เมลาโทนินช่วยลดความจำบกพร่องที่เกิดจากการเหนี่ยวนำด้วยกรดวอลโพรอิกในหนูแรทโตเต็มวัย

อนุสรา อารณะโรจน์[1], สุชาดา ครุฑศรี[1], กรรวี สุวรรณโคตร[1], ภรณ์ทิพย์ ชัยสว่าง[1], วนัสนันท์ แป้นนางรอง[1], จริยา อำคา เวลบาท[1,2]*

[1]ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

[2]กลุ่มวิจัยและพัฒนาด้านประสาท มหาวิทยาลัยขอนแก่น

Melatonin Alleviates Valproic Acid-Induced Memory Impairments in Adult Rats

Anusara Aranarochana[1], Suchada Krutsri[1], Kornrawee Suwannakot[1], Pornthip Chaisawang[1], Wanassanun Pannangrong[1], Jariya Umka Welbat[1, 2]*

[1]Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

[2] Neuroscience Research and Development Group, Khon Kaen University, Khon Kaen 40002, Thailand.

หลักการและวัตถุประสงค์: กรดวอลโพรอิก (valproic acid) เป็นยากันชักที่นิยมใช้มากที่สุดในปัจจุบัน valproic acid มีผลยับยั้งการสร้างเซลล์และการเจริญเปลี่ยนแปลงของเซลล์ โดยไปยับยั้งการทำงานของเอนไซม์ histone deacetylase และทำให้การสร้างเซลล์ประสาทต้นกำเนิดลดลง ซึ่งทำให้เกิดความจำบกพร่องในผู้ป่วยและสัตว์จำพวกหนู เมลาโทนิน (melatonin) เป็นฮอร์โมนที่หลั่งจากต่อมไพเนียลซึ่งควบคุม circadian rhythm มีการศึกษาพบว่า melatonin มีฤทธิ์ต้านอนุมูลอิสระและปกป้องประสาท melatonin ช่วยป้องกันการเกิดความจำบกพร่องในสัตว์ทดลอง ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของ melatonin ในการช่วยป้องกันภาวะความจำบกพร่องที่เกิดจาก valproic acid ในหนูแรทโตเต็มวัย

วิธีการศึกษา: การศึกษานี้ใช้หนูแรทสายพันธุ์ Spraque-Dawley เพศผู้ โดยแบ่งออกเป็น 6 กลุ่ม ประกอบด้วย กลุ่ม control, valproic acid, melatonin, preventive, recovery และ throughout หนูแรทถูกชักนำให้เกิดความจำเสื่อมด้วย valproic acid ความเข้มข้น 300 มิลลิกรัม/กิโลกรัม วันละสองครั้งเป็นเวลา 14 วัน (กลุ่ม valproic acid) หรือได้รับ melatonin (8 มิลลิกรัม/กิโลกรัม/วัน) เป็นระยะเวลา 14 วัน (กลุ่ม melatonin) หรือได้รับ valproic acid ร่วมกับ melatonin โดยได้รับ melatonin เป็นระยะเวลา 14

Background and objectives: Valproic acid is a broad-spectrum drug widely used as an anticonvulsant. A recent study has indicated that valproic acid reduces gene transcription mediated by inhibition of histone deacetylase activities and contribute to suppression of neural stem cell proliferation, which might help to explain the cause of memory impairment produced in patients and rodents. Melatonin is a hormone secreted by the pineal gland and regulates the circadian rhythm. Previous studies have reported that melatonin exerts antioxidant and neuroprotective properties. It also prevents memory impairment in animal models. The present study aimed to investigate protective effects of melatonin on memory impairment caused by valproic acid in adult rats.

Methods: Male Spraque-Dawley rats were divided into 6 groups, including control, valproic acid, melatonin, preventive, recovery and throughout groups. Rats received valproic acid (300 mg/kg) twice a day for 14 days or melatonin (8 mg/kg/day) for 14 days (melatonin group) or co-treatment of VPA and

*Corresponding author : *Jariya Umka Welbat, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen Thailand, 40002. Email: jariya@kku.ac.th*

วันระหว่างที่ได้รับหรือหลังได้รับ valproic acid (กลุ่ม preventiveและ recovery) และ 28 วัน (กลุ่ม throughout) หลังจากนั้นทำการทดสอบความจำชนิดสเปเชียล (spatial) และนอนสเปเชียล (non-spatial) โดยใช้ novel object location และ novel object recognition ตามลำดับ และนำมาคำนวณเป็นค่า discrimination index

