Peptides-Derived from Thai Rice Bran Improve Hemodynamics and Induce Vasorelaxation in Renovascular Hypertensive Rats

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Abstract

The present study aimed to investigate the protective effect of Thai rice bran peptides (RBP)-derived from byproduct of rice bran oil manufacture on development of hypertension and hemodynamic alterations in two kidney-one clip (2K-1C) hypertensive rats. Moreover, the vasorelaxant effect of RBP on aortic rings isolated from these animals was also evaluated. Male Sprague-Dawley rats were induced 2K-1C hypertension by placing a silver clip (0.2 mm i.d.) around the left renal artery, whereas sham-operated rats were served as control animals. Three days after surgery, 2K-1C rats and sham-operated rats were intragastrically administered with RBP (250 or 500 mg/kg/day) or vehicle continuously for 6 weeks. After 6 weeks of treatment, it was found that RBP significantly reduced blood pressure and improved hemodynamic status of 2K-1C rats by increasing hindlimb blood flow and decreasing hindlimb vascular resistance ($P<0.01$). In addition, RBP in a concentration-dependent manner caused relaxation of the aortic rings-precontracted with phenylephrine in 2K-1C rats, and the relaxant effect was found to depend on the endothelium. Our results suggest that peptides-derived from byproduct of rice bran oil manufacture normalizes hypertension of 2K-1C hypertensive rats through the vasodilatory actions on the vasculature of 2K-1C hypertensive rats.

Keywords: rice bran peptide, 2K-1C hypertension, vasorelaxation, hidelimb blood flow, hidelimb vascular resistance

1. Introduction

Rice (*Oryza sativa* L.) 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, and presently utilized as a food material [1]. Previous studies reported that peptide-derived from rice bran (RBP) contains large amounts of fiber and bioactive phytochemicals, for instance, tocopherols, tocotrienols, oryzanols, vitamin B complex and phenolic compounds [2].

The renin-angiotensin aldosterone system (RAAS) plays a key role in the regulation of blood pressure and electrolytes. Angiotensin converting enzyme (ACE) catalyzed angiotensin I to potent vasoconstrictor angiotensin II and inactivates vasodilator bradykinin as well [3]. It is suggested that angiotensin II, a major component of RAAS, plays a key role in development of hypertension. Therefore, inhibition of ACE activity, which inhibits the generation of angiotensin II, causes a reduction in blood pressure.

Hypertension is a chronic disease, which is facing challenges of the world population. Long term

antihypertensive drug treatment is a usual method for controlling blood pressure. In contrary, food rather than drugs are preferred for preventing diseases. Moreover, the hydrolysate peptides-derived from varieties of food protein have been found to posses the ACEI activity [4]. In vitro studies demonstrated that RBP possess antioxidant and antihypertensive effect through the angiotensin converting enzyme inhibiting activity (ACEI activity) [5, 6]. Therefore, the present study was aimed to evaluate the protective effect of RBP on development of hypertension and hemodynamic alterations in 2K-1C hypertensive rats. This animal model is imitative of renovascular hypertension in humans [7]. A direct vasodilator effect of RBP was also determined on the aortic rings isolated from 2K-1C hypertensive rats.

2. Methods

2.1 Animal and surgery

All procedures were approved by the Institutional Animal Care and Use Committee, Khon Kaen University (AEKKU17/2553). Male Sprague-Dawley rats (180-200 g) obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nokhon Pathom, Thailand. Induction ofrenovascular hypertension was carried out in rats according to the 2K-1C model originally described by Goldblatt [7]. 2K-1C rats were induced by placing a silver clip with an internal diameter of 0.20 mm around the left renal artery. In the Sham-operated control group, animals were performed in similar surgical procedures without clip placement. Animals were maintained on standard rat chow with a 12-h light12-h dark cycle and given free access to both food and water at the Northeast Laboratory Animal Center, Khon Kaen University.

