



ใบคาลินช่วยลดความจำบกพร่องที่เกิดจากการเหนี่ยวนำด้วยกรดวอลโปรอิกในหนูแรทโตเต็มวัย

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Baicalin Alleviates the Memory Impairment Caused by Valproic Acid in Adult Rats

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บทคัดย่อ

หลักการและวัตถุประสงค์: กรดวอลโปรอิก (VPA) ถูกใช้อย่างแพร่หลายในการรักษาโรคลมชักและความผิดปกติทางอารมณ์ อย่างไรก็ตาม VPA ส่งผลข้างเคียงต่อความจำทั้งในผู้ป่วยและหนูทดลอง เนื่องจาก VPA มีฤทธิ์ไปลดการสร้างเซลล์ประสาทใหม่ในสมองส่วนฮิปโปแคมปัส ใบคาลิน (baicalin) เป็นสารประกอบฟลาโวนอยด์ธรรมชาติที่สกัดได้จากพืช *Oroxylum indicum* (ในไทยรู้จักกันในชื่อ เพกา) และพืชสายพันธุ์ *Scutellaria* การศึกษาก่อนหน้านี้พบว่า ใบคาลินมีฤทธิ์ป้องกันระบบประสาทในกลุ่มโรคที่มีความเสื่อมของระบบประสาท โดยการไปเพิ่มการสร้างเซลล์ประสาท ยับยั้งความเป็นพิษและลดการตายของเซลล์ รวมทั้งสามารถยับยั้งภาวะเครียดออกซิเดชันและการอักเสบของระบบประสาท ซึ่งเป็นสาเหตุของการเกิดความจำบกพร่อง ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของใบคาลินในการป้องกันการเกิดภาวะความจำบกพร่องในหนูแรทโตเต็มวัยที่ถูกเหนี่ยวนำด้วย VPA

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague-Dawley ถูกแบ่งออกเป็น 4 กลุ่ม ได้แก่ Control, VPA, Baicalin และ VPA+Baicalin โดย VPA (300 มก./กก.) ให้โดยการฉีดเข้าช่องท้อง วันละสองครั้ง เป็นเวลา 14 วัน และใบคาลิน (50 มก./กก.) ให้โดยการฉีดเข้าช่องท้อง วันละหนึ่งครั้ง เป็นเวลา 21 วัน หนูแรทถูกทดสอบความจำชนิด spatial และ recognition โดยใช้การทดสอบ novel object location (NOL) และ novel object recognition (NOR) ตามลำดับ

ผลการศึกษา: หนูแรทในกลุ่ม control, Baicalin และ VPA+Baicalin สามารถจำแนกตำแหน่งใหม่ของวัตถุจากตำแหน่งเก่าหรือวัตถุชนิดใหม่ออกจากวัตถุเดิมได้อย่างมีนัยสำคัญทางสถิติ ในทางตรงกันข้ามหนูแรทในกลุ่ม VPA ไม่สามารถจำแนกตำแหน่งของวัตถุและชนิดของวัตถุได้

สรุป: การศึกษานี้แสดงให้เห็นว่า ใบคาลินสามารถช่วยฟื้นฟูความจำบกพร่องในหนูแรทที่เกิดจากฤทธิ์ของ VPA ได้

คำสำคัญ: ใบคาลิน, กรดวอลโปรอิก, ความจำบกพร่อง

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Abstract

Background and Objective: Valproic acid (VPA) is widely used in clinics to treat seizures and mood disorders. However, it has adverse effects on memory in patients and rats due to the ability of VPA to reduce hippocampal neurogenesis. Baicalin is a natural flavonoid compound isolated from the *Oroxylum indicum* plant (also known as Phea ka in Thai) and other *Scutellaria* species. Previous studies have reported that baicalin has neuroprotective effects on neurodegenerative diseases by promoting neurogenesis, inhibiting excitotoxicity, and reducing apoptosis, including inhibiting oxidative stress and neuroinflammation, which causes memory impairment. Therefore, the present study aimed to investigate the protective effects of Baicalin on memory impairment in adult rats induced by VPA.

Methods: Male Sprague Dawley rats were divided into 4 groups: control, VPA, Baicalin, and VPA+Baicalin. The VPA (300 mg/kg) was administered by intraperitoneal injection twice daily for 14 days, and Baicalin (50 mg/kg) was administered by intraperitoneal injection once a day for 21 days. The spatial and recognition memory were examined by novel object location (NOL) and novel object recognition (NOR) tests, respectively.

Result: Using the NOL and NOR tests, rats in control, Baicalin, and VPA+Baicalin groups significantly discriminate the objects in the novel location from the familiar location or novel object from the familiar one. On the other hand, rats in the VPA group showed no significant difference in discrimination in both tests.

Conclusion: These results demonstrated that Baicalin improves memory impairments caused by VPA in adult rats.

Keywords: Baicalin, valproic acid, memory impairment

Introduction

Valproic acid (VPA), a branched short-chain fatty acid, is an anticonvulsant and mood-stabilizer medication with an effective broad-spectrum of activity. VPA can inhibit histone deacetylase (HDAC) activity, which is an enzyme that regulates gene expression and transcription. This action contributes to the hyperacetylation of DNA, inhibition of neural stem cell proliferation by up-regulation of p21/waf1, increase in intracellular apoptosis, and reduces brain-derived neurotrophic factor (BDNF) expression^{1,2}. Cognitive impairment was observed in children and adult patients who were receiving VPA treatment^{3,4}. In addition, VPA-treated rats also showed memory impairments and a reduction of neurogenesis in the hippocampus⁵. In mammals, adult neurogenesis occurs in the subgranular zone (SGZ) of the dentate gyrus, and the rate of adult neurogenesis in the hippocampus correlates with learning and memory⁶.

Flavonoids are commonly found in many fruits and vegetables. Based on chemical structure, flavonoids can be classified into six major classes; anthocyanins, chalcones, flavanones, flavones, flavonols, and isoflavonoids⁷. Interestingly, accumulating evidence supports the benefits of flavonoid intake on learning and memory in both humans and animals^{8,9}. Baicalin (7-glucuronic acid, 5,6-dihydroxyflavone), is a flavonoid compound isolated from the roots of *Scutellaria baicalensis* Georgi, the plant used in traditional Chinese medicine¹⁰. It is also reported in *Oroxylum indicum* (L.) Kurz (known as Phea ka in Thai)¹¹. Baicalin has a variety of biological activities, including anti-apoptotic, antioxidant, and anti-inflammatory. Recent studies have demonstrated that baicalin promotes hippocampal neurogenesis and improves memory deficits in rodent models of depression, Alzheimer's disease, and cerebral ischemia¹²⁻¹⁴.

Based on the balance of evidence, we hypothesize that Baicalin might provide protection against VPA-induced memory impairment. The current study aimed to evaluate this hypothesis using the novel object location (NOL) and novel object recognition (NOR) tests to evaluate memory function.

Materials and Methods

Animals

Healthy forty-eight adult male Sprague Dawley rats (age: 4-5 weeks old, body weight: 230-250 grams) were purchased from Nomura Siam International Co., Ltd. Pathumwan, Bangkok. They were housed in groups of four per cage in transparent plastic cages with food and water freely available and maintained at $23 \pm 2^\circ \text{C}$ under a standard 12 h/12 h light/dark cycle (lights on at 6 am). Prior to the experiment, rats were given 14 days to acclimate to their housing facility. All procedures were carried out in agreement with the Guide for the Care and Use of Laboratory Animals and were approved by the Khon Kaen University Ethics Committee in Animal Research (IACUC-KKU-74/65).

Drug administration

VPA (Sigma Aldrich, Inc., St. Louis, USA) was dissolved in 0.9% saline solution at a concentration of 300 mg/kg. Baicalin (Sigma Aldrich, Inc., St. Louis, USA) was dissolved in 0.9% saline solution and incubated for 1 hour at 37°C under agitation. The solution was filtered through $0.22 \mu\text{m}$ syringe filter nylon. The selected dose was chosen based on previously published experiments^{15,16}. All test solutions were freshly prepared each day of the experiments. After the acclimation, rats were randomly divided into 4 groups (12 animals in each group) (Fig. 1):

Group I (Control group): Received saline solution (0.9% NaCl) 1 mL/kg by i.p. injection twice daily from day 1 to day 14 and following once a day until day 21.

Group II (VPA group): Received VPA 300 mg/kg by i.p. injection twice daily at 9 a.m. and 4 p.m. for 14 days.

Group III (Baicalin group): Received baicalin 50 mg/kg by i.p. injection at 9 a.m. for 21 days.

Group IV (VPA + Baicalin group): Received VPA 300 mg/kg by i.p. injection twice daily at 9 a.m. and 4 p.m. for 14 days and Baicalin 50 mg/kg by i.p. injection at 9 a.m. for 21 days.

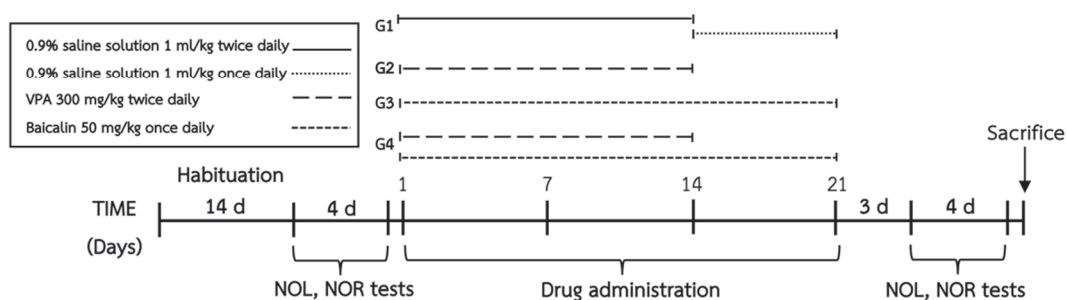


Figure 1 Timeline of drug administration and behavioral tests.

Behavioral Tests

Before and after drug administration, all rats were subjected to behavioral tests by an experimented observer in the light phase. Rats were assessed for spatial and recognition memory by NOL and NOR tests, respectively. Behavior was tracked using a digital camera suspended above the center of the arena and connected to a computer running the EthoVision® XT video-tracking software (EthoVision®, XT version 12, Noldus, Wageningen, Netherlands).

Novel object location (NOL) and novel object recognition (NOR) tests

NOL and NOR tests were performed in a 50 cm x 50 cm x 50 cm open-field black acrylic arena based on the previous study^{5,17}. Both tests consist of familiarization and choice trials. Rats were habituated to the empty arena for 30 minutes before the test. On test day of the NOL test, rats were habituated again for 3 minutes. In the familiarization trial, rats were placed in the arena with two identical objects (plastic bottles filled with water) at two different locations and allowed to freely explore for 3 minutes. Then, the rats were returned to their home cages for 15 minutes (inter-trial interval), during which the objects and arena were cleaned with 20% alcohol to neutralize odor cues. In the choice trials, one of the two objects was moved to a novel location while the other object remained in a familiar location. After that, rats were allowed to freely explore the objects for another 3 minutes.

The NOR test started the following day after the NOL test. The NOR test is identical to the NOL until the choice trial, one of the familiar objects and the novel object (triangle) were placed into the arena at the same location in the familiarization trial. Then, rats were allowed to freely explore the objects in the arena for 3 minutes.

The exploration time was considered whenever the rats explore the objects by licking, biting, sniffing, or their noses directly toward the object at fewer than 2 centimeters. The discrimination index (DI) was calculated using the formula: time spent exploring the novel location or novel object (second) - time spent exploring the familiar location or familiar object (second).

Statistical analysis

Statistical analyses were performed by using GraphPad Prism (Version 9.0; GraphPad Software Inc., San Diego, CA, USA). All data are presented in mean $\pm$ standard error of the mean (SEM). The level of statistical significance was preset to p -value < 0.05 . For evaluation of the body weight (in grams) of rats between groups, data was assessed by Two-way repeated measures ANOVA. For behavior data, the total exploration time was assessed by One-way ANOVA and DI was assessed by One-sample t -test. Post hoc analyses were performed using Bonferroni corrections.

Results

1. Effects of VPA and baicalin on body weight

Daily body weight measurements throughout the experiment are shown in Fig. 2. The Baicalin-treated group had no significant difference in comparison with the control group ($p > 0.05$), while VPA and VPA+Baicalin-treated groups showed significantly less weight gain than the control group on days 8 to 21 of treatment ($p < 0.05$ and $p < 0.01$). After the VPA administration, they continued to gain weight and almost returned to the control level.

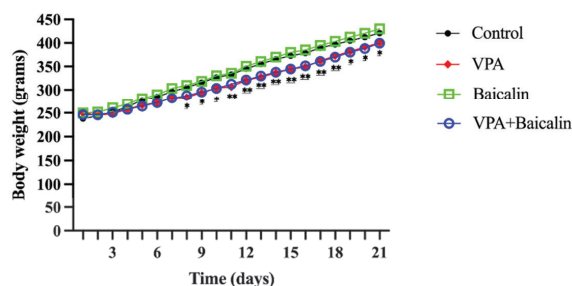


Figure 2 The effects of VPA and baicalin on body weight gain in rats for 21 days of treatment. (* $p < 0.05$ and ** $p < 0.01$ significant differences compared with the control group)

2. Effects of VPA and Baicalin on locomotor activity

In the familiarization and choice trials for the NOL and NOR tests, locomotor activity was calculated as the total time spent exploring each of the two objects. Before and after drug administration, rats in all groups showed no significant difference in both behavioral tests ($p > 0.05$, Fig. 3 and 4), indicating that VPA and baicalin did not impair animals' locomotor activity.

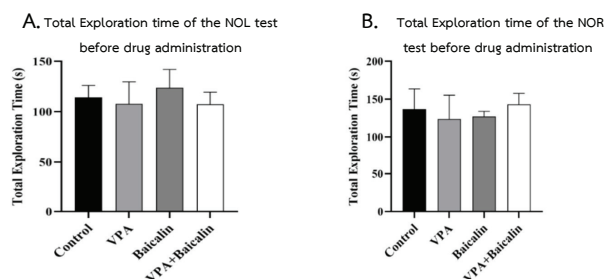


Figure 3 Total exploration time of the animals exploring each object in the NOL (A) and NOR (B) tests before drug administration.

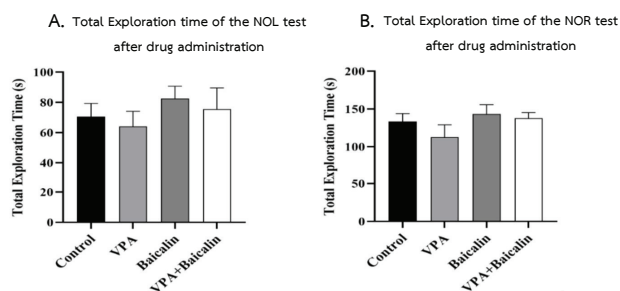


Figure 4 Total exploration time of the animals exploring each object in the NOL (A) and NOR (B) tests after drug administration.

3. Effects of VPA and Baicalin on spatial memory

The effects of VPA and baicalin on spatial memory were assessed with the NOL test. Before drug administration, rats in all groups showed significant differences in the DIs compared to zero ($p < 0.05$, Fig. 5A), indicating that rats had normal memory to discriminate novel and familiar object locations. After drug administration, the DIs of the control, baicalin, and VPA+Baicalin groups were significantly higher than zero ($p < 0.05$, Fig. 5B). While the VPA-treated group showed no significant difference from zero, suggesting spatial memory impairment. According to these findings, baicalin treatment reverses VPA-induced spatial memory loss.

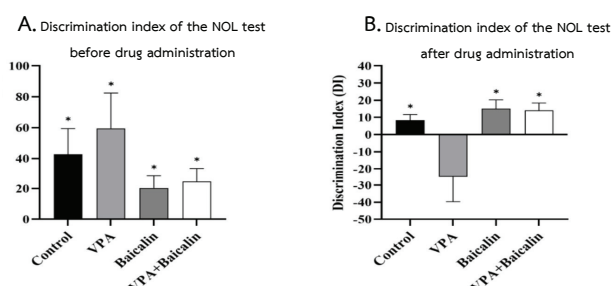


Figure 5 The discrimination index of the NOL test before (A) and after drug administration (B). (* $p < 0.05$ significant differences compared to zero)

4. Effects of VPA and Baicalin on recognition memory

The effects of VPA and Baicalin on recognition memory were assessed with the NOR test. Before drug administration, all groups of rats showed significant differences in the DIs compared to zero ($p < 0.05$, Fig. 6A), indicating that rats were able to discriminate novel and familiar objects. After drug administration, the DIs of the control, Baicalin, and VPA+Baicalin groups were significantly higher than zero ($p < 0.05$, Fig. 6B). While the VPA-treated group showed no significant difference from zero, demonstrating recognition memory impairment. According to these findings, Baicalin treatment reverses VPA-induced recognition memory loss.

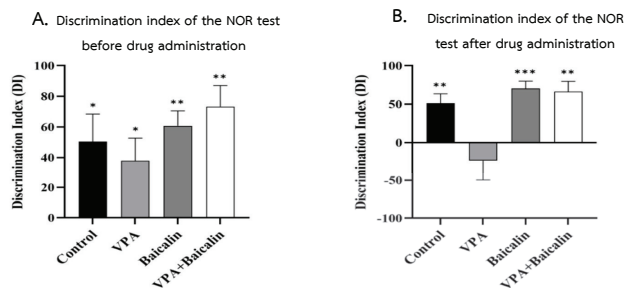


Figure 6 The discrimination index of the NOR test before (A) and after drug administration (B). (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ significant differences compared to zero)

Discussion

VPA is an anticonvulsant medication used to treat seizures and mood disorders. VPA therapy alone has been reported to produce cognitive impairment^{3,4}. The hippocampus is a prime target for VPA-induced cognitive impairment as this region is essential for spatial and recognition memory¹⁸. In the present study, we demonstrated that baicalin treatment in rats attenuated memory impairment induced by VPA without affecting locomotor activity in the NOL and NOR tests.

The body weight gain during the experimental period in the Baicalin group was not significantly different compared to the control group. This suggests that Baicalin did not affect the body weight gain of rats, which is consistent with earlier reports^{19,20}. For VPA and VPA+Baicalin-treated groups showed significantly less weight gain than the control group, there was consistent evidence that the body weight of rats exposed to VPA was significantly less weight gain than control²¹.

The effects on locomotor activities were evaluated by the total exploration time of all objects. We found that VPA and Baicalin administration did not influence locomotor activity. Therefore, animals' performance in behavioral tests was not involved with motor dysfunction.

NOL and NOR tests were selected due to their effectiveness in assessing cognitive and memory function influenced by the hippocampus. Moreover, in both tests, no positive or negative reinforcement exists but utilizes the animal's innate tendency to explore novelty²². The NOL test is a behavioral task used to evaluate spatial memory, which is a memory

related to spatial relationships between routes, locations, or configurations. The NOR test, in contrast, evaluates recognition memory, which is a memory to recognize familiar a situation that has been encountered previously^{23,24}. Our behavior analysis showed the VPA-treated rats had a negative DI value, which means that the rats were unable to discriminate between familiar and novel locations during the choice trial of the NOL test. The NOR test came up with similar findings that VPA-treated rats were unable to discriminate between familiar and novel objects. These results suggest that VPA impaired spatial and recognition memory. Our data support previous reports that VPA treatment at a dose of 300 mg/kg twice a day by intraperitoneal injection impairs both spatial and recognition memory in rats^{15,25}. However, in the present study, the rats receiving baicalin and VPA+Baicalin co-administration showed positive DI values in both NOL and NOR tests, which means that the rats could discriminate the objects placed in the novel location or object from the familiar object. Thus, the result suggests that Baicalin attenuates VPA-induced spatial and recognition memory impairment in rats. Similarly, Baicalin administration (50 mg/kg) improved spatial learning deficit in the Morris water maze test and also improved recognition memory in the NOR test after cerebral ischemic injury^{16,26}.

Previous reports have shown that VPA treatment has adverse effects in patients and rats, as a result of the overproduction of reactive oxygen species (ROS) and decreased levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GSH-Px)²⁷. Overproduction of ROS can lead to activation of program cell death pathways, reduced neurogenesis, and cognitive deficits²⁸. Baicalin is a flavonoid with two hydroxyl groups at positions 5 and 6. These hydroxyl groups act as hydrogen donors that neutralize the free radicals through the electron transfer mechanism and form relatively stable flavonoid radicals, resulting in antioxidant activity²⁹. Baicalin improved antioxidant enzyme activities (CAT, SOD, and GSH-Px), increased the Bcl-2/Bax ratio, and activated caspase-9/3³⁰. Furthermore, baicalin enhances cell proliferation and neurogenesis in the hippocampal dentate gyrus via

the Wnt/ β -catenin pathway in a mouse model of depression¹². In the present study, VPA treatment caused memory impairment in adult rats. These impairments were attenuated by Baicalin administration; it may be due to powerful antioxidant activities and improvement of neurogenesis. However, further molecular studies should be conducted to elucidate its accurate mechanism and effects.

Conclusion

Findings from this study showed Baicalin improved spatial and recognition memory impairment caused by VPA. Our study provides evidence supporting the clinical application of Baicalin in the treatment of memory deficit in patients taking VPA.

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