Rice Bran Peptide <5 kDa Fraction Expresses Anticoagulant Activity

Paisal Mongkolwongroj [1,2],
Wanida Phonanurakwong[1], Yuniyati Samoh[1], Nantarat Komanasin [1,2],
Supawan Thawornchinsombat[2,3]
[1] Faculty of Associated Medical Sciences, Khon Kaen University
[2] Cardiovascular Research Group, Khon Kaen University
[3] Faculty of Technology, Khon Kaen University
123 Mittraprab Road, Khon Kaen 40002
Tel:0-4320-2087
Email:paisal@kku.ac.th

Abstract

Antioxidant, anticancer and ACE inhibiting activities of rice bran protein/peptide have been reported. This study examines the <5 kDa fraction of rice bran peptide for its anticoagulant activity. Anticoagulant activity of Jasmin 105 rice bran peptide MW < 5kDa fraction was assessed by incubating rice bran peptide at concentrations from 0.025 to 64 mg/mL in normal pooled plasma, and Prothrombin time (PT) as well as Activated Partial Thromboplastin Time (APTT) clotting times were determined by automated coagulation analyzer, Sysmex CA 1500. Plasma without rice bran peptide and with 0.25 IU/mL of heparin were used as controls. Prolongation of APTT were clearly observed corresponding with increasing peptide concentrations, however, the anticoagulant effects on PT were seen only at high concentrations of peptide (32 and 64 mg/mL). The anticoagulant activity of the peptide is not time and temperature dependent. This study provides the evidence of anticoagulant activity of rice bran peptide in *in vitro* system, yet its role in *in vivo* remains to be investigated.

Keywords: rice bran peptide, anticoagulant activity

1. Introduction

Rice (*Oryza Sativa*) is the major food for the world's population and it is a good source of carbohydrate, protein and fat. Rice bran is an under-utilized product from the milling process which composed of the pericarp and the embryo of the rice. This byproduct, however, contains many biological active compounds that may have industrial as well as health impacts [1]. Current evidences demonstrated that rice bran oil constituents, gamma-oryzanol, phytosterols and tocopherol exhibited anti-hyperlipidemic effect in both animal and human studies [2], [3]. As for the rice bran protein, emerging data suggested that protein hemolysate/peptide may have antioxidant, anti-hypertensive, anticancer, antithrombotic, as well as angiotensin-converting enzyme (ACE) inhibiting effects [2]-[10]. Furthermore, antioxidant and ACE inhibiting effects of the rice bran peptide were found stronger in the smaller molecular weight fraction (< 5 kDa) than in the higher molecular weight peptides [11]. Interestingly, antithrombotic effect of rice bran was demonstrated by furulate derivatives acting on platelet to prevent platelet aggregation [2], [12]. Antithrombotic peptide from hydrolysis of casein has also been reported, and it inhibited both ADP-activated platelet aggregation as well as the binding of fibrinogen to platelet surface. Therefore this study aims to explore the anticoagulant activity of rice bran peptide <5kDa fraction in *in vitro* system.

2. Methods

Rice bran peptide was prepared according to the published method [11]. Briefly, defatted rice bran was alkalinized with pH adjustment and proteolysis using Protex 6L enzyme. The protein hydrolysate was obtained by centrifugation at 12,000 g for 30 minutes at 4°C. The peptide was further processed by membrane ultra filtration and <5 kDa fraction was collected and spray dried. Normal pooled plasma was prepared according to NCLLS guideline [13]. Lyophilized rice bran peptides were added into the plasma at the final concentration of 0.025, 0.05, 0.1, 0.5, 1, 2, 4, 8, 32 and 64 mg/mL. and the anticoagulant effect was assessed by 2 clotting assays namely Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT) using Thromborel S and Parthomtin, (Dade Behring) as clotting reagents. The clotting times were measured by automated coagulometer, Sysmex CA 1500, Japan. For control, plasma with no added rice bran peptide and with 0.25 IU/mL of high molecular weight heparin (Leo) was employed. To further study the anticoagulant effect of the peptide, plasma without peptide, with peptide concentration of 32 mg/mL and with heparin were continued to be incubated at 37°C for 30 minutes, 1 and 2 hours, and the clotting times were assayed accordingly.

3. Results

The results illustrated the anticoagulant effects of rice bran peptide on both PT and APTT. For PT, only high concentrations of rice bran peptide (32 and 64 mg/mL) prolonged the clotting times, and at 64mg/mL of the peptide demonstrated clotting time approximately 2 times of the control which was similar to the condition of patients who were on warfarin. While heparin at the therapeutic concentration generally did not prolong the clotting time. Anticoagulant effects of rice bran peptide were more clearly demonstrated on APTT. Prolongation of the clotting times were found at concentrations from 8 to 64 mg/mL, and the anticoagulant effects increased correspondingly with the peptide concentrations. The effect of 64 mg/mL peptide was in accordance with the therapeutic ranges of heparin. For heparin control, prolongation of APTT clotting time was demonstrated (Figure 1).
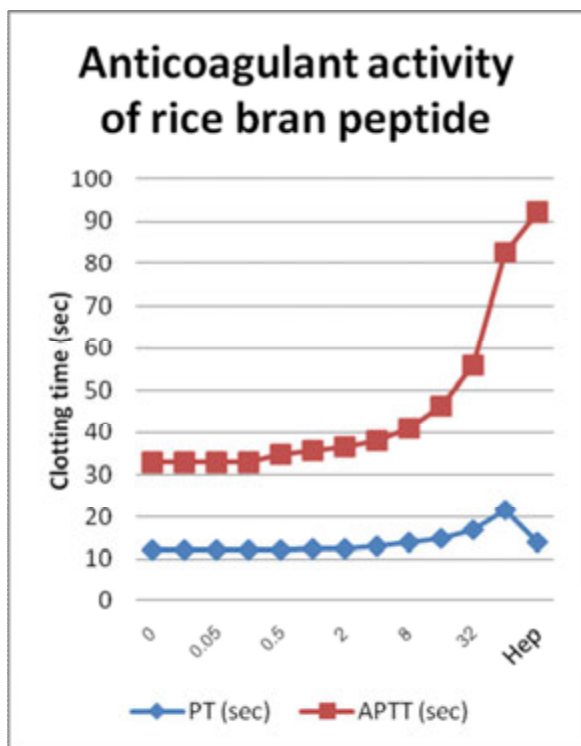

Figure 1. Anticoagulant effects of rice bran peptide on PT and APTT at various concentrations. Plasma with no rice bran peptide and with 0.25 IU/mL of heparin (Hep) were included as controls. Data were shown as mean from triplicate tests.

To understand the nature of anticoagulant effect, plasma without rice bran peptide and with 32 mg/mL rice bran peptide as well as with 0.25 IU/mL heparin were incubated at 37°C for 0, 15, 30 and 60

minutes and PT and APTT were tested. The results demonstrated no effect of time and temperature on the prolongation of both PT and APTT by rice bran peptide. The same results were found in controls without rice bran peptide and with heparin. These results suggest that anticoagulant activity of rice bran peptide was time and temperature independent as shown in Figure 2.
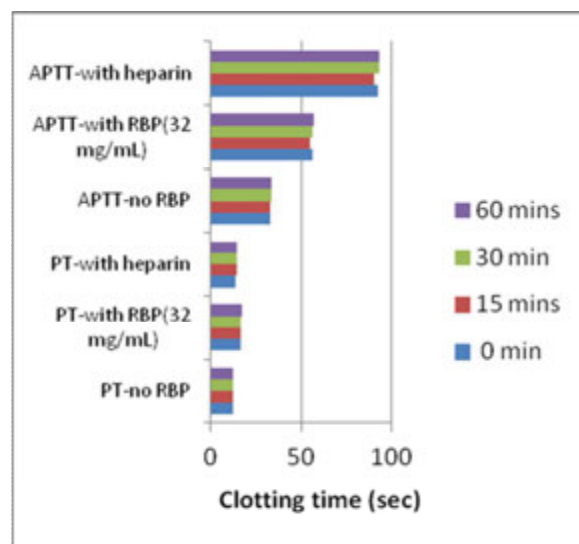

Figure 2. Effect of time and temperature on the anticoagulant activity of rice bran peptide. Data were shown as mean from triplicate tests.

4. Discussion

This study has demonstrated the anticoagulant effect of rice bran peptide <5 kDa fraction in *in vitro* system. The anticoagulant effect was very similar to heparin indicating that it may involve in multiple steps in the clotting cascade. Previous studies reported the antithrombotic effect of rice bran by preventing platelet aggregation [2]. Two bioactive peptides namely Asp-Glu-Glu and Ser-Ser-Gly-Glu from soy bean protein hydrolysates also exhibited anti-platelet aggregation activity [12]. Previous studies by Li *et al* [10] and Kannan *et al* [14],[15] demonstrated the <5 kDa rice bran peptide with amino acid sequence of Thr-Gln-Val-Tyr and Glu-Gln-Gly-Pro-Arg expressed anti-hypertensive and anti-cancer activities respectively. Interestingly, recent report by Hettiarachchy *et al* [15] further illustrated that the <5 kDa, 5-10 kDa rice bran fractions as well as the pentapeptide Glu-Gly-Pro-Arg exhibited inhibitory effects against Alzheimer and obesity. This result suggested another biological function of the rice bran peptide, and it may have potential role in management of

cardiovascular diseases, however, further study in *in vivo* system is needed.

5. Conclusion

This study reported the anticoagulant effect of rice bran peptide <5 kDa in *in vitro* system as demonstrated by prolongation of both PT and APTT, and the anti-clotting activity is time and temperature independent.

6. Acknowledgements

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7. References

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