2.2 Measurement of the vasorelaxation

Six weeks after surgery, 2K-1C and sham-operated rats were anesthetized with an intraperitoneal injection of pentobarbitone sodium (60 mg/kg BW). The arterial blood pressure and heart rate were measured through the left femoral artery. Rat was sacrificed by over dosage of the anesthetic drug. The thoracic aortas were immediately removed and cut into 3 mm in length. 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, $MgSO_4.7H_2O$ 1.18, Glucose 11, $NaHCO_3$ 25 and $CaCl_2.2H_2O$ 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 60 min. After equilibration, the aortic rings were precontracted with 1μM phenylephrine (PE). When the steady contraction was reached, 1 μM acetylcholine (ACh) was added to the bath to assess endothelium integrity. The relaxant effect of RBP on PE 1μM pre-contracted aortic rings was examined. When contraction had reached a steady-state (considered as 100% and was defined as control), RBP (0.003-3mg/ml) was subsequently added to the bath. The effect of RBP was evaluated as the percentage of relaxation of the PE-induced contraction. In some experiments, the vasorelaxant effect of RBP was examined in the denuded aortic rings. The endothelium was removed by gently rubbing the lumen side of the aortic rings and the removal of endothelium was tested by the absence of relaxation in response to 1 μM ACh.

2.3 Measurement of the hemodynamics

After three days, 2K-1C rats and sham-operated rats were intragastrically administration with RBP at dose of 500 or 250 mg/kg/day or deionization water as the vehicle for 6 weeks. The body weight and systolic blood pressure (SBP) were measured in non-anesthetized animals by an indirect tail-cuff method. (IITC model 179 blood pressure analyzer, Life science, U.S.A.).

At the end of the experiment, the animals were anesthetized with pentobarbitone sodium (60 mg/kgBW, 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 heart rate and arterial blood pressure, using the AcqKnowledge data acquisition and analysis software (BIOPAC system Inc., California, U.S.A.). The hindlimb blood flow (HBF) was continuously measured by placing an electromagnetic flow probe around the abdominal aorta connected to the electromagnetic flow meter (Carolina Medical Electronics, NC, USA). Hindlimb vascular resistance (HVR) was calculated from mean arterial pressure (MAP) divided by HBF in 100 g tissue unit.

2.4 Data analysis

Results were expressed as means ± 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 the Tukey test. *P* value of less than 0.05 was taken as significant.

3. Results

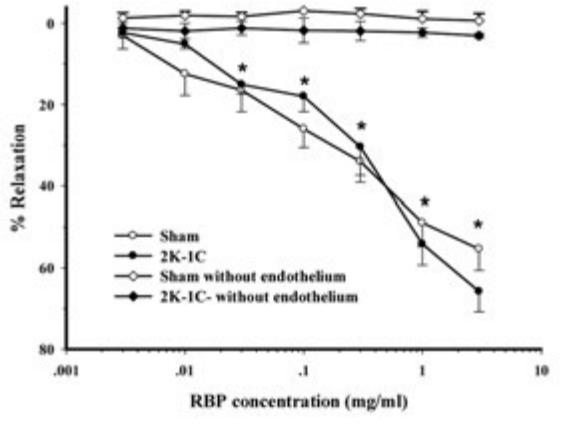


Figure 1 Concentration-response curve to RBP in aortas with or without endothelium from 2K-1C and sham-operated pre-contracted with phenylephrine. Values are means ± S.E.M., and are expressed as percentage of relaxation (n= 5-6/group). *p <0.05 versus in the presence of endothelium.

RBP at doses ranging from 0.03-3 mg/mL induced a concentration-dependent relaxation of the PE-precontracted aortic rings from 2K-1C and sham-operated rats. The concentration required to induce the half maximal effect (IC50) in 2K-1C and sham operated rats are 0.2 and 0.3 mg/mL respectively. There was no significant difference in relaxation of the aortic rings from either 2K-1C or sham-operated rats. In the aortic rings without endothelium, addition of RBP to the bath solution caused no relaxation, indicating that the relaxant effect of RBP requires the endothelium (Fig. 1).

Table 1 Effect of RBP on body weight and arterial blood pressure.

	Sham	Sham+RBP500	Hypertensive rats		
			2K-1C	2K-1C+RBP250	2K-1C+RBP500
BW (g)	322.33 +20.25	333.75 + 12.48	326.4 + 25.27	342.50 + 12.28	322.67 + 20.22
SBP (mmHg)	122.74 + 7.18	128.42 + 5.57	195.47 + 11.38*	152.37 + 8.89*#	144.95+5.82*#
DBP (mmHg)	77.22 + 4.81	80.32 + 5.10	129.27 + 4.28*	97.59 + 8.28*#	89.06 + 7.39#
MAP (mmHg)	95.93 + 5.34	100.43 + 4.65	157.78 + 7.25*	122.59 + 8.05*#	115.25 +7.35*#
HR (beat/min)	350.49 ± 4.29	345.74 ± 4.41	375.83 ± 13.94	371.05 ± 27.28	365.84 ± 5.06

Values are means ± S.E.M. (n= 5-6/group). BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. *p<0.05 versus sham, #p <0.05 versus 2K-1C.

