Effect of Rice Bran Protein Hydrolysates on Oxidative Stress and Vascular Dysfunction in High-Carbohydrate, High-Fat Diet-Induced Metabolic Syndrome Rats

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Background and objective: Prolonged oxidative stress associated in metabolic syndrome (MS) serves as a key signaling event in alteration of the cardiovascular function. It has been demonstrated that peptides-derived from Hom-Mali Thai rice bran protein hydrolysates (RBPH) possess strong antioxidant with angiotensin converting enzyme (ACE) inhibitory activity. The present study aimed to investigate whether RBPH supplementation alleviates oxidative stress and vascular dysfunction in a rat model of MS induced by a high-fat, high- carbohydrate (HFHC) diet.

Method: Male Sprague-Dawley rats were induced MS by feeding a HFHC diet with 15% fructose in the drinking water for 16 weeks. Concomitantly, the normal control rats were fed with standard chow diet and distilled water. Rats in the MS and control groups were orally administered with RBPH (250 or 500 mg/kg) or vehicle for the last six weeks of experiments.

Result: Rats fed with HFHC diet for 16 weeks developed hypertension, vascular dysfunction, impaired glucose tolerance and oxidative stress. Supplementation with RBPH attenuated hypertension, improved vascular function and decreased blood glucose. RBPH also alleviated oxidative stress by suppression of vascular superoxide production as compared to the MS controls ($p<0.05$).

Conclusion: These findings suggest that RBPH might have auxiliary therapeutic potential for improvement of vascular function in MS-induced by a HFHC diet. The plausible mechanism of RBPH might partly via its antioxidant and antihypertensive effects.

Key words: Rice bran protein hydrolysates, Oxidative stress, Vascular dysfunction, High-carbohydrate, high-fat diet

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Introduction

Metabolic syndrome (MS) is characterized by accumulation of visceral fat associated with the clustering of metabolic and pathophysiological cardiovascular risk factors, including impaired glucose tolerance, dyslipidemia, and hypertension[1]. The prevalence of MS is rapidly increasing worldwide not only in industrialized countries but also in developing countries. MS has become a serious public health problem[2,3]. The prevalence of MS in Thai population was reported to be 32.6% (men 28.7%, women 36.4%)[4] according to the National Cholesterol Education Program Adult Treatment Panel III (NECP-ATPIII) criteria, with modification of waist circumference (WC) for Asians. A growing body of evidences suggest that increased oxidative stress to adipocytes plays a role in the pathogenesis of cardiovascular disease in MS[5]. Increased oxidative stress to adipocytes causes dysregulated expression of

inflammation-related adipocytokines in MS. Imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines is responsible for oxidative stress especially to endothelial cells and underlies the pathogenesis of the obesity-associated insulin resistance, impaired glucose tolerance (IGT), type-2 diabetes mellitus (T2DM), hypertension, dyslipidemia, and vascular disease[5]. Therefore, oxidative stress appears to possess at least in part of the insulin resistance, the endothelial dysfunction and the appearance of vascular complications in MS.

Rice (*Oryza sativa* L.) is one of the most common staple diets for humans, especially in Asian countries including Thailand and Asian Countries. Rice bran (RB) is a by-product of the rice milling which is derived from the outer layer of the rice grain. Rice bran contains large amounts of fiber and bioactive phytochemicals, such as tochopherols, tochotrienols, oryzanols, vitamin B complex and phenolic compounds[6]. Data from *in vitro* study have shown that rice bran peptides derived from rice bran protein hydrolysates (RBPH) possess strong ACE inhibitory and antioxidant effects.[7] It is of interest to explore whether RBPH could reduce the symptoms of MS and improve vascular function. The present study was aimed to investigate the antioxidative and vascular protective effects of RBPH on HFHC diet-induced MS in rats.

Methods

1. Animals and experimental procedures

The experimental protocol of this study has been reviewed and approved by the Animal Ethics Committee of Khon Kaen University (AEKKU/19/2555). Male Sprague-Dawley rats (220-250g) were obtained from the National Laboratory Animal Center, Mahidol University. Animals were kept at the Northeast Laboratory Animal Center, Khon Kaen University.

After an adaptation periods of 7 days, rats were randomly divided into five groups (n= 5-8 each). Rats in group 1and 2 were fed with standard chow diet (CP) and distilled water. Rats in group 3-5 were induced MS by feeding with high fat (20%) and high carbohydrate (52.5%) diet (HFHC) with 15% fructose in drinking water for 16 weeks. RBPH (250 or 500 mg/kg/day) dissolved in distilled water (DI) was administered once- daily for the final 6 weeks of the 16 weeks protocol via oral gavage. The body weight, food and water intakes were recorded daily. Fasting blood glucose (FBG) and blood pressure were measured every two weeks by using a tail cuff plethysmography (IITC model 179 blood pressure analyzer, Life science, USA). After 16 weeks of experiment, animals were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). The arterial blood pressure and heart rate were measured through femoral artery by a previously described method [8]. Vascular responsiveness was determined by using vasoactive agents, including phenylephrine (Phe; 0.01, 0.03, 0.1 μmol/kg), acetylcholine (ACh; 3, 10, 30 nmol/kg) and sodium nitropusside (SNP; 1, 3, 10 nmol/kg). At the end of experiment, the carotid arteries were immediately excised for measurement of superoxide ($O_2^{\cdot-}$) production by using lucigenin-enhanced chemiluminescence technique [8].

