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Lycopene attenuates insulin signaling deficits, oxidative stress, neuroinflammation, and cognitive impairment in fructose-drinking insulin resistant rats

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ABSTRACT

Fructose intake is linked with the increasing prevalence of insulin resistance, and insulin resistance links Alzheimer's disease with impaired insulin signaling, oxidative damage, neuroinflammation, and cognitive impairment. As a member of the carotenoid family of phytochemicals, lycopene is used as a potent free scavenger, and has been demonstrated to be effective in anti-oxidative stress and anti-inflammatory reaction in the models of AD and other neurodegenerative diseases. Here, we investigated the effect of lycopene on learning and memory impairment and the possible underlying molecular events in fructose-drinking insulin resistant rats. We found that long-term fructose-drinking causes insulin resistance, impaired insulin signaling, oxidative stress, neuroinflammation, down-regulated activity of cholinergic system, and cognitive impairment, which could be significantly ameliorated by oral lycopene administration. The results from this study provide experimental evidence for using lycopene in the treatment of brain damage caused by fructose-drinking insulin resistance.

1. Introduction

Lycopene, a member of the carotenoid family, is found in tomatoes and other fruits with red color (Kuhad et al., 2008). Lycopene inhibits lipid peroxidation and oxidative DNA damage as a highly efficient antioxidant and free radical scavenger (Kuhad et al., 2008; Sandhir et al., 2010). Additionally, *in vitro* and *in vivo* studies have suggested that lycopene may have other multiple properties such as anti-amyloid (Qu et al., 2011), anti-apoptosis (Fujita et al., 2013), anti-inflammation (Ip et al., 2013; Palozza et al., 2011), anti-ischemia (Hsiao et al., 2004), and anti-tumor (Gunasekera et al., 2007). Owing to its high liposolubility lycopene can pass through blood–brain barrier (Khachik et al., 2002). Furthermore lycopene has been shown to reverse neurobehavioral deficits in rats (Rao and Balachandran, 2002; Kuhad et al., 2008). Thus lycopene may be potentially protective for brain disorders.

About 60–70% of type 2 diabetes mellitus (T2DM) patients have central neurologic complications, and insulin resistance is to be the core cause (Manschot et al., 2008). Insulin resistance is a risk factor for Alzheimer's disease (AD) (Craft, 2005; Gordon et al., 2005; Stranahan et al., 2008). The proposition of the term “Type 3 diabetes” for AD is based on the similar alteration patterns of signaling components in insulin signaling transduction pathway: insulin receptor (IR), insulin-like growth factor-1 receptor (IGF-1R), phosphoinositide 3-kinase (PI3K), and protein kinase B (PKB/AKT) (Steen et al., 2005; Pei et al., 1997, 1999). Binding of insulin to the IR and IGF-1R activates the tyrosine kinase domain on the IR and IGF-1R, which results in tyrosine phosphorylation of multiple IR substrate that consequently activates several downstream signaling pathways including PI3K/AKT (Nemoto et al., 2010).

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Lycopene is reported to protect diabetes-associated brain pathologies by down-regulation of diabetes-stimulated oxidative stress and inflammatory response (Kuhad et al., 2008). The carotenoid inhibits pivotal oxidative and pro-inflammatory mediators ROS (Di Mascio et al., 1989) and cytokines (Palozza et al., 2011), and affects signal transduction pathways or mediators that regulates oxidative and inflammatory processes: PI3K/AKT pathway, transcription factors such as NF-kB and peroxisome proliferators-activated receptor γ (PPARγ) pathway (Simone et al., 2011).

To better understand the potential therapeutic role of lycopene, we have investigated its effect in modulating molecular pathways involved in impaired insulin signaling, oxidative stress, neuroinflammation, deficits in cholinergic function, and impairment in learning and memory ability induced by long-term fructose intake in rats in the present study. Here, we reported that the insulin signaling changes is induced by fructose-drinking brain resistance and this inducing effect can be protected by lycopene in brains.

2. Materials and methods

2.1. Animals and experimental design

Six-week-old male Wistar rats were obtained from the Experimenal Animal Center of Shandong University, Jinan, China. The animals were housed (3 rats/cage) at 22 ± 2 °C under diurnal cycle (light–dark: 08:00–20:00). The rats were given food and water ad libitum. The animals were cared in accordance with the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation. The experiment was approved by the Provincial Hospital Council on Animal Care Committee, Shandong University, Jinan, China.

