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Riceberry bran extract prevents renal dysfunction and impaired renal organic anion transporter 3 (Oat3) function by modulating the PKC/Nrf2 pathway in gentamicin-induced nephrotoxicity in rats

Phatchawan Arjinajarn[a], Anchalee Pongchaidecha[b], Nuttawud Chueakula[b], Krit Jaikumkao[b], Varanuj Chatsudthipong[c], Sugunya Mahatheeranont[d], Orranuch Norkaew[d], Nipon Chattipakorn[b,e], Anusorn Lungkaphin[b,*]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
[b] Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
[c] Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand
[d] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
[e] Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

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ABSTRACT

Purpose: This study investigated the protective effects of Riceberry bran extract (RBBE) on renal function, and the function and expression of renal organic anion transporter 3 (Oat3) in gentamicin-induced nephrotoxicity in rats and explored the mechanisms for its protective effects.
Material and methods: Male Sprague Dawley rats ($n = 42$) were divided into six groups to receive normal saline, gentamicin (100 mg/kg), co-treatment of gentamicin and RBBE (at dose of 250, 500 and 1000 mg/kg), and RBBE (at dose of 1000 mg/kg) only, for consecutive fifteen days. Renal function, oxidative and antioxidative markers, the function and expression of Oat3 and histological changes in the kidney were evaluated.
Results: Elevation of BUN, serum creatinine levels and reduction in urine creatinine and creatinine clearance indicated decreased renal function in the gentamicin-treated rats. The decrease of [^{3}H]ES uptake in the renal cortical slices of these rats, reflecting the attenuation of Oat3 transport function that was accompanied by decreased expression of Oat3. Moreover, increased MDA level and reduced superoxide dismutase (SOD) and glutathione (GSH) activities were found in gentamicin-treated rats compared to the control group. These changes were associated with the upregulated PKCα, Nrf-2, Keap 1, NQO-1 and HO-1 expressions in kidneys. RBBE treatment improved the renal function and Oat3 transport function and expression in gentamicin-treated rats. The oxidative status was also restored by RBBE treatment.
Conclusion: RBBE protects kidney injury by its antioxidant effect, subsequently leading to modulation of the PKC/Nrf2 antioxidant defense pathway.

Introduction

Gentamicin is widely used and is an effective aminoglycoside antibiotic used in the treatment of life-threatening gram-negative bacterial infections (Ali, 2003). However, its clinical usefulness is limited due to the development of nephrotoxicity. Renal toxicity from gentamicin administration is related to a selective accumulation of the drug in the renal proximal tubular cells which leads to cell injury, and results in renal dysfunction (Quiros et al., 2011). In vivo and in vitro studies have shown that gentamicin administration can enhance the generation of reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide and superoxide anions mostly in the mitochondria, which subsequently damage some

Abbreviations: **ARE**, antioxidant response element; **ARF**, acute renal failure; **BUN**, blood urea nitrogen; **CAT**, catalase; **ES**, estrone sulfate; **GCL**, glutamate-cysteine ligase; **GC–MS**, gas chromatography-mass spectrometry; **GFR**, glomerular filtration rate; **GM**, gentamicin; **GSH**, glutathione; **GSH-Px**, glutathione peroxidase; **HO-1**, heme oxygenase-1; **HRP**, horseradish peroxidase; **Keap1**, kelch-like ECH associated protein 1; **MAP kinase**, mitogen-activated protein kinase; **MDA**, malondialdehyde; **NQO-1 NAD(P)H**, quinine oxidoreductase-1; **Nrf2**, nuclear factor erythroid-2-related factor-2; **Oat**, organic anion transporter; **PKC**, protein kinase C; **RBBE**, Riceberry bran extract; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase; **TBARS**, thiobarbituric acid reactive substances.

* Corresponding author. Fax. 6653 945365.
E-mail addresses: onanusorn@yahoo.com, anusorn.lungka@cmu.ac.th (A. Lungkaphin).

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macromolecules and induce cellular injury and necrosis (Du and Yang, 1994). However, the mechanisms in which increased oxidative stress induced renal dysfunction are still unclear.

Nuclear factor erythroid-2-related factor-2 (Nrf2) is a redox-sensitive transcription factor, which plays a crucial role in cellular defense against oxidative stress (Ishii et al., 2000). It activates the antioxidant response element (ARE) to enhance the expression of phase II detoxification enzymes and antioxidant proteins including catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), heme oxygenase-1 (HO-1) and NAD(P)H:quinine oxidoreductase-1 (NQO-1) (Kim and Vaziri, 2010). Nrf2 has been reported to protect rat kidneys against oxidative stress induced by gentamicin through the activation of antioxidant and phase II enzymes such as HO-1 (Subramanian et al., 2015).

Renal excretion of organic anions largely occurs at the proximal tubule through the organic anion transporter (Oat) which is one of the major routes for body drug clearance/detoxification. To date, several renal Oat isoforms have been cloned and identified. Among these, only Oat1 and Oat3 have been shown to play a major role in the cellular uptake of organic anions across the basolateral membrane of the renal proximal tubules. Moreover, among the Oat isoforms, Oat3 exhibits the highest mRNA expression level in the human kidneys (Motohashi et al., 2002). Previous studies clearly showed that acute renal failure (ARF) induced by gentamicin treatment decreased the renal excretion of organic anion compounds, and the renal expressions and functions of Oat1 and Oat3 were decreased in gentamicin-treated rats (Guo et al., 2013). The generation of free radicals and oxidative stress condition in proximal tubular epithelial cells has been shown to account for the mechanisms of several xenobiotic toxicities (Cuzzocrea et al., 2002; Du and Yang, 1994). Of interest, hydroxyl radical scavengers and iron chelators have been shown to reduce the severity of gentamicin-induced function and histological tubular damage (Ali, 2003;Guo et al., 2013).

Several natural compounds are demonstrated to exert antioxidants and provide protection against free radical-induced tissue damage. Rice is the staple food for people in the Asia-Pacific region. Riceberry rice (Oryza sativa L.), a Thai black purple rice, has been recently developed by crossing Chao Hom Nin Rice and Khao-Dawk Mali 105 (Kongkachuichai et al., 2013). The bran of Riceberry has been shown to possess high antioxidative activity. It contains a large number of antioxidant compounds, such as anthocyanins (cyanidin 3-glucoside and peonidin-3-glucoside), β-carotene, γ-oryzanol and vitamin E complex (tocopherols and tocotrienols) (Leardkamolkarn et al., 2011). Recent studies reported the antioxidant, anticancer and antidiabetic effects of Riceberry bran extract (RBBE) (Prangthip et al., 2013; Leardkamolkarn et al., 2011). Despite these benefits, the effect of RBBE on renal function in gentamicin-induced nephrotoxicity has not been investigated. We investigated the protective effects of RBBE against gentamicin-induced renal dysfunction and the reduction of Oat3 function and expression in the rat kidney. We hypothesized that the renoprotective effect of RBBE against gentamicin-induced nephrotoxicity mediates through its antioxidative action by modulating the PKC/Nrf2 pathway.

Materials and methods

Chemicals and reagents

Gentamicin was obtained from the Pharmaceutical Organization, INC (Bangkok, Thailand). Malondialdehyde (MDA) assay kit was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Superoxide dismutase (SOD) and Glutathione (GSH) assay kits were purchased from BioAssay Systems (Hayward, CA, USA). [^{3}H]ES was purchased from Perkin Elmer (Norwalk, CT, USA). Polyclonal antibody against Oat3 was purchased from Cosmo Bio Co. Ltd. (Tokyo, Japan). Antibodies against nuclear factor erythroid-2-related factor-2 (Nrf2) and protein kinase Cα (PKCα) (C-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against heme oxygenase-1 (HO-1) and NAD(P)H:quinine oxidoreductase-1 (NQO-1) were obtained from Abcam (Cambridge, UK). Monoclonal antibody against Lamin B1 and anti-β-actin antibody were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Monoclonal mouse anti-Na^+-K^+-ATPase and kelch-like ECH-associated protein 1(Keap 1) were obtained from Millipore (Billerica, MA, USA). Horseradish peroxidase (HRP) conjugated goat anti-rabbit and anti-mouse secondary antibodies were purchased from Amersham (Arlington Heights, IL, USA). All solvents used for extraction of chemical components from the Riceberry bran; methanol, dichloromethane, and hexane, were analytical grade obtained from Fluka (Buchs, Switzerland). Methanol and water, HPLC grade solvents, were purchased from Merck (Darmstadt, Germany). De-ionized water was obtained from a Mili-Q UV-Plus water purification system (Milipore Corp, USA). Formic acid was purchased from Fluka (Buchs, Switzerland). Standard grade vitamin E was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Standard anthocyanins; cyanidin-3-O-glucoside and peonidin-3-O-glucoside, were purchased from Apin Chemicals Ltd. (Oxfordshire, UK). All other chemicals were purchased from a commercial source at the analytical pure grade.

Preparation of Riceberry bran extract

Thai black non-glutinous rice, cv. Riceberry, grown in 2013, was obtained as harvested paddy from an experimental field in Kasetsart University, NakornPathom Province located in Central Thailand. The paddy was dried by modified hot air at a temperature of 40 °C until their moisture content was reduced to approximately 14% by weight. On the day of the experiment, the rice sample was dehusked and milled in a local milling system (Natrawee Technology, Chachoengsao, Thailand) for 30 s to obtain approximately 10% (w/w) fresh rice bran. One kilogram of the Riceberry bran was extracted with methanol (3 l) at room temperature for 24 h. After solvent evaporation, a dark red viscous crude extract of 55.8 g was obtained for further analysis by gas chromatography-mass spectrometry (GC-MS).

GC–MS analysis

A gas chromatograph-mass spectrometer (Agilent 6890 and HP 5973 mass-selective detector, Agilent Technologies, Palo Alto, CA) equipped with a fused silica capillary column having phase HP-5MS with dimension 30 m × 0.25 mm i.d. and 0.25 mm film thickness (Agilent Technologies) was utilized for analysis of chemical components obtained from crude methanol extract of the Riceberry bran. The sample was injected with a split ratio of 20:1. The injection port temperature was 250 °C. The column temperature program started at 60 °C upon injection. The temperature was increased at a rate of 3 °C/min to 285 °C. Purified helium gas at a flow rate of 1 ml/min was used as the GC carrier gas. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV; ion source temperature, 230 °C; quadrupole temperature, 150 °C; mass range m/z 50–550; scan rate, 0.68 s/scan; EM voltage, 1456 V. The GC-MS transfer line was set to 280 °C. Identification of organic components in both extracts was performed by matching their mass spectra with reference spectra in the Wiley 7n Mass Spectral Library and the NIST 08 Mass Spectral Library, both purchased from Agilent Technologies. In addition, Kováts indices and retention times of known standards, for some available compounds, were used to aid structural confirmation. Quantitative analysis of each component in percent was