2. Statistical analysis: Data are expressed as means ± S.E.M. Comparisons between groups were evaluated by one-way and two-way analysis of variance (ANOVA) and followed by Student Newman-Keul's test for post-hoc test. All statistical analyses were performed using Sigmastat software version 3.1. A p-value of <0.05 was considered as statistically significant.

Results

The HFHC-fed rats showed the symptoms of MS, including hypertension, dyslipidemia, hyperglycemia and impaired glucose tolerance. The baseline values of systolic blood pressure (SBP) at the beginning were similar among all experimental groups. After receiving HFHC diets for four weeks, SBP was significantly increased when compared with those treated with standard chow diet ($p<0.05$; Fig. 1). Treatment with RBPH at dose 250 or 500 mg/kg/day for 6 weeks significantly decreased blood pressure of MS rats, especially in a high dose of RBPH. Although the SBP of MS rats treated with RBPH was higher than normal controls, a reduction in blood pressure after treatment with RBPH indicates the antihypertensive property of RBPH. Moreover, we

also found that RBPH significantly decreased FBG of MS rats ($p<0.05$; Fig. 2), suggesting the hypoglycemic effect of RBPH.

A marked increase in $O_2^{\cdot-}$ production was observed in the vascular tissues of MS rats (Fig. 3). RBPH at dose 500 mg/kg/day significantly reduced the level of $O_2^{\cdot-}$ production ($p<0.05$; Fig. 3). A significant reduction in vascular responses to Phe and ACh was found in rats treated with HFHC diet ($p<0.05$; Fig. 4). Interestingly, RBPH at tested doses significantly restored the vascular responsiveness ($p<0.05$; Fig. 4). However, there was no significant change in relaxation response to nitroprusside, suggesting endothelial-dependent vasorelaxation is impaired in MS rats and restored by feeding with RBPH.

Conclusion

Results of this study demonstrated that HFHC diet together with high fructose in drinking water induced MS, increased oxidative stress and vascular dysfunction in rats. RBPH alleviated the symptoms of MS by decrease of blood pressure, FBG, vascular $O_2^{\cdot-}$ production and attenuation of vascular dysfunction. This study indicates that RBPH from Thai rice may provide a useful dietary supplement to decrease MS by alleviation of oxidative stress and improvement in vascular function.

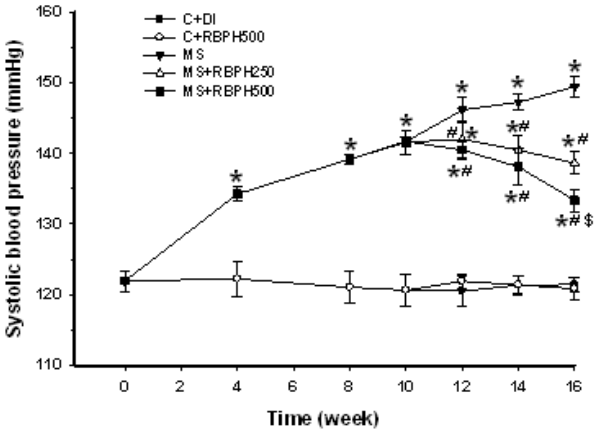

Fig. 1 Effect of RBPH on systolic blood pressure during the experimental period of 16 weeks. Data expressed as mean ± S.E.M. (N=5-8/group). * $p<0.05$ vs. control, $^{\#}p<0.05$ vs. MS, $^{\$}p<0.05$ vs. MS with RBPH 250 mg/kg.

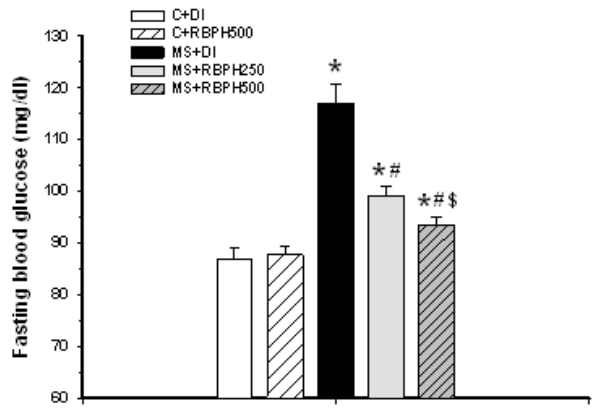

Fig. 2 Effect of RBPH on fasting blood glucose at 16 weeks of treatment. Data expressed as mean ± S.E.M. (N=5-8/group). * $p<0.05$ vs. control, $^{\#}p<0.05$ vs. MS, $^{\$}p<0.05$ vs. MS with RBPH 250 mg/kg.

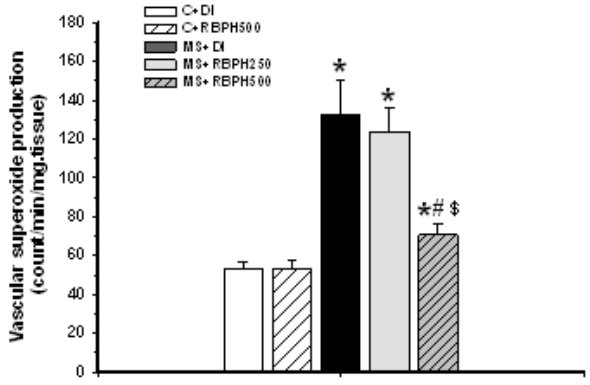

Fig. 3 Effect of RBPH on $O_2^{\cdot-}$ production in carotid arteries of MS rats. Data expressed as mean ± S.E.M. (N=5-8/group). *$p<0.05$ vs. control, $^{\#}p<0.05$ vs. MS, $^{\$}p<0.05$ vs. MS with RBPH 250 mg/kg.

Acknowledgments

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Proceeding

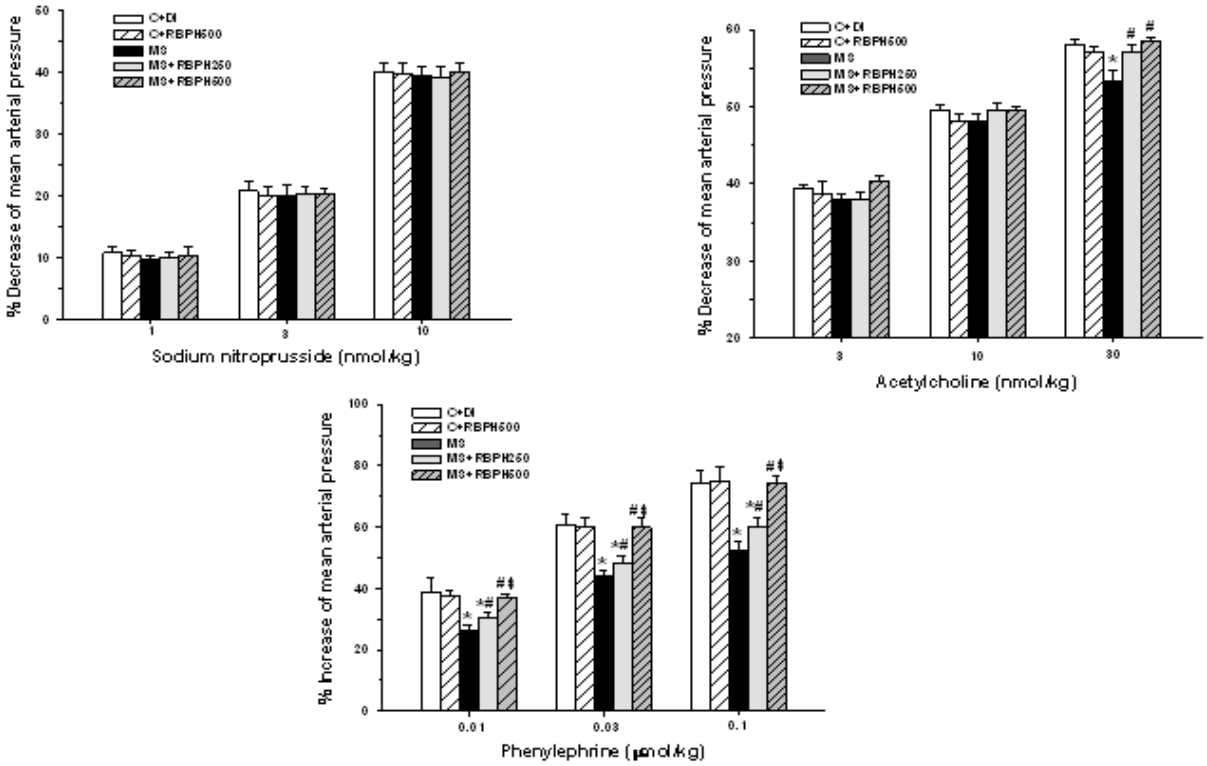

Fig. 4 Effect of RBPH on arterial pressure responses to vasoactive substances (SNP, ACh, Phe) of rats in all experimental groups. Data expressed as mean ± S.E.M. (N=5/group). *p<0.05 vs. control, #p<0.05 vs. MS group, $p<0.05 vs. MS with RBPH 250 mg/kg.

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