After adaptation for a week, the rats were randomly divided into 6 groups (8 in each group). Group I comprised of control rats were fed with normal drinking water; Group II rats received 10% fructose solution in drinking water for 16 weeks to develop insulin resistance; Groups III rats were administered 10% fructose solution in drinking water for 16 weeks and lycopene (4 mg/kg; oral gavage) for the last 10 weeks of the 16-week period. Group IV rats received lycopene only (4 mg/kg; oral gavage). Lycopene was dissolved in double distilled water with 5% Tween 80. Non-treated rats (Groups I and II) were administered with the vehicle of lycopene at the same volume for the last 10 weeks.

2.2. Drugs and regents

Lycopene was purchased from Sigma (St. Louis, MO, USA). Immunoreagent kit used for fasting insulin (FINS) levels was from Beijing Furui Biotechnology (Beijing, China). TNF-α and IL-1β ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA). While NF-κB ELISA kit was purchased from Imagenex (San Diego, USA). Anti- PPARγ antibody was from Abcam (Hong Kong, China), the primary antibodies against IR and IGF-1were from Santa Cruz (CA, USA), the primary antibodies against PI3K, p-AKT, and AKT were from Cell Signaling Technology (MA, USA), anti-β-actin, and the secondary anti-rabbit or anti-mouse antibodies were from Zhongshan Jinqiao Biotechnology (Beijing, China). Polyvinylidene difluoride (PVDF) membrane was from Biorad (CA, USA). Enhanced chemiluminescence kit was from Millipore (MA, USA).

2.3. Preparation of supernatants

A total of 3 rats (one in group II and one in group III) were excluded from the study because of death or diseases specified above before the beginning of Morris water maze test, all other rats were killed by decapitation under anesthesia before collected blood from the tail vein after behavioral experiments. The brains were immediately removed from each rat on the dry ice for the isolation of cerebral and hippocampus using guidelines from rat brain atlas. 250 mg brains tissues (cerebral cortex and hippocampus) were separated from one brain hemisphere, and incubated with 1 ml of ice cold 1 × hypotonic buffer supplemented with 0.1 mM 1 mM DTT (dithiothreitol) and 1% detergent solution for 30 min on ice. After incubation, the brain homogenates were centrifuged for 10 min at 8000 × *g* at 4 °C. The supernatant (Cytoplasmic Fraction) was transferred into a separate tube and stored at 4 °C. The nuclear pellet was re-suspended in 100 μl nuclear lysis buffer by pipetting up and down. The samples were vortexed vigorously and the suspension was incubated at 4 °C for 30 min. The suspension was vortexed again for 30 s and centrifuged at 12,000 × *g* for 1 min at 4 °C in a microcentrifuge. The supernatant from nuclear fraction (Nuclear Supernatant Fraction) was transferred into a pre-chilled microcentrifuge tube. Cytoplasmic and nuclear fractions separated from the brain homogenate were stored at −80 °C until processed for biochemical estimations for biochemical estimations and for quantification of TNF-a, IL-1b, and NF-κb (Siqueira et al., 2005). The hippocampus and cerebral cortex from another hemisphere with a volume of 0.5 cm^3 were collected and frozen immediately in liquid nitrogen for further western blot analyses.

2.4. Detection of fasting glucose and insulin levels in plasma

The homeostasis model assessment-estimated insulin resistance (HOMA-IR) is a method used to quantify insulin resistance and pancreatic beta cells function (Matthews et al., 1985). The HOMA-IR index was measured at the end of the experiments for assessing insulin sensitivity. The rats after behavior tests were fasted for 12 h, 3 ml blood samples collected from abdominal aorta for each case 2 days before animals were killed, and then centrifuged at 1200 × *g* for detection of fasting blood glucose (FBG) and fasting insulin (FINS) levels. The FBG levels were determined by a glucose-oxidase biochemistry analyzer and FINS levels were measured by homogeneous phase competitive immunoradiometric assay with immunoreagent kit using GC-911c immunoradiometric counter (Enterprises Group of USTC, Hefei, China). HOMA-IR was calculated as formula: HOMA-IR = FBG × FINS/22.5 (Luo et al., 2011).

2.5. ROS, lipid peroxidation, and protein carbonyl content determination

ROS levels were quantified via the 2′-7′-dichlorofluorescein-diacetate (DCFH-DA) assay previously described using cytoplasmic fractions (Siqueira et al., 2005). The final results were corrected for protein concentration and then expressed as percentage of the corresponding values in control group. The malondialdehyde (MDA) content, a measure of lipid peroxidation (LPO), was assayed in the form of thiobarbituric acidreactive substances according to the method of Wills (1965). Protein carbonal content (PCC), a marker of oxidized proteins, was measured spectrophotometrically (Levine et al., 1990).

2.6. SOD, CAT, GSH, and GPx assay

SOD activity was assayed by monitoring the inhibition of the reduction of nitro blue tetrazolium by cytoplasmic fractions at 560 nm according to the method of Winterboun (1975). CAT activity was assayed as the rate of decrease in absorbance of H2O2 at 240 nm/min/mg protein in the presence of CAT according to the method of Aebi (1984). GSH content was assayed by spectrophotometry using 5, 5′-dithiobis (2-nitrobenzoic acid) according to the method of Jollow et al. (1974). GPx activity was determined with 5-5′-dithiobis-p-nitrobenzoic acid according to the method of Hafeman (1974).

2.7. TNF-α, IL-1β, and NF-κB ELISA

The quantification of TNF-α was done in the cytosolic fractions of different brain regions according to manufacturer's instructions with a sensitivity of ~5.6 pg ml^{-1}, intra-assay CV, ~6.2% and inter-assay CV, ~9.2%. The quantification of IL-1β was done in the cytosolic fractions of different brain regions according to manufacturer's instructions with a sensitivity of ~6.6 pg ml^{-1}, intra-assay CV, ~2.9% and inter-assay CV, ~1.7%. The sample values were read off the standard curve and the results were expressed as pg/mg of protein. NF-κB free p65 was measured by the NF-κB/p65 ActivELISA kit in the nuclear fraction of both brain regions according to manufacturer's instructions with a sensitivity of ~10.8 pg ml^{-1}, intra-assay CV, ~6.9% and inter-assay CV, ~3.6%. The results were expressed as ng/mg of protein.

2.8. Western blot

The supernatant proteins were isolated from another hemisphere cerebral cortex and hippocampus with lysis buffer containing 50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol and protease inhibitor (1 mM PMSF). Protein concentration was estimated by using BCA protein assay kit (Beyotime, Shanghai, China) with BSA as a standard. Equal amounts protein (80 μg) were separated on 10% SDS-PAGE, and then transferred to PVDF with transfer buffer composed of 25 mM Tris–HCl (pH 8.9), 192 mM glycine, and 20% methanol. Membranes were blocked by 5% BSA in PBS (containing 0.05% Tween 20) for 1 h at room temperature and then incubated overnight at 4 °C with specific polyclonal antibodies against IR (1:1000), IGF-1R (1:1000), PI3K (1:500), p-AKT (1:1000), AKT (1:1000), and PPARγ (1:1000), Secondary antibodies are anti-mouse IgG (1:1000) and anti-rabbit (1:5000), respectively. Antibody complexes were detected by chemiluminescence using the ECL kit (Santa Cruz, CA, USA). The relative expression of proteins was quantified densitometrically using the software Alphamager 2200 (AlphaInnotech Corporation, California, USA) and calculated according to the reference bands of β-actin. The expression of target proteins was semi-qualified as the following formula: Relative coefficient = target protein concentration/β-actin concentration.

2.9. AChE and ACh assay

The quantitative measurement of AChE levels were estimated by spectrophotometry using 5, 5′-dithiobis (2-nitro benzoic acid) by cytoplasmic fractions according to the method of Ellman et al. (1961), results were calculated using molar extinction coefficient of chromophore (1.36 × 10^4 M^{-1} cm^{-1}) and expressed as percentage of control. The Ach content was estimated by the method of Yang et al. (1995).

2.10. Experiments of behavioral test

The Morris water maze (MWM) test, which is consisted of 5-day training (visible and invisible platform training sessions) and a probe trial on day 6, was used to