นิพนธ์ต้นฉบับ . Original Article

ผลป้องกันทางระบบประสาทของกรดคาเฟอิคต่อความบกพร่องของความจำที่ถูกเหนี่ยวนำด้วยแอล-เมไธโอนีนในหนูแรทโตเต็มวัย

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The Neuroprotective Effect of Caffeic Acid against L-Methionine Induced Memory Deficits in Adult Rats

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หลักการและวัตถุประสงค์: แอล-เมไธโอนีนพบในผลิตภัณฑ์ธรรมชาติเช่น เนื้อ นม และธัญพืช แอล-เมไธโอนีนเป็นกรดอมิโนและสามารถถูกเปลี่ยนไปเป็นสารโฮโมซิสเทอีนโดยการเพิ่มกลุ่มเมทิล การสะสมของโฮโมซิสเทอีนที่มากเกินเป็นสาเหตุของของภาวะไฮเปอร์โฮโมซิสเทอีน และนำไปสู่ภาวะความจำบกพร่อง กรดคาเฟอิคเป็นสารประกอบฟีโนลิกและพบในกาแฟและชา การศึกษาก่อนหน้าได้รายงานผลป้องกันทางระบบประสาทของกรดคาเฟอิคต่อความจำที่เสื่อมลง ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของกรดคาเฟอิคในการป้องกันความบกพร่องของความจำต่อการเหนี่ยวนำของแอลเมไธโอนีน

วิธีการศึกษา: หนูแรท เพศผู้สายพันธุ์ Sprague Dawley จำนวน 60 ตัว ถูกแบ่งออกเป็น 6 กลุ่ม ได้แก่ control, L-met, caffeic acid 20, caffeic acid 40, caffeic acid 20+L-met และ caffeic acid 40+L-met โดยกลุ่ม control ได้รับโพรพิลีนไกลคอลและสารละลายเซลลูโลส โดยการป้อน ในกลุ่ม L-met, caffeic acid 20, caffeic acid 40, caffeic acid 20+L-met และ caffeic acid 40+L-met ได้รับกรดคาเฟอิค (20 และ 40 มิลลิกรัม/กิโลกรัม) และแอล-เมไธโอนีน (1.7 กรัม/กิโลกรัม) โดยการป้อน เป็นเวลา 28 วัน วันละ 1 ครั้ง การเปลี่ยนแปลงน้ำหนักและการเคลื่อนไหว นอกจากนั้นความจำความจำเกี่ยวกับพื้นที่และความจำโดยรู้จำถูกตรวจโดยใช้การทดสอบ novel object location (NOL) และ novel object recognition (NOR) ถูกตรวจสอบตามลำดับ

ผลการศึกษา: ผลลัพธ์แสดงว่ากลุ่มที่ได้รับกรดคาเฟอิค (20 และ 40 มิลลิกรัม) และแอล-เมไธโอนีนไม่มีผลด้านลบต่อน้ำ

Background and objective: L-methionine is found in natural products such as meat, milk and cereal grain. It is a non-essential amino acid and can be changed to homocysteine by methylation. Excess accumulation of homocysteine is a course of hyperhomocysteinemia and leads to memory deficit. Caffeic acid is a phenolic compound found in coffee and tea. Previous studies reported the neuroprotective effect of caffeic acid on memory and cognitive functions. Therefore, this study aimed to determine the neuroprotective effect of caffeic acid against L-methionine induced memory deficit.

Methods: Sixty male Sprague-Dawley rats were divided into 6 groups: control, L-met, caffeic acid 20, caffeic acid 40, caffeic acid 20+L-met, caffeic acid 40+L-met groups. The control group received propylene glycol and carboxymethyl cellulose by oral gavage once a day for 28 hours. The caffeic acid 20, caffeic acid 40, caffeic acid 20+L-met, caffeic acid 40+L-met groups, caffeic acid (20 and 40 mg/kg) and L-methionine (1.7 g/kg) groups were administered by oral gavage once a day for 28 days. Changes of body weight and locomotor activity were examined. Moreover, spatial and recognition memory were determined using the novel object location (NOL) and novel object recognition (NOR) tests, respectively.

