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Betanin attenuates oxidative stress and inflammatory reaction in kidney of paraquat-treated rat

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ABSTRACT

The effects of natural pigment betanin on oxidative stress and inflammation in kidney of paraquat-treated rat were investigated. Paraquat was injected intraperitoneally into rats to induce renal damage. The rats were randomly divided into four groups: a control group, a paraquat group, and two paraquat groups that were treated with betanin at 25 and 100 mg/kg/d three days before and two days after paraquat administration. Treatment with betanin alleviated the paraquat-incurred acute kidney injury, evidenced by histological improvement, reduced serum and urine markers for kidney injury. Betanin antagonized the paraquat-induced inflammation, indicated by reduced expression of inducible nitric oxide synthase and cyclooxygenase, blunted activation of nuclear factor kappa B, and diminished lysosomal protease activities. Betanin also decreased oxidative stress elicited by paraquat. In conclusion, betanin may have a protective effect against paraquat-induced acute kidney damage. The mechanisms of the protection appear to be the inhibition of oxidative stress and inflammation.

1. Introduction

Reactive oxygen species (ROS) and the so-called oxidative stress, i.e., an imbalance between ROS production and the anti-oxidative ability of biological systems, are involved in many diseases, thus antioxidants are very important therapeutic agents (Preiser, 2012). The anti-oxidative function of natural pigments has attracted attention in recent years (Sreekanth et al., 2007). Betalains are pigments present in red beetroot (*Beta vulgaris* var. *rubra*) and several other species such as amaranthus and cactus pear, and are widely used in food products (Nemzer et al., 2011; Vulić et al., 2014; Wootton-Beard and Ryan, 2011). Among betalain components, betanin (betanidin 5-*O*-β-D-glucoside, CAS number: 7659-95-2, red) is the most abundant (Gliszczyńska-Świgło et al., 2006). Several experiments have shown that betanin and betalains are excellent antioxidant components (Winkler et al., 2005). Phenolic and cyclic amine groups within betanin are good electron donors, endowing it with exceptionally high capacity for scavenging free-radicals. We previously reported some benefits of betanin against herbicide paraquat-induced liver damage in rat via protecting mitochondria (Han et al., 2014).

Paraquat is highly toxic to humans and animals when exposed via ingestion, skin contact, or inhalation. It has caused numerous human deaths since its introduction into agriculture. The toxicity of paraquat is attributable to its induction of ROS during redox cycling (Black et al., 2008). Although lung is the primary organ affected, paraquat can exert toxic effects in multiple other organs. Paraquat is excreted mainly through kidney. It accumulates within the kidneys and produces acute and severe nephrotoxicity, manifested with an abrupt decline in renal filtration function, resulting in substantial morbidity and mortality. Presently there is no effective treatment targeted directly at the etiological factor of such renal damage (Wang et al., 2013). Antioxidant treatment may provide some benefits by alleviating the lesion and promoting the recovery.

In the present study, we explored whether betanin alleviates the paraquat-induced acute nephrotoxicity. We focused on the anti-inflammatory roles of betanin, in particular, its effects on paraquat-induced production of ROS and its products (Lin et al., 2006).

2. Materials and methods

2.1. Animal study procedure

The animal experiments were in compliance with the Guide for the Care and Use of Laboratory Animals of Shenyang Agriculture University. Healthy male Sprague-Dawley rats (220 ± 20 g) were obtained from Liaoning Provincial Laboratory Animal Public Service Center (China, Benxi City). As described in our previous report (Han et al., 2014), the animals were divided randomly into four groups with 10 rats in

Abbreviations: i.p., intraperitoneally; i.g., by gavage; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH, glutathione; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; T-TBS, tris-buffered saline; ROS, reactive oxygen species; BUN, blood urea nitrogen; NGAL, urine neutrophil gelatinase-associated lipocalin; iNOS, inducible nitric oxide synthase; COX, cyclooxygenase; NF-κB, nuclear factor kappa B.

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each group: control group, paraquat group, betanin 25 mg/kg group and betanin 100 mg/kg groups. Betanin (TCI, Japan) was administered by intra-gastric gavage (i.g., distilled water as vehicle) for five consecutive days. On day 3 of betanin administration, all rats except those in the control group were intraperitoneally (i.p.) injected with 20 mg/kg paraquat (Sigma, USA). All rats were euthanized 72 h after paraquat administration. Serum and urine samples were collected before termination. Kidneys were harvested for further investigation.

2.2. Serum and urine biochemistry measurements

Serum creatinine, blood urea nitrogen (BUN), urine neutrophil gelatinase-associated lipocalin (NGAL) and microproteinuria were analyzed with respective commercial kits (Beijing DingguoChangsheng Biotechnology Co. Ltd., China). Serum nitrate/nitrite was determined by Griess reaction (Guevara et al., 1998).

2.3. Histophathology

Formalin-fixed kidneys were embedded in paraffin and prepared in 3-μm-thick sections. Sections were stained with hematoxylin–eosin and blindly assessed for kidney injury using an arbitrary scale (Rifaioglu et al., 2013): Score 0: No pathological changes. Score 1: Slight degenerative changes in tubuli and glomeruli with cortical involvement less than 25%. Score 2: Mild degenerative changes with cortical involvement of 25–50%. Score 3: Tubular and glomerular necrosis at different foci throughout the cortex with cortical involvement of 50–75%. Score 4: Extensive and marked necrosis throughout the cortex with cortical involvement of more than 75%.

2.4. Evaluation of oxidative stress

Kidney tissues were homogenized (10%, w/v) in ice-cold 1.15% KCl, 0.01 M sodium, potassium phosphate buffer solution (pH 7.4). The homogenates were centrifuged at 10 000 × *g* for 20 min at 4 °C, and the supernatant was collected. Superoxide dismutase (SOD) activity was measured with the photochemical method (Beauchamp and Fridovich, 1971), Catalase (CAT) activity was determined by the method of Claiborne (1985), and the lipid peroxidation was determined as described previously (Saleem et al., 2000).

2.5. Western blot

Western blotting was performed as previously described (Zhang et al., 2013). Briefly, approximately 20 μg of protein sample was resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidenedifluoride membranes. Membranes were incubated in tris-buffered saline (T-TBS) containing 3% non-fat dry milk with the appropriate primary antibody specific for inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 (Cell Signaling Technology, USA) overnight at 4 °C. The blot was washed and incubated with goat anti-mouse IgG conjugated to peroxidase (Cell Signaling Technology). Antibody binding was detected by chemoluminescence staining using an ECL detection kit (Bio-Rad, Hercules, USA), and the density of each band was quantified using the Gel Doc XR system (Bio-Rad).

2.6. Nuclear factor kappa B (NF-κB) activation

NF-κB (p65) DNA-binding activity was used as NF-κB activationindicator. Nuclear protein was extracted from kidney tissue. NF-κB (p65) DNA-binding activity was assessed using a commercial ELISA kit (Cell Signaling Technology) following the manufacturer's protocol.

2.7. Lysosomal hydrolases activity measurement

Lysosomal fraction of kidney tissue was isolated by the method of Wattiaux et al. (1977). The β-d-glucuronidase activity was determined by the method of Kawai and Anno (1971). β-d-Galactosidase activity was determined using the method of Conchie et al. (1967). The activities of Nacetyl-β-d-glucosaminidase (Moore and Morris, 1982) and cathepsin-D (Sapolsky et al., 1973) were also measured.

2.8. Protein concentration determination

Protein concentration in all samples was determined by the method of Bradford (Bradford, 1976), using bovine serum albumin as the standard.

2.9. Statistical analysis

Statistical analysis was performed using SPSS version 18.0 (IBM, Armonk, NY, USA). Data are expressed as the mean ± SD. One-way analysis of variance was used to compare data from multiple groups, followed by the Student–Newman–Keuls test. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

No animals died in the present study. Compared to the control animals, rats in the paraquat group exhibited anorexia, weakness, withering, and weight loss after paraquat exposure. These features were less obvious with treatment by betanin, especially in the 100 mg/kg group.

3.1. Betanin alleviated paraquat-incurred acute kidney injuries

Serum and urine markers for kidney acute injuries, including serum creatinine, BUN, urine NGAL and microprotein were measured. As shown in Fig. 1, all the parameters were significantly higher in paraquat group than the control group, suggesting renal injuries by paraquat. Betanin treatment resulted in significant and dose-dependent reduction of these parameters ($p < 0.05$).

Upon gross anatomy evaluation, betanin treatment resulted in attenuation of hyperemia and swelling elicited by paraquat, and reduced increased kidney/body weight ratio by paraquat. Upon histology examination, serious renal injuries were observed in paraquat group, with tubular and glomerular degeneration, hemorrhage and even necrosis.The histology injury score significantly increased in the paraquat group compared to the control group ($p < 0.05$), consistent with previous reports that paraquat induces acute nephrotoxicity (Lock and Ishmael, 1979; Rifaioglu et al., 2013; Wang et al., 2013). Betanin treatment preserved glomerular and tubular structure, and ameliorated hemorrhage, resulting in significant reduction of histology score (Fig. 2). Together these results demonstrated that betanin protects kidney from paraquat-induced injuries.

3.2. Betanin alleviated paraquat-incurred oxidative stress in kidney

In the present study, we assessed the effect of betanin on paraquat-induced oxidative stress. ROS-induced modifications of cellular constituents such as lipid peroxide (TBARS) and changes in antioxidative systems including SOD and CAT are common indicators of oxidative stress. In the present results, rats from the betanin groups showed significantly reduced oxidative stress elicited by paraquat, demonstrated by lower TBARS level, and higher SOD and CAT activities, as illustrated in Fig. 3.

3.3. Betanin alleviated paraquat-incurred kidney inflammation

Oxidative stress and inflammation are closely related. Oxidative stress and ROS trigger inflammation through immune recognition of molecules released from dying cells; meanwhile the activated neutrophils, macrophages and granulocytes release ROS (Epstein and Weiss, 1989). ROS and other factors stimulate inflammatory leukocytes, resulting in activation of NF-κB, which coordinates the expression of genes for inflammatory and innate immune responses and genes encoding inflammatory chemokines and cytokines (Saklatvala et al., 2003) such as COX-2 and iNOS. COX-2 and iNOS have been shown to play pivotal roles in the development of certain inflammatory diseases (Murakami and Ohigashi, 2007). COX catalyzes the conversion of arachidonic acid to prostaglandin H_2 and is the molecular target for analgesic and anti-inflammatory remedies. Among COX isoforms, COX-2 is inducible in most mammalian tissues in response to physical, chemical and biological stimuli (Murakami and Ohigashi, 2007). iNOS catalyzes the oxidative removal of the guanidino nitrogen derived from arginine with stoichiometric formation of nitric oxide (NO). With proinflammatory stimuli, iNOS protein is highly induced to produce NO, which is rapidly reduced to nitrite, resulting in elevated nitrate/nitrite in serum (Fletcher et al., 1998).

In the present study, expression of inflammatory cytokines, NF-κB activation in kidney, as well as serum nitrate/nitrite level were