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Vasoprotective effects of rice bran water extract on rats fed with high-fat diet

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ABSTRACT

Objective: To elucidate the protective effects of rice bran water extract on the expression of endothelial nitric oxide synthase (eNOS), nuclear factor-kappa B (NF-κB), and a cluster of differentiation 36 (CD36) in the vasculature of high-fat diet-fed rats.

Methods: Male Sprague-Dawley rats were divided into three groups. Group I served as control, Group II was treated with high-fat diet, and Group III was treated with high-fat diet and rice bran water extract at 2205 mg/kg/day. After four weeks, the metabolic parameters, malondialdehyde as a marker of oxidative stress, and histological features of the aorta were evaluated. The levels of transcripts and proteins in aorta were determined by real-time PCR and Western blot analysis, respectively.

Results: In comparison with the Group II, rice bran water extract administration resulted in a significant reduction in body weight, visceral fat tissue weights, blood glucose levels, and serum total-cholesterol and free fatty acid levels in Group III. Serum triglyceride levels tended to decrease in the Group III. Also, rice bran water extract administration obviously decreased malondialdehyde levels in both serum and aorta. Interestingly, rice bran water extract treatment demonstrated a significant up-regulation of eNOS expression and down-regulation of NF-κB p65 and CD36 expressions. Nonetheless, all groups showed normal histology of aorta.

Conclusions: Rice bran water extract exhibited vasoprotective effects in the high-fat diet-induced obesity condition by modulating the expression of eNOS, NF-κB, and CD36 and metabolic parameters.

1. Introduction

Metabolic syndrome is a common cluster of metabolic disturbances prevalent worldwide [1]. It is composed of several vascular risk factors including abdominal (visceral) obesity, dyslipidemia, hyperglycemia, and hypertension. In addition, oxidative stress is one of the important pathomechanisms that have been suggested to play a role in the development of metabolic syndrome, coronary artery disease, and hypertension [2]. The excess of reactive oxygen species can induce oxidative damage to biomolecules (*e.g.*, lipids, proteins, and nucleic acids). Among the various lipid oxidative damages, malondialdehyde (MDA) is a key end-product of lipid peroxidation and serves as a biomarker of oxidative stress in an animal model of atherosclerosis and patients with cardiovascular disease [3,4].

Although the pathogenesis of vascular disease in metabolic syndrome is complex, the decreased nitric oxide (NO), enhanced inflammatory responses, and lipid accumulation are involved in the early stages of vascular disease. Endothelial nitric oxide synthase (eNOS), nuclear factor-kappa B (NF-κB), and a cluster of differentiation 36 (CD36) play major roles in vascular NO synthesis,

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inflammation, and lipid uptake, respectively [5–7]. At the molecular level, both animal and human studies have proposed that down-regulation of eNOS expression, as well as up-regulation of NF-κB p65 subunit (NF-κB p65) and CD36 expressions are significant factors of vascular disease [8–13]. Moreover, overexpression of eNOS and inhibition of CD36 and NF-κB signaling have been revealed to protect rodents from the development of vascular disease [14–16]. Thus, the regulation of eNOS, NF-κB, and CD36 expressions in the vasculature is considered to be an important preventive mechanism for cardiovascular disease.

Consumption of rice bran has been shown to be associated with beneficial effects on metabolic and cardiovascular diseases [17,18]. Rice bran contains several nutrients and phytochemicals such as carbohydrates, proteins, fibers, polyphenols, and γ-oryzanols [17]. Our preliminary study demonstrated that rice bran water extract from the Khao Dawk Mali 105 rice variety (*Oryza sativa* Linn.) significantly reduced insulin resistance, as well as abdominal and hepatic fat deposition in rats fed with a high-fat diet for four weeks. Although our results suggested that Khao Dawk Mali 105 rice bran water extract could reduce the vascular risk factors, vasoprotection at the molecular level has not been elucidated yet. Thus, the present study aimed at verifying the effects of rice bran water extract on the expressions of eNOS, NF-κB, and CD36 in the vasculature of high-fat diet-fed rats so as to provide a model for prevention of metabolic syndrome.