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หนักตัวและเกี่ยวกับการเคลื่อนที่ ความบกพร่องของความจำเกี่ยวกับพื้นที่และความจำโดยรู้จำพบในกลุ่มแอล-เมไธโอนีน แต่ไม่พบในกลุ่มอื่น

สรุป: การศึกษาครั้งนี้พบว่ากรดคาเฟอิค (20 และ 40 มิลลิกรัม) สามารถป้องกันภาวะความจำบกพร่องที่เหนี่ยวนำโดย แอล-เมไธโอนีนได้

คำสำคัญ: กรดคาเฟอิค; แอล-เมไธโอนีน; ความจำบกพร่อง

Results: The results demonstrated that caffeic acid (20 and 40 mg) and L-methionine did not have a negative effect on the body weight and locomotor activity. Impairments of spatial and recognition memories were found in the L-met group, but was not detected in the other groups

Conclusion: This study reveals that caffeic acid (20 and 40 mg) could potentially protect against L-methionine induced memory deficits.

Keywords: Caffeic acid; L-methionine; Memory impairment.

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Introduction

L-methionine, a non-essential amino acid, can be found in general food such as fish, meat, poultry, cheese and milk[1]. L-methionine is transferred to the body with a meal and cannot be synthesized in the human body[2]. However, L-methionine can be changed to homocysteine by cell metabolism and high homocysteine level in the blood stream leads to hyperhomocysteinemia. Hyperhomocysteinemia can induce oxidative stress and toxicity in the cell via decreasing of nitric oxide in vascular endothelial cells. Besides, hyperhomocysteinemia has the ability to increase lipid peroxidation that is associated with the inflammatory process and causes memory deficit. These effects are associated with learning and memory deficits[3–5].

Caffeic acid (3, 4-dihydroxycinnamic acid) is a polyphenol and categorized into hydroxycinnamic acid groups. Caffeic acid can be found in plant extracts, such as tomato, mulberry, strawberry, tea, red wine, and coffee[6]. Caffeic acid has the ability to improve memory and cognitive functions by antioxidant properties. Caffeic acid also has an antioxidant potential to scavenge free radicals[7,8]. The neuroprotective effect of caffeic acid can ameliorate the disruption of the brain from oxidative stress generated from hydrogen peroxide[9]. Therefore, this study aims to investigate the neuroprotective effect of caffeic acid on memory deficits caused by L-methionine in adult rats using the novel object recognition (NOL) and novel object location (NOR) tests.

Materials and Methods

Animals

Sixty Adult Sprague-Dawley male rats, age 4-6 weeks old, body weight 200-220 g from Nomura Siam International Co., Ltd. Pathumwan, Bangkok, Thailand. The animals were controlled in the environment maintained in a 12:12 h light: dark cycle and the temperature was maintained at 23-25 °C. Food and water were always available for animals during the experiment. The experiment protocol and all handling procedures were approved by the Khon Kaen University Ethics Committee in Animal Research (AEKKU 26/63).

Drug administration

The animals were divided into 6 groups (10 rats per group) and allowed to habituate in an animal facility for one week before starting the experiment. The control group: rats were administered with 1 ml/kg propylene glycol (Ajax Finechem Pty Ltd., Australia) and 0.5% w/v carboxymethyl cellulose (CMC) 1 ml/kg (Sigma Aldrich Chemical Co., Saint Louis, MO, USA). The L-met group: rats were administered with L-methionine (1.7 g/Kg) suspended in 0.5% w/v CMC (Sigma Aldrich Chemical Co., Saint Louis, MO, USA) 10. The caffeic acid 20 and 40 groups: rats were administered with caffeic acid (Sigma Aldrich Chemical Co., Saint Louis, MO, USA) 20 and 40 mg/Kg dissolved in propylene glycol. The caffeic acid 20 and 40 + L-met groups: rats were administered L-methionine 1.7 g/Kg and caffeic acid 20 and 40 mg/Kg. All animals were treated one time per day for 28 days. Three days after drug administration, the NOL and NOR tests were conducted to assess spatial and recognition memory (Figure 1).

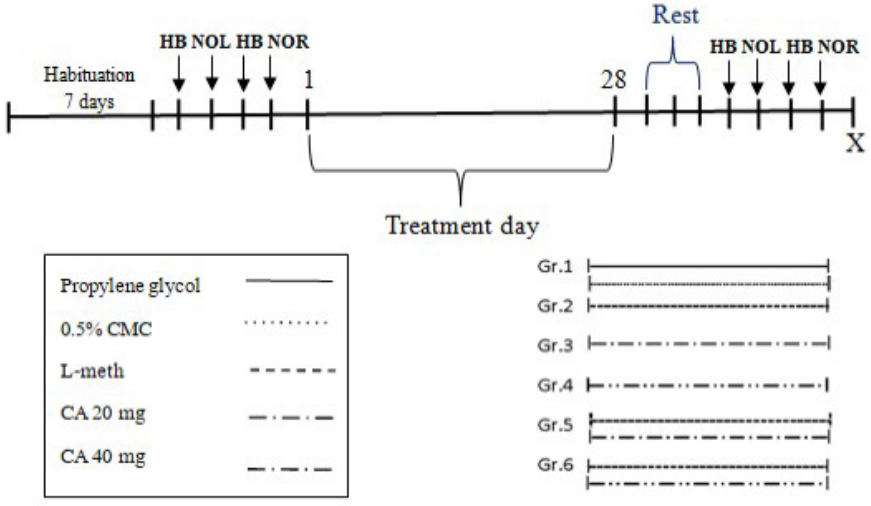

Figure 1 Timeline of drug administration and behavioral testing.

