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Purple sweet potato color ameliorates kidney damage via inhibiting oxidative stress mediated NLRP3 inflammasome activation in high fat diet mice

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ABSTRACT

Inflammation plays a crucial role in the pathogenesis of obesity. Purple sweet potato color (PSPC) has potential anti-inflammation efficacy. We evaluated the effect of PSPC on kidney injury induced by high fat diet (HFD) and explored the mechanism underlying these effects. The results showed that PSPC (700 mg/kg per day) reduced body weight, ratio of urine albumin to creatinine, inflammatory cell infiltration, and Collagen IV accumulation in mice fed an HFD (60% fat food) for 20 weeks. PSPC significantly reduced the expression level of kidney NLRP3 inflammasome including NLRP3 and ASC and Caspase-1, and resulted in decline of IL-1β. Moreover, PSPC inhibited the activation of I kappa B kinase β (IKKβ) and the nuclear translocation of nuclear factor kappa beta (NF-κB). Additionally, PSPC decreased the expression level of oxidative stress-associated AGE receptor (RAGE) and thioredoxin interacting protein (TXNIP) in the upstream of NLRP3 inflammasome. These data imply that the beneficial effects of PSPC on HFD-induced kidney dysfunction and damage are mediated through NLRP3 signaling pathways, suggesting a potential target for the prevention of obesity.

1. Introduction

Inflammation, as a major cause of organ damage, plays a crucial role in a variety of pathological processes of metabolic disorder diseases, such as gout, scurvy, and diabetic ketoacidosis. Emerging evidence suggests that inflammation-mediated kidney damage is involved in pathogenesis of obesity disease. Recently, several studies have shown chronic overfeeding, such as a high fat diet (HFD), leads to immune cell infiltration in different tissues, resulting in the production of inflammatory gene expression including cytokines, chemokines, and other mediators (Dong et al., 2014; Stemmer et al., 2012), which are implicated in oxidative stress and inflammatory response associated with obesity. HFD-induced activation of NOD-like receptor 3 (NLRP3) inflammasome and the alteration of its upstream or downstream signaling molecules, such as nuclear factor kappa beta (NF-κB) and the receptor of advanced glycation end products (RAGE), have also been demonstrated to be involved in obesity process, resulting in kidney injury and insulin resistance (Solini et al., 2013; Vandanmagsar et al., 2011; Harcourt et al., 2011). Furthermore, manipulation of inflammation-related genes can prevent and reverse kidney damage induced by HFD. For example, traditional fermented soybean products-doenjang and cheonggukjang blunted the kidney inflammatory response by suppressing NF-κB-related activities of inflammatory proteins in rats fed a HFD (Choi et al., 2011). Medical plant Magnolia extract (BL153) improved urine protein and kidney structure by reducing the expression of inflammation markers tumor necrosis factor-α (TNF-α) and plasminogen activator inhibitor-1 (PAI-1) in

Abbreviations: ACR, urine albumin/creatinine ratio; AGEs, advanced glycation end products; ASC, apoptosis associated speck-like protein containing a CARD; Col IV, Collagen IV; COX-2, cyclooxygenase-2; HFD, high fat diet; IL-1β, interleukin-1β; iNOS, induce nitric oxide synthase; IκBα, I kappa B α; IKKβ, I kappa B kinase β; PSPC, purple sweet potato color; NF-κB, nuclear factor kappa beta; NLRP3, NOD-like receptor 3; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; RAGE, the receptor of advanced glycation end products; T2D, type 2 diabetes; TNF-α, tumor necrosis factor-α; TXNIP, thioredoxin interacting protein.

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HFD-treated mice (Cui et al., 2013). Collectively, the natural productions contribute to attenuating kidney damage induced by HFD; however, the novel natural products and their protective mechanisms still need to be determined.