2. Materials and methods

2.1. Preparation and characterization of rice bran water extract

The bran of Khao Dawk Mali 105 rice variety was purchased from the local mill in Surin Province, Thailand. Rice was grown in the organic farm approved by the Organic Agriculture Certification of the Department of Agricultural Extension (Bangkok, Thailand). Freshly milled rice bran was stabilized at 130 °C for 90 s. About 2000 g of stabilized rice bran was boiled in 8000 mL of distilled water for 1 h at 70 °C. After centrifugation at 8000 r/min for 10 min, the supernatant was freeze-dried into powdered extract by using a freeze dryer (Lyophilization Systems Inc., USA). The procedure of preparation was described in details by Qureshi *et al.* [17]. The proximate analysis, total phenolic compounds, and γ-oryzanol contents of rice bran water extract were also determined using the official method of Association of Official Analytical Chemists, Folin–Ciocalteu method, and high-performance liquid chromatography [19], respectively.

2.2. Experimental diets and animals

The standard chow (C.P. mice feed, Thailand) consisted of 13%, 55%, and 32% of total energy derived from fat, carbohydrate, and protein, respectively. The high-fat diet was modified from the diet that induces obesity in which 65% of total energy was derived from fat [20]. The major ingredients of the high-fat diet included pork belly, pork liver, margarine, sugar, wheat flour, standard chow, and a whole egg (hen). The high-fat diet consisted of 65%, 24%, and 11% of total energy derived from fat, carbohydrate, and protein, respectively.

Male Sprague-Dawley rats (6–8 weeks old and weighting 180–220 g) were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. All experimental procedures involving animals were conducted in accordance to Association for Assessment and Accreditation of Laboratory Animal Care and approved by the Animal Ethics Committee of the Faculty of Medicine, Thammasat University, Pathum Thani, Thailand (AE 002/2013). Animals were maintained under controlled temperature of (24 ± 1) °C with 60% humidity and a 12 h light and 12 h dark cycle. After a week of acclimatization, the rats were randomly divided into three groups of eight rats each. Rats in Group I were fed with standard chow (control group). Rats in Group II were fed with high-fat diet alone. Rats in Group III were fed with high-fat diet and orally gavaged with a fixed dose of rice bran water extract (2205 mg/kg/day, dissolved in distilled water). Our preliminary work indicated that rice bran water extract at the dose of 2205 mg/kg/day was effective in improving the metabolic disturbances in high-fat diet-fed rats. Thus, this dose was chosen for the present research. Rats in all three groups were fed with water and experimental diets *ad libitum* throughout four weeks of the experiment. Body weight, food intake, and energy intake were measured daily. At the end of treatment, the animals were sacrificed with an overdose of pentobarbital sodium (intraperitoneal injection), and their blood was collected by a cardiac puncture. Tissues were removed, weighed, and properly kept for histological study or biochemical assays.

2.3. Blood biochemical assessment

The blood glucose levels were determined with a glucometer (Accu-Chek Performa, Roche Diagnostics, Switzerland). The concentrations of total-cholesterol (total-C), high-density lipoprotein-cholesterol (HDL-C), and triglyceride (TG) in the serum were analyzed using enzymatic colorimetric method (Fluitest test kits, Analyticon Biotechnologies AG, Germany). The serum low-density lipoprotein-cholesterol (LDL-C) level was determined using the Friedewald equation [21]: LDL-C = Total-C – HDL-C – (TG/5). The concentrations of free fatty acid (FFA) were measured using enzymatic colorimetric method (FFA assay kit, Wako, Japan).

2.4. Measurement of serum and aortic MDA

MDA levels were analyzed as a biomarker for oxidative damage. The concentrations of MDA in the serum and aortic tissues were determined spectrophotometrically at 532 nm according to a previously published method with some modifications [22], using 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, USA) as a standard. For tissue samples, total protein levels were used for normalization of MDA levels and determined by Bradford protein assay kit (Bio-Rad, USA) according to the manufacturer's instructions. Serum and aortic MDA levels were expressed as nmol/dL and nmol/mg of protein, respectively.

2.5. Real-time PCR analysis

Total RNA from the aortas was extracted using TRIzol reagent (Invitrogen, USA), according to the manufacturer's recommendations. Total RNA concentration and purity were determined by the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Subsequently, RNA (200 ng) was reverse transcribed into cDNA using the cDNA reverse transcription kit (Applied Biosystems, USA) according to the manufacturer's instructions. Quantitative PCR was performed using the TaqMan reagent kit and StepOnePlus real-time PCR system (Applied Biosystems, USA). The relative mRNA levels of eNOS (assay ID Rn02132634_m1), NF-κB p65 (assay ID Rn01502266_m1), and CD36 (assay ID Rn02115479_g1) were analyzed by the $2^{-\Delta\Delta CT}$ method. The