Behavioral tests

Before and after drug administration, behavioral tests were performed using the NOL and NOR tests. Both tasks consisted of a square black arena (width x length x high = 50 x 50 x 50 cm.) and plastic bottles. Movement patterns were monitored by an overhead video camera connected to EthoVision® XT software (EthoVision®, XT Version 12, Noldus, Wageningen, Netherlands).

NOL and NOR tests

The NOL test consists of habituation, familiarization and choice trials. In the habituation, the animals were placed in the center of an empty arena for 30 minutes. After 24-hour habituation, the animals were placed in the center of the arena without any object for 3 minutes. In the familiarization, the animals were placed in the center of the arena to explore two similar objects that were placed in different corners in the arena for 3 minutes and then the animals were moved back to their home cages for 15 minutes. In the choice trial, one object was put in a novel location (NL) and the other one was placed in the same location (familiar location; FL). The animals were allowed to freely explore the objects in the arena for 3 minutes.

In the NOR test, the habituation and familiarization trials are the same as the procedure of the NOL test. In the choice trial, the animals were placed in the center of the arena with one of the familiar objects (FO) and a novel object (NO). The animals were allowed to freely explore the objects in the arena for 3 minutes. The exploration time in both tests was recorded using EthoVision® XT software to determinate locomotor activity of the animals and evaluate discrimination index (DIs) that is defined as the ability to recognize between the novel and familiar locations or objects. Normally, animals will spend more time investigating an object in a novel location or object than a familiar location or object in the tests. Therefore, the DIs should be significantly greater than zero.

Statistical analysis

All statistical analyses were analyzed using GraphPad Prism (Version 7.0; GraphPad Software Inc., San Diego, CA, USA). The data were expressed as mean ± standard error of mean (SEM). $p<0.05$ was examined to show statistical significance. Two-way ANOVA was used to analyze the body weight and one-way ANOVA was used to determine total exploration time. Discrimination index was compared using one sample t-test.

Results

Effects of caffeic acid and L-methionine on body weight

The body weight of animals was monitored daily during the experiment. The body weight data of the caffeic acid 20, caffeic acid 40, L-methionine, caffeic acid 20+L-methionine and caffeic acid 40+L-methionine groups showed no significant differences when compared to the control group ($p<0.05$) as shown in Figure 2. This result indicates that caffeic acid and L-methionine did not have a negative effect on body weight.

Effects of Caffeic acid and L-methionine in the behavioral tests

- Before drug administration

Total exploration time is defined as total object

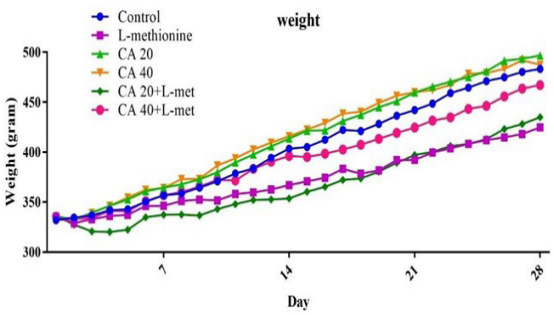

Figure 2 Body weight of animals in each group during the drug administration

exploration time during the familiarization and choice trials. The data showed no significant differences in terms of total exploration time in both the NOL and NOR tests among the treatment groups ($p>0.05$, one-way ANOVA, Fig. 3A and 3B), indicating that animals had no impairment in their locomotor activity in the NOL and NOR tests.

In both the NOL and the NOR tests, the DIs of animals in the all groups were significantly higher than zero ($p<0.05$, one-way ANOVA, Fig. 4A and 4B).

- After drug administration

The total exploration times in the NOL and NOR tests were not significantly different in all groups ($p>0.05$, one-way ANOVA, fig. 5A and 5B).

After drug administration, the DIs of the control, caffeic acid 20 and 40 mg, caffeic acid 20+L-met and caffeic acid 40+L-met groups were significantly higher than zero except the L-methionine group ($p<0.05$, one-way ANOVA, Fig. 6A and 6B).