Purple sweet potato (*Ipomoea batatas*) color (PSPC), as a kind of anthocyanins, is a natural stable polyphenolic pigment and could be directly absorbed to the blood without cytoxicity (Kano et al., 2005). Emerging studies demonstrate that PSPC possesses potential efficacy in the prevention of different inflammation-induced diseases via its *in vivo* effective anti-oxidant activities. The previous data in our lab have showed that PSPC could significantly ameliorate learning memory or liver injury by improving oxidative damage and inflammatory response of brain or liver in D-galactose-mediated aging mice (Shan et al., 2009; Zhang et al., 2009). Recently, PSPC is found to accelerate improvement in the prevention and treatment of HFD-mediated mouse obesity by reducing liver inflammation via AMPK signaling inhibition (Hwang et al., 2011a, 2011b) or by raising liver insulin sensitivity via blocking NF-κB signaling (Zhang et al., 2013). These results suggest that exploring the protective mechanism of PSPC on the metabolic tissues associated with obesity disease, such as kidney, may accelerate the progress in the treatment of obesity disease. In the present study, we studied kidney response of PSPC-administrated mice to HFD feeding by testing oxidative stress and inflammatory response, and the regulatory mechanism of PSPC' preventing kidney damage by measuring the role of NLRP3 inflammasome in the protective process.

2. Materials and methods

2.1. Animal and treatment

Eight-week-old male ICR strain mice (32.12 ± 2.14 g) were purchased from the Branch of National Breeder Center of Rodents (Beijing, China). The mice were housed in a room under the conditions of constant temperature (23 ± 1 °C) and humidity (60%), and a 12 h light/dark schedule (lights on 8:00–20:00), and the mice were given free access to food and water. After one week of acclimatization to the conditions, mice were randomly divided into four groups including Control group, HFD, HFD/PSPC, PSPC. Mice were fed on either normal diet containing 11.4% fat, or HFD of 60% fat. In addition, mice were either administrated with PSPC [including two major components, cyanidin acyl glucosides and peonidin acyl glucosides (>90%), Qingdao Pengyuan Natural Pigment Research Institute, China] at dose of 700 mg/kg per day or its solvent (0.1% Tween 80 water solution) by oral gavage for 20 weeks as the described previously (Zhang et al., 2013). All procedures in the experiment consisted with Chinese legislation on the use and care of laboratory animals and were approved by the respective university committee for animal experiments. After 20 weeks, mice were sacrificed and kidney tissues were used for experiments or stored at −80 °C for later use.

Body weight of mice was measured every four weeks after 6 h fasting. Urine samples were collected from the mice housed in metabolic cages for 24 h to detect urine creatinine and urine protein content.

2.2. Determination of albumin and creatinine in urine

After the drug treatment, the excretion of urine protein was detected using urine albumin-to-creatinine ratio (ACR) in 24 h urine collections. The concentration of urine creatinine and albumin was assessed using the commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China). The absorbed value was detected by UV2501.

2.3. Glucose and insulin tolerance tests

After 20-week dietary intervention, glucose tolerance test and insulin tolerance test were carried out respectively. Glucose tolerance test was performed in fasted mice (12 h) with oral treatment of 1.5 g/kg glucose, then blood glucose values were detected immediately before and 30, 60, 90 and 120 min. For insulin resistance test was performed in fasted mice with intraperitoneal injection of insulin (1 U/kg body weight), and blood glucose values were detected immediately before and 30, 60, 90 and 120 min. Blood samples were obtained by tail venipuncture, and blood glucose values were detected by the Ascensia Elite glucose meter (Bayer Corporation, Mishawaka, IN, USA).

2.4. Histological evaluations

The mice were perfused transcardially with 100 ml of 0.9% sterile saline, and kidney tissues were removed immediately and fixed in a fresh solution of 4% paraformaldehyde (PH 7.4) at 4 °C for 4 h, then incubated overnight at 4 °C in 100 mM sodium phosphate buffers (PH 7.4) containing 15%, 20%, and 30% sucrose, respectively; and embedded in optimal cutting temperature (OCT) compound (Leica, CA, Germany). 12 μM cryosections were collected on 3-animopropyl-trimethoxysilane-coated slides (Sigma–Aldrich). The kidney sections were stained with hematoxylin and eosin, and measured by an expert in kidney pathology (S.M.) blinded to the type of treatment received by the animals.

