นิพนธ์ต้นฉบับ • Original Article

ผลของโปรตีนไฮโดรไลเซทชนิด A-2 จากข้าวสีต่อภาวะความจำบกพร่องในหนูแรทที่ถูกฉีดด้วยบีต้าอไมลอยด์

วนัสนันท์ แป้นนางรอง[1,2*], จริยา อำคา เวลบาท[1,3], บังอร ศรีพานิชกุลชัย[2]
[1]ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น ประเทศไทย
[2]ศูนย์วิจัยและพัฒนาผลิตภัณฑ์สุขภาพจากสมุนไพร มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น ประเทศไทย
[3]กลุ่มวิจัยและพัฒนาด้านประสาทวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น ประเทศไทย

Effect of Protein Hydrolysate Type A-2 from Pigmented Rice on Memory Deficits in Ab-injected rats

Wanassanan Pannangrong[1,2*], Jariya Umka Welbat[1,3], Bungorn Sripanidkulchai[2]
[1]Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[2]Center for Research and Development of Herbal Health Products, Khon Kaen University, Khon Kaen, Thailand
[3]Neuroscience Research and Development Group, Khon Kaen University, Khon Kaen, Thailand

หลักการและวัตถุประสงค์: เปปไทด์จากอาหารที่มีฤทธิ์ทางชีวภาพสามารถส่งเสริมสุขภาพหรือลดความเสี่ยงต่อการเกิดโรคได้ สารต้านอนุมูลอิสระจากธรรมชาติถูกนำเสนอว่าเป็นอีกทางเลือกหนึ่งในการรักษาโรคอัลไซเมอร์ โดยมีรายงานว่าข้าวสีมีสารต้านอนุมูลอิสระสูง การศึกษาครั้งนี้เพื่อทดสอบผลของโปรตีนไฮโดรไลเซทชนิด A-2 จากข้าวสีต่อภาวะความจำบกพร่องในหนูทดลองที่ถูกฉีดด้วยบีต้าอไมลอยด์

วิธีการศึกษา: โปรตีนสกัดชนิด A-2 จากข้าวสี ขนาด 10, 20 และ 40 มก./กก. ถูกป้อนให้กับหนูทดลองพันธุ์ Wistar วัยเจริญพันธุ์ เพศผู้ เป็นเวลา 9 สัปดาห์ หลังจากป้อนสาร 56 วัน หนูทดลองถูกฉีดด้วยบีต้าอไมลอยด์เข้าโพรงสมองทั้ง 2 ข้าง หลังจากนั้น 10 วัน จึงประเมินทักษะการเรียนรู้และความจำด้วย Novel object recognition

ผลการศึกษา: พบว่าบีต้าอไมลอยด์มีผลให้เกิดการสูญเสียการเรียนรู้และความจำโดยทำให้ค่าดัชนีการแยกแยะวัตถุลดลง เมื่อเปรียบเทียบกับกลุ่มควบคุมที่ถูกฉีดด้วยบีต้าอไมลอยด์ ขณะที่หนูถูกป้อนโปรตีนสกัดชนิด A-2 ขนาด 20 และ 40 มก/กก น้ำหนัก มีค่าดัชนีการแยกแยะวัตถุที่ 5 นาที และ 24 ชั่วโมงเพิ่มขึ้นชัดเจน บ่งบอกว่าหนูที่ได้รับโปรตีนไฮโดรไลเซทชนิด A-2 จากข้าวสีมีการเรียนรู้ ความจำทั้งระยะสั้นและระยะยาวแบบ recognition ดี

Background and Objective: Bioactive food-derived peptides possess the ability to promote wellness or reduce the risk of diseases. The natural antioxidants have been proposed as alternative therapeutic agents for Alzheimer's disease. It also has been reported that pigmented rice (*Oryza sativa* L.) is a natural antioxidants. Therefore, The present study aimed to determine the neuroprotective effect of protein hydrolysate type A-2 from pigmented rice against β-amyloid (Aβ) injected rats.

Methods: Male adult Wistar rats were orally given aqueous protein hydrolysate type A-2 extract of pigmented rice (HPR) at various doses ranging from 10, 20, and 40 mg/kg BW for 9 weeks. At day 56, Aβ25-35 was injected via both sides of lateral ventricles. After 10 days of Aβ injection, rats were tested for cognitive performance using Novel object recognition tasks.

Results: Aβ25-35 obviously exhibited cognitive deficits by decreasing the discriminative index. When compare to the V plus Aβ group, HPR20-, HPR40-treated rats showed a significantly higher discriminative index during 5 minutes and 24 hours delay testing phase, reflecting the increase of learning, short-term and long-term recognition memory of HPR type A-2.

*Corresponding Author: Wanassanun Pannangrong Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. Tel: +66-43-363690; e-mail: wankun@kku.ac.th

สรุป: ผลการศึกษาครั้งนี้แสดงข้อมูลเบื้องต้นว่ามีความเป็นไปได้ที่สารสกัดไฮโดรไลเซทชนิด A-2 จากข้าวสีช่วยป้องกันระบบประสาทด้านการสูญเสียการเรียนรู้และความจำ

คำสำคัญ: บีต้าอไมลอยด์, โปรตีนไฮโดรไลเซท, ความจำชนิดรีคอกนิชั่น

Conclusions: These findings do provide initial evidence that HPR type A-2 may be benefit to be used for neuroprotective effect.

Keywords: β-amyloid, Protein hydrolysate, Recognition memory

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Introduction

"Bioactive peptides" produced from natural sources have been widely investigated. The studies presented the possible roles of food-derived bioactive peptides in decreasing the risk of different kinds of diseases, especially neurodegenerative disease.[1] Most of the protein isolates and hydrolysates are commonly being prepared from protein-rich sources like soy and whey.[2,3] There have been relatively few studies that have focused on examining the bioactive peptide properties of rice. Rice (*Oryza sativa* L.) is the main food of Thai population and a cheap product having nutrients including vitamins, minerals and fiber. Pigmented rice is a type of rice grown in Thailand recently utilized as a healthy food. An outer layer of pigmented rice is a good source of fat, protein and antioxidants. Serveral studies reported that peptide-derived from pigmented rice bran contains large amounts of fiber and bioactive phytochemicals such as, tocopherols, tocotrienols, oryzanols, vitamin B complex and phenolic compounds.[4-6] A study showed the pigmented rice could significantly prevent memory impairment and hippocampal neurodegeneration in hippocampus.[7] The objective of this study was to investigate the effects of protein hydrolysate type A-2 from pigmented rice on learning and memory in β-amyloid injected rats.

Materials and Methods

Plant materials and reagents

Hydrolysate peptide type A-2 from pigmented rice was studied and prepared by Prof. Dr. Bungorn Sripanidkulchai and the Center for Research and Development of Herbal Health Products (CRD-HHP) at Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

Animals and treatments

All experiments were conducted under the National-Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Khon Kaen University (Approval No. 0514.1.12.2/76). Male Wistar rats weighing 180-220 grams were used in this study. Rats were maintained on a 12-h light/dark cycle with free access to commercial food pellets and drinking water. All rats were trained with the novel object recognition (NOR) test for five days to assess their learning ability and were divided into six groups (n = 9): Sham vehicle, vehicle plus Aβ, donepezil plus Aβ, HPR10 plus Aβ, HPR20 plus Aβ and HPR40 plus Aβ. All treatments were performed by gastric gavage with biomedical needles at 8.00 to 9.00 a.m. for 56 consecutive days. At day 56, the rats in groups 2-6 were injected with Aβ25-35 into both sides of the lateral ventricle, whereas the vehicle rats in the first group received the sham injections. Ten days after Aβ25-35 injection, the rats were tested for the behavioral performance using NOR tasks (Figure 1).

Aβ25-35 Treatment

All surgical procedures were conducted under aseptic conditions and sodium pentobarbital (35 mg/kg b.w., i.p., Sigma-Aldrich, Germany) anesthesia. Rats were maintained in a stereotaxic holder. A mid-line sagittal incision was made in the scalp and two holes were drilled in the skull over the lateral ventricles using the following coordinates: AP = -0.8 mm, L = ± 1.5 from the bregma.[8-10] The dura was perforated with the needle of the microsyringe, which was inserted 3.8

mm beneath the dura mater. Two microliters of either aggregated Aβ25-35 were injected into both sides of lateral ventricles. After surgery, the animals were returned to their home cages.

Novel object recognition (NOR)

The NOR test was used to examine hippocampus-dependent memory.[11] Briefly, rats were habituated in an open-field arena for 5 minutes (min) before training. During the training phase, two identical objects were placed in two locations and each rat was allowed to explore the objects for 5 min. In the testing phase, one of the objects was replaced with a novel object. Rats were placed again in the apparatus to explore the objects for 5 min after a retention interval of 5 min and a 24 h delay (Figure 1). Each group's ability to recognize the novel object was determined using a discrimination index (DI) calculated for each animal using the formula[12]: N - F/N + F, which corresponds to the difference between the time exploring the novel (N) and the familiar object (F), corrected for total time exploring both objects. The result can vary between +1 and -1, where a positive score indicates more time spent with the novel object, a negative score indicates more time spent with the familiar object, and a zero score indicates a null preference.

Statistical analysis

All data were expressed as mean standard error of the mean (S.E.M.). Statistical analysis of the experimental data was carried out using GraphPad Prism (version 5). Significance of differences among groups were analyzed using a one-way ANOVA and a Newman-Keuls post hoc test. The criterion for statistical significance was $P < 0.05$.

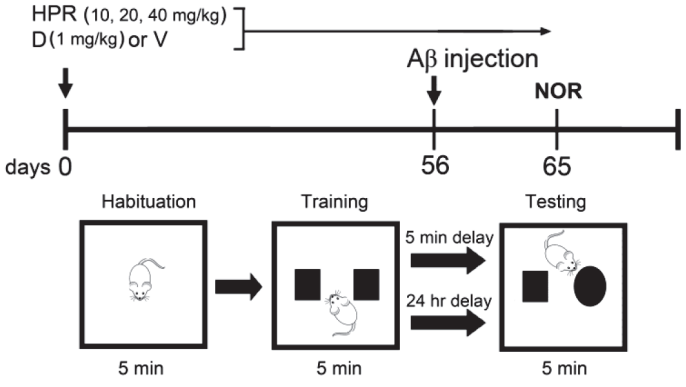

Figure 1 Timeline of drug administration and behavioral tests. (Aβ: β-amyloid (25-35), D: donepezil, HPR: protein hydrolysate type A-2 from pigmented rice, NOR: novel object recognition, V: vehicle)

Results

Effect of hydrolysate peptide type A-2 on recognition memory

During training session, the results showed no significant difference in the time spent exploring the two identical objects (Figure 2). In the first and second period (delay 5 min, 24 h), Aβ25-35 exhibited cognitive deficits by decreasing the discriminative index (Figure 3, 4). When compare to the V+Aβ group, HPR20-, HPR40-treated rats showed a significantly higher discriminative index during 5 min and 24 hours delay testing phase.

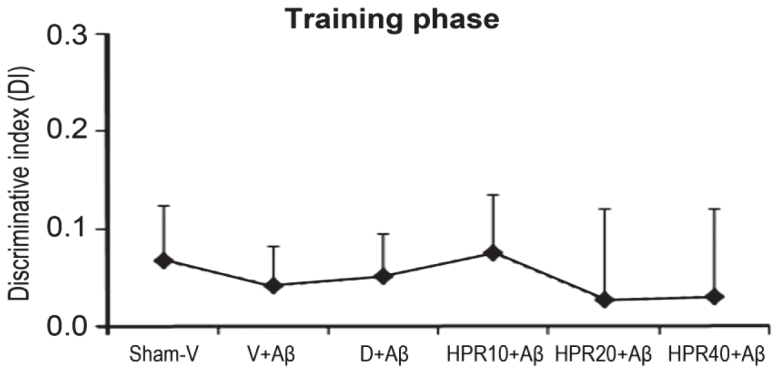

Figure 2 Effects of HPR type A-2 on memory performance in Aβ-induced rats as measured by the discrimination index (DI) in the training phase of NOR task. Data are presented as mean ± S.E.M. (n = 9/group).

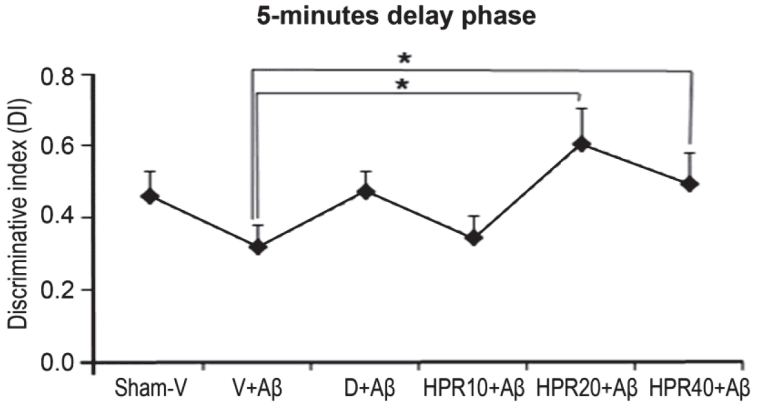

Figure 3 Effects of HPR type A-2 on memory performance in Aβ-induced rats as measured by the discrimination index (DI) after a five-minutes delay of NOR task. Data are presented as mean ± S.E.M. (n = 9/group), * = significant differences from vehicle+Aβ group at $p < 0.05$.

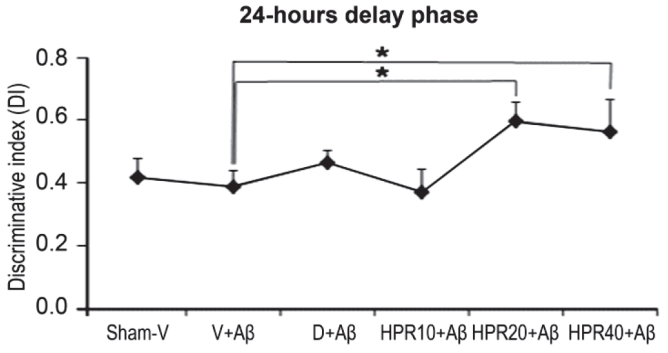

Figure 4 Effects of HPR type A-2 on memory performance in Aβ-induced rats as measured by the discrimination index (DI) after 24-hours delay of NOR task. Data are presented as mean ± S.E.M. (n = 9/group), * = significant differences from vehicle+Aβ group at $p < 0.05$.

Discussion

Previous studies have shown that pigmented rice could enhance the cognitive performance as well as reverse the deleterious effects of brain ageing.[7] The present study used the novelty of exploration to investigate the effect of HPR type A-2 on recognition based on the amount of time that rats spend exploring the presented two objects.[13] Recognition memory is the ability of an individual to recognize the previous events or objects. The novel environment is matched with the previously stored memory for the identification.[14] In line with previous studies, we observed that Aβ caused deterioration in the NOR test both in short- and long-term memory in rat.[15,16] When HPR type A-2 plus Aβ was administered, it took longer for the rats to recognize the novel object compared to the familiar object indicating that HPR type A-2 had a neuroprotective effect on the Aβ25-35-injected rats. However, this neuroprotective effect was not detected at low doses (10 mg/kg BW). This is probably due to this dose of HPR type A-2 extract is not sufficiency to search the therapeutic level. During training periods, rats in all groups did not show significant difference among the two identical objects. An alternative possibility is that since both objects were new objects, rats can not separate the objects (Figure 2).

Conclusion

These findings do provide initial evidence that HPR type A-2 may be benefit to be used for neuroprotective effect.

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