Discussion

In the present study, the results show that caffeic acid has the ability to prevent memory impairment caused by L-methionine in adult rats. Caffeic acid (20 and 40 mg/kg) and L-methionine did not have a negative effect on the body weight when compared with the control group. The adult neurogenesis in the subgranular zone of hippocampal dentate gyrus has an important role with spatial and

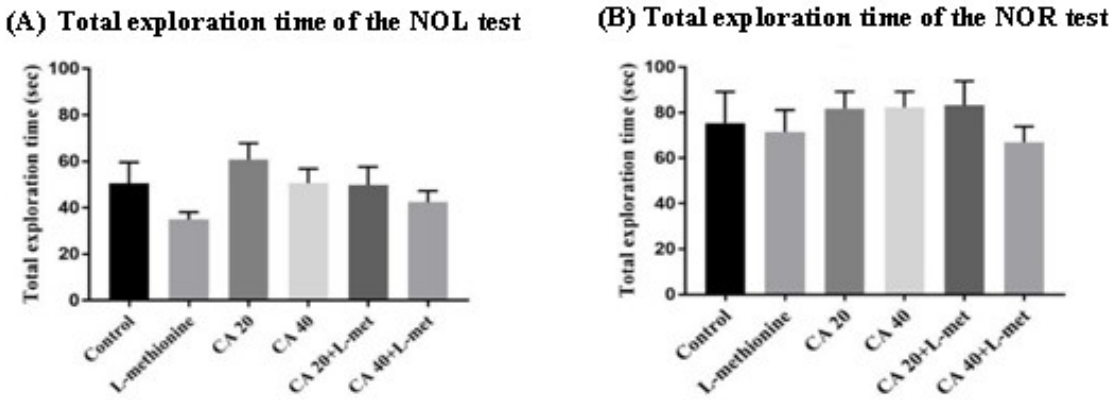

Figure 3 Total exploration time of all animals in the NOL (A) and NOR (B) tests. In both the NOL and NOR tests, the DIs of animals in the all groups were significantly higher than zero ($p<0.05$, one-way ANOVA, Fig. 4A and 4B).

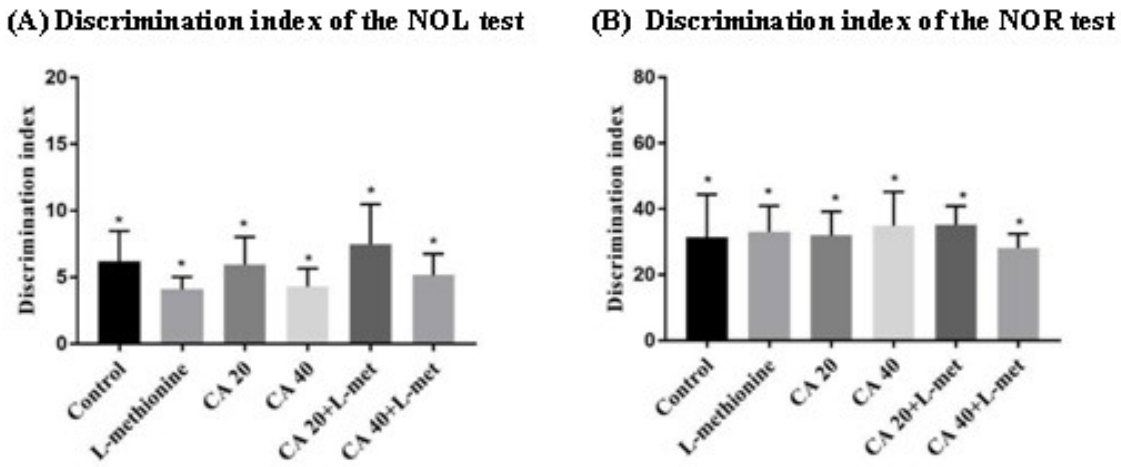

Figure 4 The DIs of the animals in the NOL (A) and NOR (B) tests before drug administration (*$p<0.05$ significant difference compared to zero).

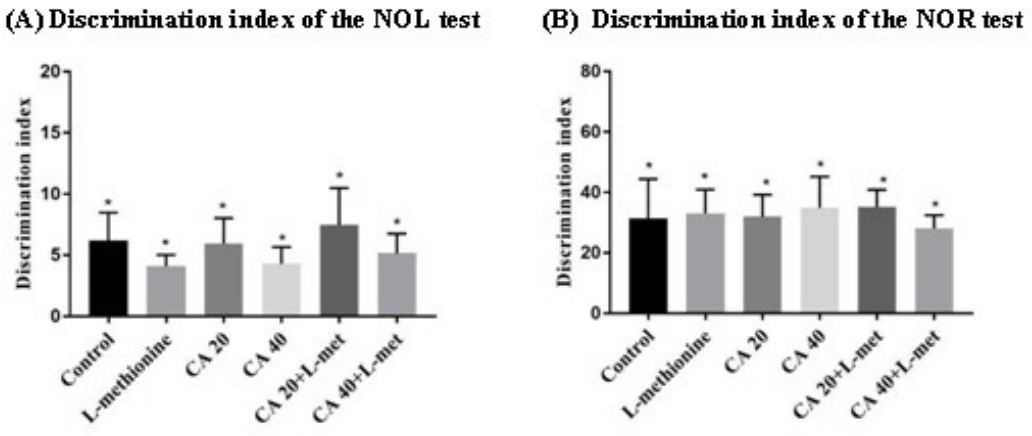

Figure 5 Total exploration time of the animals in the NOL (A) and NOR (B) tests after drug administration.

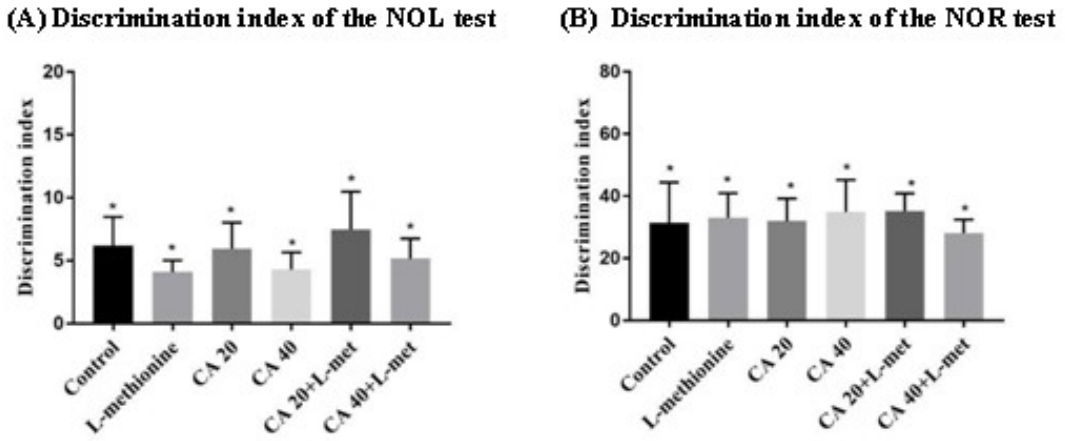

Figure 6 The DIs of the animals in the NOL (A) and NOR (B) tests after drug administration (*p<0.05 significant differences compared to zero).

recognition memory[11–14].

First of all, the aim of this study was to investigate the effect of caffeic acid on memory deficits caused by L-methionine in a rat model. The behavioral tests presented that L-methionine (1.7g/kg) administration led to impairments in spatial and recognition memory in adult rats. The NOL and NOR tests were conducted to assess changes of animal behavior in this study [15–17]. The NOL and NOR tests consist of habituation and testing with two objects in an open-field arena. On the test day of the NOL and NOR tests, the results were detected when one of the objects was moved to a new location or changed to a novel object17. The NOL and NOR tests used to evaluate spatial memory and recognition memory are associated with hippocampal neurogenesis[18]. After drug administration, the result of the L-methionine group in this study showed a failure to discriminate between objects placed in the familiar and novel location in the NOL test. Identically in the NOR test, animals in the L-methionine group could not discriminate between the familiar and novel objects. Consistent findings with the previous studies reveal that L-methionine induced memory and cognitive impairments[19]. Caffeic acid has the ability to reduce the effect of L-methionine induced memory impairments. Caffeic acid plays an important role to inhibit p53 signaling protein through down-regulation of the process of L-methionine conversion to homocysteine that consequently causes hyperhomocysteinemia. This leads to DNA damage that initiates decrease of neurogenesis related to memory deficits[20, 21]. The animals receiving both caffeic acid and L-methionine were able to discriminate between the object placed in a novel location and a novel object as confirmed by the positive DIs that were significantly higher than zero. Previous studies have reported the benefit of caffeic acid that can improve memory and cognitive functions by eliminating free radicals and anti-lipid peroxidation22. The total exploration time was measured to determine locomotor activity before and after drug

administration, demonstrating that caffeic acid and L-methionine did not harm movement ability[17].

Conclusion

Finally, the present study reveals that caffeic acid can improve memory impairment caused by L-methionine in adult rats. The result of this study postulates the potential of caffeic acid to improve spatial and recognition memory deficits in an animal model.

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