ผลการศึกษา: จากการศึกษานี้ในการทดสอบ novel object location และ novel object recognition ก่อนการให้สารต่างๆ พบว่าค่า total exploration time และค่า discrimination index เมื่อเปรียบเทียบกันในหนูแรทแต่ละกลุ่มไม่แตกต่างกัน (p>0.05) การศึกษาหลังจากได้รับสารต่างๆ พบว่าค่า total exploration time เมื่อเปรียบเทียบกันในหนูแรทแต่ละกลุ่มไม่แตกต่างกัน (p>0.05) และค่า discrimination index ในหนูแรทกลุ่ม control, melatonin, preventive, และ throughout มีค่าสูงกว่าค่าศูนย์อย่างมีนัยสำคัญทางสถิติ (p<0.05) เมื่อเทียบกับค่าศูนย์ แต่ในกลุ่ม valproic acid และกลุ่ม recovery มีค่าไม่แตกต่างจากค่าศูนย์ อย่างมีนัยสำคัญทางสถิติ (p>0.05)

สรุป: melatonin สามารถป้องกันและฟื้นฟูความจำชนิด spatial และ non-spatial ที่บกพร่องเกิดจากกรดวอลโพรอิกในหนูแรทโตเต็มวัย

คำสำคัญ: เมลาโทนิน, กรดวอลโพรอิก, ความจำบกพร่อง, ความจำชนิดสเปเชียล, ความจำชนิดนอนสเปเชียล

melatonin by receiving melatonin during or after valproic acid administration for 14 days (preventive and recovery groups) and 28 days (throughout group). Novel object location and novel object recognition tests were used to assess spatial and non-spatial memories, respectively. Data from the behavioral tests were calculated and converted to discrimination index.

Results: In novel object location and novel object recognition tests before drug administration, the total exploration times and discrimination index showed no significant different among groups (p>0.05). After drug administration, the total exploration times showed no significant different among groups (p>0.05). In contrast, the discrimination index was significantly higher than zero in control, melatonin, preventive, and throughout groups (p<0.05) but not significantly different from zero in valproic acid-treated and recovery groups (p>0.05).

Conclusion: Melatonin can prevent and improve spatial and non-spatial memory impairments caused by valproic in adult rats.

Keyword: melatonin, valproic acid, memory impairment, spatial memory, non-spatial memory

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Introduction

Valproic acid (VPA) is clinically used as an anticonvulsant to prevent seizures in both adults and children[1]. High-doses of valproic acid reduce histone deacetylase activity and cause DNA hyperacetylation, which contribute to suppression of cell proliferation involved in neurogenesis[2-5]. Treatment of VPA causes an increase in Reactive oxygen species (ROS) formation that induces oxidative stress and damage. The oxidative damage following VPA exposure stimulates intracellular apoptosis[6-9]. VPA leads to aberration of hippocampal neurogenesis, which is associated with specific memory deficits[10-12]. The rats received intraperitoneal (i.p.) injection of VPA (300 ml/kg) twice daily show spatial memory impairment. This behavioral change is relevant with a reduction in cell proliferation in the hippocampus[13]. Melatonin is a neuro-hormone that is mainly produced by the pineal gland during light-dark cycle to regulate the mammalian biological processes[14]. Melatonin and its metabolites act as a powerful direct free radical scavenger and potent antioxidant agent by enhancing antioxidative defenses[15,16]. A previous study has demonstrated that administration of melatonin (8 mg/kg) promotes cell survival by increasing the number of new neurons derived from hippocampal neural precursor cells[17]. In addition, melatonin and its metabolites play a protective role against radiation-induced impairment of cognitive functions in the hippocampal dentate gyrus by attenuation of cellular death[18].

However, there is no evidence to support the effects of melatonin on memory impairments caused by VPA. Therefore, the aim of this study was to

investigate the effects of VPA and melatonin co-administration on memory deficits by using novel object location and novel object recognition tests.

Materials and Methods

Animals

Seventy-two adult male Sprague Dawley rats (age: 4-5 weeks, weight: 180-220 grams, from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpatom) were used in this study. The experimental protocol was approved by the Khon Kean University Ethics Committee in Animal Research (project number: ACUC-KKU 8/2559). All rats were fed and controlled under standard laboratory condition with 25-30 °C and lighting (dark/light cycle alternating every 12 hours). Rats were weighed daily from arrival and allowed to habituate in the animal facility for one week prior to the start of the procedures. Rats were divided into 6 groups (12 rats in each group): control, valproic acid, melatonin, melatonin co-administration (preventive, recovery and throughout) groups.

Treatments

The control group received ethanol (final concentration was less than 1%) by intraperitoneal injection once a day at 7 p.m. for 28 days and 0.9% normal saline solution by intraperitoneal injection, twice a day at 10 a.m. and 3 p.m. for 14 days. The valproic acid group received valproic acid 300 mg/kg intraperitoneal injection twice a day at 10 a.m. and 3 p.m. for 14 days. For melatonin administration, rats received melatonin 8 mg/kg by intraperitoneal in jection once a day at 7 p.m. for 14 days during valproic acid treatment in preventive group, 14 days after valproic acid treatment in recovery group and 28 days during and after valproic acid treatment in throughout group (Fig. 1).