Immunohistochemical staining was performed following a commercial kit (Zhongshan Golden Bridge of Biotechnology, Beijing, China). Firstly, endogenous peroxidase activity in the tissue sections was blocked with 3% H_2O_2 for 10 min, and nonspecific binding sites were blocked with 5% goat serum for 1 h. Then the sections were incubated with rabbit anti-Collagen IV (Col IV, 1:200, Santa Cruz Biotechnology) at 4 °C overnight. And, a biotinylated goat anti-rabbit IgG secondary antibody was added for incubation of 20 min. Subsequently, the avidin–biotin–horseradish peroxidase was applied for 20 min. Horseradish peroxidase was reacted with DAB and H_2O_2 for 5 min to produce the yellow deposit. The stained sections were washed in distilled water and sealed by water or neutral resins. The stained sections were captured using a leica 200 microscope (leica 4000, German).

2.5. Tissue homogenates

For biochemical analysis, mice were deeply anaesthetized and sacrificed. The kidney tissues were immediately separated and homogenized in ice cold 1/10 (w/v) 50 mM phosphate buffer saline solution (PBS, pH 7.2) with 10 strokes at 1200 rpm in a Potter homogenizer (Kontes, Vineland. NJ, USA). Homogenates were centrifuged at 12,000*g* for 10 min to obtain the supernatants for detecting the level of advanced glycation end products (AGEs) and reactive oxygen species (ROS).

For ROS assay, The kidney tissue homogenates were diluted at 1:20 (v:v) with ice-cold Locke's buffer (154 mM NaCl, 5.6 mM KCl, 3.6 mM NaHCO3, 2.0 mM CaCl2, 10 mM D-glucose, and 5 mM 4-(hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, PH 7.4) to obtain the concentration of 5 mg tissue/ml.

For western blotting analysis, the kidneys were homogenized in 1/5 (w/v) ice-cold lysis buffer (25 mM HEPES, PH 7.4, 125 mM NaCl, 25 mM NaF, 1 mM EDTA, 1 mM ethylene glycol tetraacetic acid, 1% NP-40, 1 mM Na3VO4, and the protease inhibitor mixture described above). The tissue homogenates were sonicated ten times for 10 s with 30 s intervals using a sonicator and centrifuged at 14,000*g* for 40 min at 4 °C, and then the supernatants were collected and stored at −70 °C for western blotting analysis. The expression levels of NF-κB p65 in cytoplasm and nuclear extracts of kidney tissues were assessed by western blotting, which was obtained by a nuclear/cytoplasm fractionation kit (BioVision, Inc., USA). Protein contents in the supernatants were detected by the bicinchoninic acid assay kit (Pierece Biotechnology, Inc., Rockford, IL, USA).

2.6. Determination of redox status

2.6.1. ROS assay

ROS was measured as described (Zhang et al., 2013). In brief, the reaction mixture (1 ml) containing Locke's buffer (pH 7.4), 0.2 ml of homogenate, and 10 μl of DCFH-DA (5 mM) was incubated for 15 min at room temperature to allow incorporation of DCFH-DA into any membrane-bound vesicles and cleavage of the diacetate group by esterases. After 30 min of further incubation, the conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorometer with excitation at 484 nm and emission at 530 nm. Blanks were included to correct for background fluorescence (conversion of DCFH-DA in the absence of homogenate). ROS formation was quantified from a DCF standard curve. Data are expressed as pmol of DCF formed per minute per mg of protein.

2.6.2. AGEs assay

Advanced glycation end products (AGEs) are formed due to nonenzymatic glycation of macromolecules, especially proteins leading to their irreversible oxidation is increased under the hyperglycemia of diabetes mellitus and obesity. Therefore, AGEs content could reflect the degree of tissue oxidative damage. AGEs content was measured according to the protocols of AGEs assay kit (Lu et al., 2010).

2.7. Western blotting analysis

Western blotting analysis was performed as previously described (Shan et al., 2009). The supernatants were then separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed by western blotting. For western blotting, samples (30 μg protein) were separated by denaturing SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Roche Diagnostics Corporation, Indianapolis, IN) by electrophoretic transfer. The membrane was blocked with 5% nonfat milk or 5% BSA and 0.1% Tween-20 in TBS and incubated overnight with one of the following primary antibodies: rabbit anti-RAGE and rabbit anti-NLRP3; rabbit anti-I kappa B kinase β (IKKβ), rabbit anti-p-IKKβ and

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