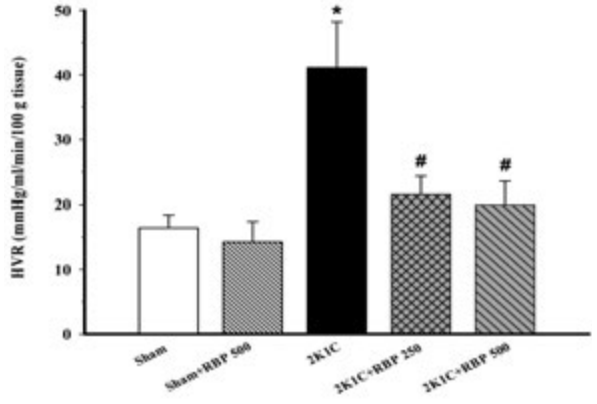
Before clipping the left renal artery, there was no significant difference in the basal values of blood pressure and body weight among all experimental groups (data not show). Six weeks after surgery, SBP, diastolic blood pressure (DBP), and mean arterial pressure (MAP) were markedly increased in 2K-1C rats when compared with sham operated rats as shown in Table 1. Moreover, there was a dramatically decreased in HBF in 2K-1C rats (Fig. 2A). Changes in MAP and HBF after renal clipping cause increased HVR up to 2.5-fold (Fig. 2B). Interestingly, administration of RBP at doses of 250 and 500 mg/kg significantly reduced SBP, DBP, MAP and HVR whereas HBF was increased in 2K-1C rats as compared to 2K-1C untreated controls, suggesting that RBP normalizes hypertension and prevents hemodynamics disturbance in this model of hypertension.

4. Discussion

The development of hypertension in 2K-1C renovascular hypertensive rats is very similar to that of renovascular hypertension in humans [7]. In this study, hypertension and hemodynamic disturbances were presented in renal artery clipped rats. Oral administration of peptides derived from rice bran protein for six weeks reduced blood pressure, decreased HVR and increased HBF of renovascular hypertensive rats. Moreover, RBP dose dependently induced aortic smooth muscle cells relaxation of 2K-1C rats.

Previous studies demonstrated that peptides hydrolysates from different types of food protein posses *in vitro* ACE inhibiting effect [4]. Interestingly, some of these peptides showed an *in vivo* ACE inhibiting activity and antihypertensive property in spontaneous hypertensive rats (SHR) and hypertensive humans [8].

(A)



(B)

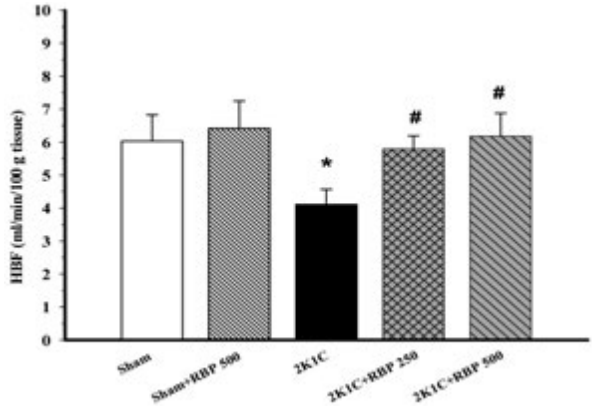


Figure 2 Effect of RBP on HBF (A) and HVR (B). Data express as means ± S.E.M. (N= 5-6/group). **p*<0.05 versus sham, #*p*<0.05 versus 2K-1C.

Results of our study support previous findings that RBP exhibit antihypertensive activity with ACE-inhibitory and vasodilator effects.

5. Conclusion

The principal finding of this study is that RBP induces relaxation of the thoracic aortas from 2K-1C and sham-operated rats, and the relaxant effect acts through the endothelium-dependent pathway. Long-term treatment of RBP attenuates hypertension and improves hemodynamic changes in renovascular hypertensive rats. The overall findings of this study suggest the beneficial effect of peptides-derived from a by-product of rice bran oil manufacture on reduction in blood pressure and adjustment of hemodynamic status in renovascular hypertensive rats. The plausible mechanism of action of RBP might be through the vasodilator effect on the vasculature of the renovascular hypertension.

Acknowledgments

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