Behavioural testing

Novel object location

Novel object location test was performed before and after drug administration. One day before testing, rats were habituated in an arena for 30 minutes. The test comprised familiarization and choice trials. In familiarization trial, two identical objects were placed in separate locations in the arena and each animal was allowed to explore the objects for 3 minutes. Then, rats were returned to their cages for 15 minutes. Meanwhile, the objects and the arenas were cleaned with 20% ethanol to eliminate olfactory cues. After that, one object was placed in the familiar location whereas the other was moved to a novel location. During the choice trial, rats were placed back to the arena and explored the objects for 3 minutes. Time spent exploring in the test was recorded by VDO camcorder Version-052, OKER (Crown computer Co., Ltd, Bangkok, Thailand). Exploration time of the test was calculated and converted to discrimination index.

Novel object recognition

One day before novel object recognition testing, rats were habituated in an arena for 30 minutes. Next day, in familiarization trial, two identical objects were placed in separate locations in the arena and each rat was allowed to explore the objects for 3 minutes. Then, the rats were returned to their cages for 15 minutes. Meanwhile, the objects and the arenas were cleaned with 20% ethanol to eliminate olfactory cues. After that, one of the familiar objects and a novel object were placed in the positions. During the choice trial, the rats were returned to the arena and explored objects for 3 minutes. Time spent exploring in the objects was recorded by VDO camcorder Version-052, OKER (Crown computer Co., Ltd, Bangkok, Thailand). Exploration time of the test was calculated and converted to discrimination index.

Discrimination index is defined as the difference in exploration time between the two objects in the choice trial. It was calculated by exploration time of the novel location or novel object (second) minus with exploration time of the familiar location or familiar object (second)[13].

Statistical analysis

All parameters were calculated using GraphPad Prism (Ver. 5.0) software and a probability level of

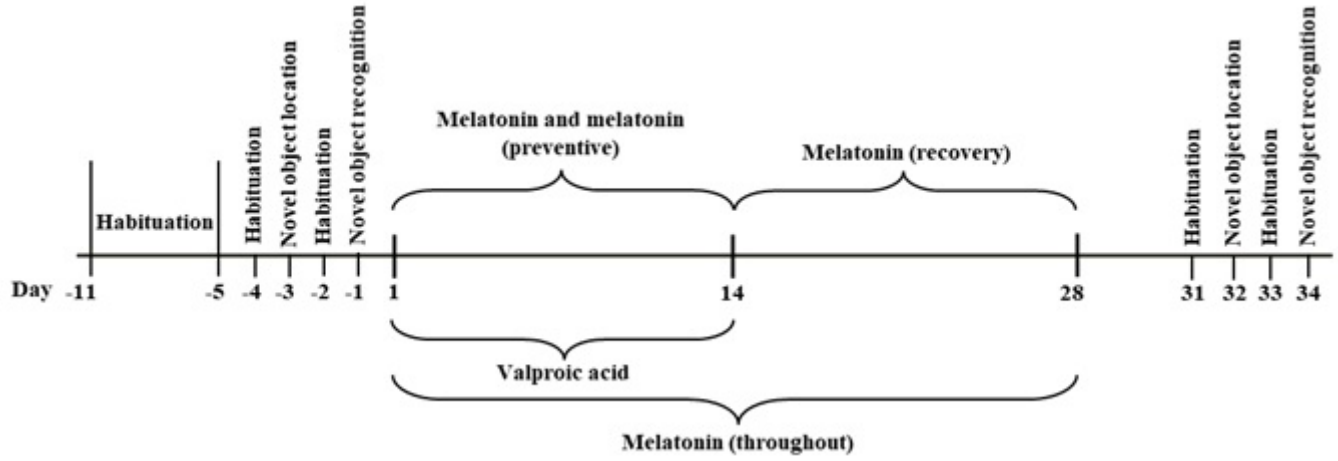

Figure 1 Timeline of animal drug administration and behavioral testing in control, valproic acid, melatonin, preventive, recovery and throughout groups.

$p<0.05$ was considered statistically significant. Two-way ANOVA was used to determine animal weight among groups. One-way ANOVA was used to compare total exploration time of NOL and NOR tests and one sample t-test was used to compare DI of NOL and NOR tests.

Results

Effects of treatments on body weight

The body weight of rats was monitored daily all over the study. Both treatment and time had a significant effect on body weight ($p<0.0001$, Fig. 2). Rats receiving melatonin alone showed no significant difference as compared to control ($p>0.05$). