic acid), a natural pentacyclic triterpenoid, has been reported to possess many biological activities, including antioxidant, anti-inflammatory, trypanocidal, antirheumatic, antiviral and antitumoral properties. Recent reports from our laboratory have also confirmed the neuroprotective effects of UA against oxidative stress and inflammatory

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; BBB, blood–brain barrier; BCA, bicinchoninic acid; Ca^{2+}/calmodulin-dependent protein kinase II, Ca2+/calmodulin-dependent protein kinase II; CCD, charge-coupled device; CD11b, cluster of differentiation molecule 11b; COX-2, cyclooxygenase-2; DAB, diaminobenzidine; D-gal, D-galactose; eIF2α, eukaryotic translation initiation factor 2α; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; ER stress, endoplasmic reticulum stress; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; GTT, glucose tolerance test; HFD, high-fat diet; HSD, honestly significant difference; i.c.v., intracerebroventricular; IKK, IκB kinase; IGT, impaired glucose tolerance; IL-2, interleukin-2; iNOS, inducible nitric oxide synthase; IOD, integral optical density; IRS1, insulin receptor substrate 1; ITT, insulin tolerance test; MWM, Morris water maze; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; NP-40, Nonidet P-40; OCT, optimal cutting temperature; OD, optical density; PERK, pancreatic endoplasmic reticulum resident kinase; PI3K, phosphoinositide 3-kinase; PMSF, phenylmethylsulphonyl fluoride; PSD-95, postsynaptic density protein 95; PTP1B, protein tyrosine phosphatase 1B; PVDF, polyvinylidene difluoride; RIPA, radioimmunoprecipitation assay; ROI, region of interest; SD, standard deviation; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; TBS, Tris-buffered saline; TNF-α, tumor necrosis factor α; T2DM, type 2 diabetes mellitus; UA, ursolic acid.

* Corresponding author. Fax: +86 516 83500348.
E-mail addresses: ylzheng@xznu.edu.cn, yzheng170@yahoo.com.cn (Y.-l. Zheng).

[1] These authors contributed equally to this work.

0889-1591/$ - see front matter Crown Copyright © 2011 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2011.06.009

responses induced by D-galactose (D-gal) (Lu et al., 2010e, 2007). In addition, studies have shown that UA can improve insulin sensitivity through blockage of nuclear factor-κB (NF-κB)-mediated inflammatory signaling, suppression of protein tyrosine phosphatase 1B (PTP1B) expression and enhancement of insulin receptor phosphorylation (Huang et al., 2005; Jayaprakasam et al., 2005; Pérez Gutiérrez et al., 2009; Zhang et al., 2006). However, no studies have been designed to investigate whether UA has a protective effect against cognitive impairments induced by brain inflammation and insulin resistance in mice given a high-fat diet (HFD). The hippocampus is a part of the limbic system and plays a key role in modulating learning and memory processes, which is sensitive to inflammation. Hippocampus-dependent learning and memory formation has been a useful model for determining the role of inflammation in cognition. Based on these considerations, we explored the aforementioned issue and investigated the potential mechanisms underlying UA action in the hippocampus of mice fed a HFD.

2. Materials and methods

2.1. Animals and administration

Four-week-old male C57BL/6J mice were purchased from the Branch of National Breeder Center of Rodents (Shanghai). Prior to experiments, mice were given free access to food and water and were kept under constant temperature (23 ± 1 °C) and humidity (60%). After one week of acclimatization to the laboratory conditions, mice were randomly divided into six groups and kept on a 12 h light/dark cycle (lights on 08:30–20:30). Mice were administered either a normal diet (control), containing 11.4% fat, or a HFD, consisting of 60% fat. Additionally, mice received daily intracerebroventricular (i.c.v.) injections of either PI-103 (0.5 μg, a specific PI3K inhibitor; Cayman Chemical, Ann Arbor, MI, USA) or its solvent (99% sterile saline/1% DMSO) followed by daily oral administration of UA (10 mg/kg/day; Sigma–Aldrich, MO, USA) or its solvent (sterile, distilled water containing 0.1% Tween-80), 0.5 h after the i.c.v. infusion. Treatments were administered to the following groups of mice for 20 weeks: Group 1 received the normal diet, daily i.c.v. infusions of the PI-103 solvent and daily oral administration of the UA solvent; Group 2 received the HFD, the PI-103 solvent and the UA solvent; Group 3 was administered the HFD, the PI-103 solvent and UA; Group 4 received the HFD, PI-103 and UA; Group 5 received the normal diet, PI-103 and the UA solvent; and Group 6 received the normal diet, the PI-103 solvent and UA. The infusions of PI-103 (0.1 μg/μl) were delivered using a microinjector (KD Scientific Inc., Holliston, MA, USA) into both cerebral ventricles (distance from bregma: anteroposterior, 0.5 mm; mediolateral, 1.0 mm; depth, 2.0 mm) at a rate of 2.5 μl/min for 20 weeks (Paxinos and Franklin, 2001). The drug dosage and period used in these experiments were based on previous reports and our pilot study (data not shown) (Lu et al., 2010e, 2007; Nakamura et al., 2010; Winzell and Ahrén, 2004; Zhang et al., 2008). The dosage of UA used for this study has no effect on the learning and memory ability of the control mice. In addition, the dosage of PI-103 has an specific inhibitory effect on the expression of p110α. All experiments were performed in compliance with Chinese legislation on the use and care of laboratory animals and were approved by the respective university committees. After the behavioral testing, mice were sacrificed and brain tissues were immediately collected for experiments or stored at −70 °C for later use.

2.2. Behavioral tests

Behavioral tests were conducted in the 20th week, after 19 weeks on the HFD. The step-through test and the Morris water maze (MWM) test were performed as described previously (Lu et al., 2010a,b,c).

2.3. Step-through test

The step-through passive avoidance apparatus consisted of an illuminated chamber (11.5 cm × 9.5 cm × 11 cm, lit with a 25 W lamp) attached to a darkened chamber (23.5 cm × 9.5 cm × 11 cm) containing a metal floor that could deliver a mild electric shock (0.3 mA, 50 Hz, 5 s). A guillotine door separated the two compartments. Briefly, mice were placed in a dimly lit room containing the apparatus for 0.5 h before training to acclimatize them to the new environment. Each mouse was placed into the illuminated chamber, facing away from the door to the dark chamber, and allowed to acclimatize to the chamber for 1 min. As soon as the mouse entered the dark chamber, the door was closed, triggering an electric shock. The mouse was immediately removed from the chamber and returned to its cage. The latency (time required to enter the dark compartment) was recorded. The retention test was conducted 24 h later. In this test, the mouse was again placed in the illuminated chamber and subjected to the same protocol but without the electric shock. The latency to enter the dark compartment was measured. The upper time limit was set to 300 s.

2.4. MWM test

The experimental apparatus consisted of a circular water tank (100 cm in diameter, 35 cm in height), filled with water (23 ± 1 °C) to a depth of 15.5 cm, which was rendered opaque by adding milk powder. A platform (4.5 cm in diameter, 14.5 cm in height) was submerged 1 cm below the water surface and placed at the midpoint of one quadrant. The pool was located in a test room, which contained various prominent visual cues. Each mouse received four training periods per day for four consecutive days. Latency to escape from the water maze (finding the submerged escape platform) was calculated for each trial. On day 5, the probe test was performed by removing platform and allowing each mouse to swim freely for 60 s. The time that mice spent swimming in the target quadrant (where the platform was located during hidden platform training) was measured. For the probe trials, the number of times the mice crossed where the platform had been located was also measured and calculated. All data were recorded with a computerized video system.

2.5. Tissue homogenates

After 20 weeks on a HFD, mice were deeply anesthetized with sodium pentobarbital (65 mg/kg, intraperitoneally) and sacrificed. For western blot analysis, which was performed as described previously (Lu et al., 2010d, 2009. 2006), the hippocampus was homogenized in 1/3 (w/v) ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Tris-buffered saline (TBS), 1% Nonidet P-40 (NP-40), 0.5% sodium deoxycholate, 0.1% SDS and 0.004% sodium azide) containing 30 μl of 10 mg/ml phenylmethylsulphonyl fluoride (PMSF), 30 μl of Na_3VO_4, 30 μl of NaF and 30 μl of protease inhibitor cocktail per gram of tissue. The homogenates were sonicated four times for 30 s with 20 s intervals using a sonicator and centrifuged at 15000*g* for 10 min at 4 °C. The supernatant was collected and stored at −70 °C for western blot studies. Western blotting was used to determine NF-κB p65 expression in cytoplasmic and nuclear extracts of brain tissues, which were obtained using a nuclear/cytoplasmic isolation kit (Pierce Biotechnology, Inc., Rockford, IL, USA). The protein levels of the supernatants were determined using a bicinchoninic acid (BCA) assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA).