

Antihypertensive and Antioxidative Effects of Rice Bran Peptides in a Rat Model of Nitric Oxide-Deficient Hypertension

**ผลต้านความดันเลือดสูงและต้านภาวะเครียดออกซิเดชันของเปปไทด์รำข้าวในหนูแรท
รูปแบบความดันเลือดสูงจากการขาดไนตริกออกไซด์**

Orachorn Boonla (อรัชพร บุญลา)* Dr. Upa Kukongviriyapan (ดร.อุพา คู่คงวิริยพันธุ์)**

Dr. Pongrat Pakdeechote (ดร.พวงรัตน์ ภักดีโชติ)*** Dr. Veerapol Kukongviriyapan (ดร.วีรพล คู่คงวิริยพันธุ์)****

Dr. Patchareewan Pannangpetch (ดร.พัชรวิทย์ ปั่นแห่งเพ็ชร)*****

Dr. Supawan Thawornchinsombat (ดร.ศุภวรรณ ถาวรชินสมบัติ)*****

ABSTRACT

The present study aimed to investigate the effect of rice bran peptides (RBP) on reduction of hypertension, oxidative stress and endothelial dysfunction in NO deficient hypertensive rats. Hypertension was induced in male Sprague-Dawley rats by administration of N^G -nitro-L-arginine methyl ester (L-NAME) at 50 mg/kg body weight/day in drinking water for 3 weeks. Animals were randomly assigned into 5 groups: normal control + deionized water (DI), normal control + RBP 100 mg/kg/day, L-NAME + DI, L-NAME + RBP 50 mg/kg/day, and L-NAME + RBP 100 mg/kg/day, respectively. It is found that RBP in a dose-dependent manner significantly reduced blood pressure, superoxide production, oxidative stress, and enhanced vasorelaxation to acetylcholine in L-NAME treated rats as compared to L-NAME treated controls ($P < 0.05$). Results of this study suggest that RBP possesses antihypertensive effect through a several factors that contribute to decreased oxidative stress and improved endothelial function.

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบผลของเปปไทด์รำข้าว (RBP) ต่อการลดความดันเลือด ลดภาวะเครียดออกซิเดชัน และลดภาวะเซลล์เอ็นโดทีเลียมทำงานผิดปกติในหนูแรทความดันเลือดสูงจากการขาดไนตริกออกไซด์ ภาวะความดันเลือดสูงจะเหนี่ยวนำในหนูแรท เพศผู้ พันธุ์ Sprague-Dawley ด้วยสารแอลเนม (L-NAME) ขนาด 50 มก./กก. น้ำหนักตัว/วัน ผสมในน้ำดื่ม เป็นเวลา 3 สัปดาห์ หนูทดลองถูกสุ่มและแบ่งออกเป็น 5 กลุ่ม ได้แก่ หนูทดลองปกติที่ได้รับน้ำปราศจากไอออน (deionized water; DI), หนูทดลองปกติที่ได้รับ RBP 100 มก./กก./วัน, หนูความดันเลือดสูง L-NAME ที่ได้รับ DI, หนูความดันเลือดสูง L-NAME ที่ได้รับ RBP 50 มก./กก./วัน, และ หนูความดันเลือดสูง L-NAME ที่ได้รับ RBP 100 มก./กก./วัน ผลการศึกษาพบว่า RBP ตามขนาดความเข้มข้นที่ให้แก่หนูความดันเลือดสูง L-NAME สามารถลดความดันเลือด ลดการสร้าง superoxide anion ลดภาวะเครียดออกซิเดชัน และเพิ่มการคลายตัวของหลอดเลือดต่อ acetylcholine ผลการศึกษานี้บ่งชี้ว่า RBP มีฤทธิ์ต้านภาวะความดันเลือดสูงโดยมีผลผ่านหลายปัจจัยที่ส่งผลลดภาวะเครียดออกซิเดชันและเพิ่มการทำงานของเซลล์เอ็นโดทีเลียม

Key Words: L-NAME hypertension, Oxidative stress, Rice bran peptides

คำสำคัญ: แอลเนมความดันเลือดสูง ภาวะเครียดออกซิเดชัน เปปไทด์รำข้าว

* Student, Doctor of Philosophy Program in Medical Physiology, Department of Physiology, Faculty of Medicine, Khon Kaen University

** Associate Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University

*** Assistant Professor, Department of Physiology, Faculty of Medicine, Khon Kaen University

**** Associate Professor, Department of Pharmacology, Faculty of Medicine, Khon Kaen University

***** Assistant Professor, Faculty of Technology, Khon Kaen University

Introduction

Rice is the main staple food of the Asian population, and also the largest staple product exporter of Thailand. Rice bran, an outer layer of brown rice obtained from a byproduct derived from rice milling industry, is a good source of fat and protein (Sereewatthanawut et al., 2008). Previous studies reported that rice bran contains a high nutritive value, for instance, tocopherols, tocotrienols, oryzanols, vitamin B complex and phenolic compounds (Min et al., 2011; Zhang et al., 2010). The benefits such as rice bran oil consumption decreased total serum cholesterol concentrations in patients with type 2 diabetes (Lai et al., 2012). Beside the rice bran oil, peptides-derived from rice bran (RBP) has been investigated in the *in vitro* and found the effects on inhibiting angiotension converting enzyme (ACE) activity and scavenging free radicals (Kokkaew and Thawornchinsombut, 2011). Moreover, RBP also exert the vasorelaxant effect on the aortic rings isolated from 2K-1C hypertensive rat (Tuangpholkrung et al., 2012).

It has been reported that chronic inhibition of NO by administration of L-NAME, a NO synthase inhibitor, causes hypertension, increases endothelial dysfunction and oxidative stress (Kitamoto et al., 2000; Nakmareong et al., 2011; Priviero et al., 2007). In addition, many documents reported that the development of hypertension is associated with a reduction of NO and excessive reactive oxygen species (ROS) production. Therefore, protection of NO depletion and reduced ROS generation might be the possible therapeutic strategy that could prevent hypertension.

Objectives of the study

The present study investigated the protective effect of RBP on reduction of hypertension, oxidative stress and endothelial dysfunction in L-NAME-induced hypertensive rats.

Methodology

RBP was obtained from Dr. Supawan Thawornchinsombat, Faculty of Technology, Khon Kaen University, Thailand. Male Sprague-Dawley, weighing 220- 250 g, were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Animals were kept in the Northeast Laboratory Animal Center, Khon Kaen University, Thailand, under standard conditions (light/dark cycle; 12 h, humidity; 30-60%, temperature; $23 \pm 2^\circ\text{C}$, air changing; 10-15 cycle/h). All procedures were approved by the Institutional Animal Ethics Committee of Khon Kaen University. Hypertension was induced in rats by administering L-NAME (50 mg/kg/day) in drinking water for 3 weeks. RBP (50 or 100 mg/kg/day) was intragastrically administered to animals simultaneously with or without L-NAME. Rats were divided into 5 groups ($n = 6$): normal control treated with DI as vehicle, normal control treated with RBP (100 mg/kg/day), hypertension treated with DI, hypertension treated with RBP (50 mg/kg/day), and hypertension treated with RBP (100 mg/kg/day).

Blood pressure measurement and biochemical assay

Systolic blood pressure (SBP) was measured weekly by using rat tail-cuff plethysmography (Blood pressure analyzer, model 179; IITC, Woodland Hills, California, USA).

At the end of experimental period, rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). A tracheotomy was performed for spontaneous breathing and the left femoral artery was cannulated with a polyethylene catheter connected to a pressure transducer for continuous monitoring of arterial blood pressure (BP) and heart rate (HR) and using the AcqKnowledge data acquisition (BIOPAC system Inc., California, U.S.A.). After measuring the baseline values of HR and BP for 30 min, rats were sacrificed by overdose of an anesthetic drug. Blood samples were collected from the abdominal aorta for assessment of plasma malondialdehyde (MDA), a lipid peroxidation marker (Nakmareong et al., 2011). The carotid arteries were rapidly excised for measurement of superoxide production by using Lucigenin-enhanced chemiluminescence technique (Nakmareong et al., 2011). The thoracic aorta was excised for evaluating the endothelium-dependent relaxation to acetylcholine (ACh) and sodium nitroprusside (SNP).

Preparation of the isolated aortic rings

The thoracic aortas were immediately cleaned of surrounding fat and connective tissues, cut into 3-4 mm rings. Each ring was suspended between the two stainless steel hooks in water-jacketed bath containing physiological salt solution (PSS) at 37°C containing (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.18, Glucose 11, NaHCO_3 25 and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.5 (pH 7.4); bubbled with 95% O_2 and 5% CO_2 . A tension of 1 g was initially applied to the ring which was equilibrated for 90 min. The system was connected to an isometric force transducer recording system (PowerLab System, AD Instruments, Australia). To examine endothelium-dependent and endothelium-independent relaxation, the concentration

responses to ACh and SNP ranging from 10^{-9} to 10^{-5} M were tested after pre-contraction with phenylephrine (PE) (1 μM).

Data analysis

Results were expressed as means $\pm$ S.E.M., and n indicates the number of animals. The differences among experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc comparison test. *P* value of less than 0.05 was taken as significant.

Results

RBP attenuated blood pressure of L-NAME hypertensive rats.

At the beginning of the experiments, baseline SBP was similar in all experimental groups (Table 1; $p < 0.05$). SBP progressively increased in L-NAME hypertensive rats throughout the study period of 3 weeks. A daily RBP supplementation (50 or 100 mg/kg) showed a significant reduction of SBP in L-NAME treated rats treated with RBP ($P < 0.05$). There was no change in SBP in normal control and control +RBP groups (Table 1). The mean arterial pressure (MAP) of L-NAME rats treated with RBP was significantly lower than L-NAME controls (Figure 1; $P < 0.05$). These results indicate the antihypertensive effect of RBP. Whereas no significant differences in MAP were seen in normal control and normal control treated with RBP, suggesting that RBP had no hypotensive effect in normotensive rats (Figure 1).

RBP alleviated oxidative stress in L-NAME hypertensive rats

The levels of superoxide production in carotid arteries and plasma MDA were significantly increased in L-NAME treated rats when compared with normal

controls (Figure 2A and B; $P<0.05$), suggesting the occurrence of oxidative stress in L-NAME hypertensive rats. RBP (50 and 100 mg/kg) ameliorated oxidative stress by reducing $O_2^{\bullet-}$ production and decreasing the MDA levels (Figure 2A and B; $P<0.05$). Interestingly, it is found that a reduction in oxidative stress is associated with a decrease in SBP of L-NAME rats treated with RBP.

Table 1 Systolic blood pressure measured by tail-cuff method throughout the study period in all experimental groups.

Group	Systolic blood pressure (mmHg)			
	Baseline	1 week	2 week	3 week
Control	115.67 $\pm$ 3.82	128.50 $\pm$ 6.33	129.00 $\pm$ 1.78	123.50 $\pm$ 4.71
Control+RBP100	119.73 $\pm$ 3.84	122.67 $\pm$ 3.10	122.60 $\pm$ 2.48	121.00 $\pm$ 2.93
L-NAME	118.17 $\pm$ 3.84	155.50 $\pm$ 6.38*	176.83 $\pm$ 4.01*	203.50 $\pm$ 7.55*
L-NAME+RBP50	112.47 $\pm$ 5.58	135.40 $\pm$ 5.67*	146.58 $\pm$ 3.07*†	155.00 $\pm$ 4.15*†
L-NAME+RBP100	113.80 $\pm$ 1.33	117.75 $\pm$ 4.39 [‡]	145.83 $\pm$ 3.78*†	148.25 $\pm$ 4.63*†

Data are shown as means $\pm$ S.E.M. (n= 6 /group), * $p<0.05$ vs. control groups, † $p<0.05$ vs. L-NAME group and # $p<0.05$ vs. L-NAME+RBP 50 group.

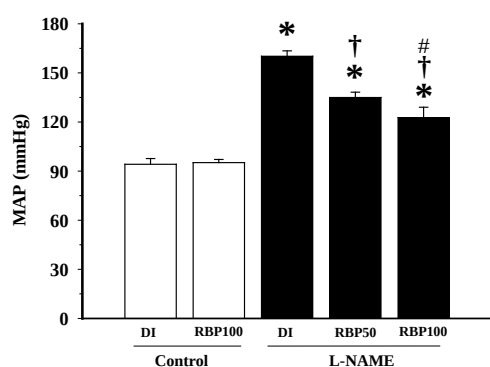


Figure 1 Effect of RBP on mean arterial pressure in all experimental groups. Data are shown as means $\pm$ S.E.M. (n= 6 /group), * $p<0.05$ vs. control groups, † $p<0.05$ vs. L-NAME group and # $p<0.05$ vs. L-NAME +RBP 50 group.

RBP improved endothelium-dependent relaxation to ACh in aortic ring.

A significant blunted response to ACh was found in the aortic rings isolated from L-NAME treated rats when compared with arteries from the normal controls (Figure 3; $p<0.05$), indicating the impairment of the endothelium-dependent relaxation after L-NAME treatment. Increased vasorelaxation to ACh was found in the aortas of L-NAME rats treated with RBP at dose of 100 mg/kg (Figure 3; $P<0.05$). There were no significant differences in endothelium-independent relaxation induced by SNP in all study groups (data not show).

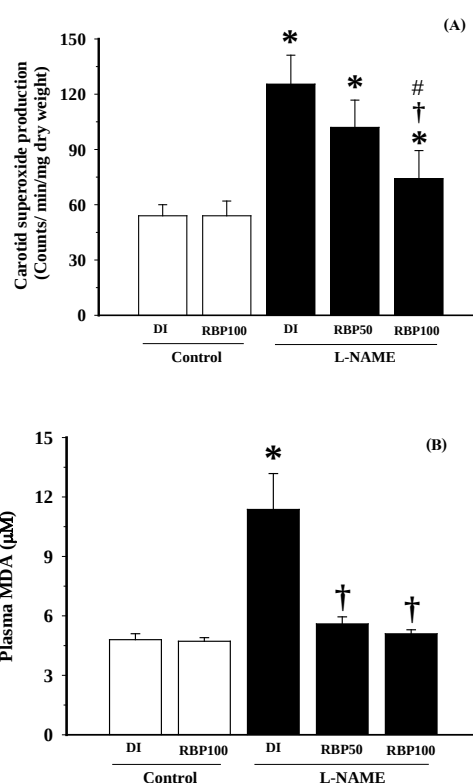


Figure 2 Effect of RBP on superoxide production in the carotid arteries (A) and plasma MDA (B) in all experimental groups. Data are shown as means $\pm$ S.E.M. (n= 6 /group), * $p<0.05$ vs. control groups, † $p<0.05$ vs. L-NAME group and # $p<0.05$ vs. L-NAME +RBP 50 group.

Discussion and Conclusions

In the present study, we found the disruption of NO generation initiated by L-NAME. Rats received L-NAME showed a significantly increased blood pressure, increased oxidative stress, and impaired endothelium-dependent relaxation. Administration of RBP 50 and 100 mg/kg/day attenuated hypertension and reduced oxidative stress in L-NAME treated rats. Moreover, L-NAME rats treated with RBP 100 mg/kg/day improved endothelial function by enhancing endothelium-dependent relaxation to ACh.

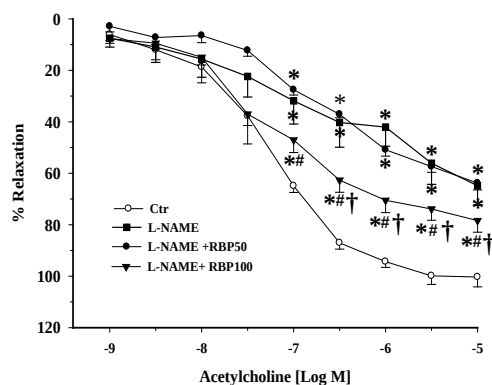


Figure 3 Effect of RBP on endothelium-dependent relaxation induced by ACh in aortic rings of rats in all experimental groups. Data are shown as means $\pm$ S.E.M. ($n = 6$ /group), $*p < 0.05$ vs. control groups, $\dagger p < 0.05$ vs. L-NAME group and $\#p < 0.05$ vs. L-NAME + RBP 50 group.

Previous studies have reported that L-NAME-induced high BP is correlated with the overproduction of vascular superoxide. Several lines of evidence support the concept that increased vascular superoxide production is associated with increased expression of NADPH oxidase, a major source of vascular superoxide generation in hypertensive rats (Gonzalez et al., 2000; Sarr et al., 2006). Excessive production of superoxide rapidly scavenges NO

resulting formation of peroxynitrite (ONOO⁻) which reduced NO bioavailability (Torok, 2008) and causing oxidative damage and endothelial dysfunction. Interestingly, we demonstrated that RBP reduced superoxide production and also improved endothelium-dependent relaxation to ACh. These effects are likely to be related to a restoration of NO bioavailability. Furthermore, the relaxant response to sodium nitroprusside, which is an NO donor, was comparable among experimental groups. These results confirm impairment of endothelial-dependent relaxation in L-NAME hypertensive rats.

It is well known that endothelium plays an important role in modulating the vascular tone and blood pressure. Therefore, increased NO bioactivity and improved endothelial-dependent relaxation during treatment with RBP could, in part, ameliorate hypertension in rats with NO synthesis inhibition. Moreover, previous studies demonstrated that many peptides derived by hydrolysate from food protein possess ACE-inhibiting activity and antioxidant effect, such as corn (Yang et al., 2007), whey protein (Tavares et al., 2012), sardine muscle (Otani et al., 2009) and also peptide derived from rice grain (Li et al., 2007) and rice bran (Kokkaew and Thawornchinsombut, 2011). Thus, the ACE-inhibiting activity might explain the antihypertensive effect of RBP in this animal model.

In conclusion, overall findings of this study suggest that RBP possesses antihypertensive effect through several factors that contribute to decreased oxidative stress and improved endothelial function.

Acknowledgements

This study was supported by grants from the Graduate School of Khon Kaen University (Grant No. 55212103) and the Graduate grant from the National Research Council of Thailand. Orachorn Boonla was supported by the Royal Golden Jubilee Ph.D. program, The Thailand Research Fund.

References

- Gonzalez, W., Fontaine, V., Pueyo, ME., Laquay, N., Messika-Zeitoun, D., Philippe, M., Arnal, JF., Jacob, MP., Michel, JB. 2000. Molecular plasticity of vascular wall during N(G)-nitro-L-arginine methyl ester-induced hypertension: modulation of proinflammatory signals. *Hypertension*. (36): 103-109.
- Kitamoto, S., Egashira, K., Kataoka, C., Usui, M., Koyanagi, M., Takemoto, M., Takeshita, A. 2000. Chronic inhibition of nitric oxide synthesis in rats increases aortic superoxide anion production via the action of angiotensin II. *J. Hypertens*. (18): 1795-1800.
- Kokkaew, H., Thawornchinsombut, S. 2011. Effect of stimulated gastrointestinal digestion on the ACE inhibitory activity of peptides derived from Thai Hom-Mali rice bran. *Journal of Physiological and Biomedical Sciences*. (24): 171.
- Lai, MH., Chen, YT., Chen, YY., Chang, JH., Cheng, HH. 2012. Effects of rice bran oil on the blood lipids profiles and insulin resistance in type 2 diabetes patients. *J. Clin. Biochem. Nutr*. (51): 15-18.
- Li, GH., Qu, MR., Wan, JZ., You, JM. 2007. Antihypertensive effect of rice protein hydrolysate with in vitro angiotensin I-converting enzyme inhibitory activity in spontaneously hypertensive rats. *Asia. Pac. J. Clin. Nutr*. (16 Suppl 1): 275-280.
- Min, B., McClung, AM., Chen, MH. 2011. Phytochemicals and antioxidant capacities in rice brans of different color. *J. Food. Sci*. (76): C117-126.
- Nakmareong, S., Kukongviriyapan, U., Pakdeechote, P., Donpunha, W., Kukongviriyapan, V., Kongyingyoes, B., Sompamit, K., Phisalaphong, C. 2011. Antioxidant and vascular protective effects of curcumin and tetrahydrocurcumin in rats with L-NAME-induced hypertension. *Naunyn Schmiedebergs Arch Pharmacol*. (383): 519-529.
- Otani, L., Ninomiya, T., Murakami, M., Osajima, K., Kato, H., Murakami, T. 2009. Sardine peptide with angiotensin I-converting enzyme inhibitory activity improves glucose tolerance in stroke-prone spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem*. (73): 2203-2209.
- Priviero, FB., Teixeira, CE., Claudino, MA., De Nucci, G., Zanesco, A., Antunes, E. 2007. Vascular effects of long-term propranolol administration after chronic nitric oxide blockade. *Eur. J. Pharmacol*. (571): 189-196.

- Sarr, M., Chataigneau, M., Martins, S., Schott, C., El Bedoui, J., Oak, MH., Muller, B., Chataigneau, T., Schini-Kerth, VB. 2006. Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. *Cardiovasc. Res.* (71): 794-802.
- Sereewatthanawut, I., Prapintip, S., Watchiraruij, K., Goto, M., Sasaki, M., Shotipruk, A. 2008. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *Bioresour. Technol.* (99): 555-561.
- Tavares, T., Sevilla, MA., Montero, MJ., Carron, R., Malcata, FX. 2012. Acute effect of whey peptides upon blood pressure of hypertensive rats, and relationship with their angiotensin-converting enzyme inhibitory activity. *Mol. Nutr. Food. Res.* (56): 316-324.
- Torok, J. 2008. Participation of nitric oxide in different models of experimental hypertension. *Physiol. Res.* (57): 813-825.
- Tuangpholkrun, P., Boonla, O., Pakdeechote, P., Kukongviriyapan, V., Pannangpetch, P., Thawornchinsombut, S., Kukongviriyapan, U. 2012. Endothelium-dependent vasodilating effect of rice bran peptides. *Journal of Physiological and Biomedical Sciences.* (25): 59.
- Yang, Y., Tao, G., Liu, P., Liu, J. 2007. Peptide with angiotensin I-converting enzyme inhibitory activity from hydrolyzed corn gluten meal. *J. Agric. Food. Chem.* (55): 7891-7895.
- Zhang, MW., Zhang, RF., Zhang, FX., Liu, RH. 2010. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J. Agric. Food. Chem.* (58): 7580-7587.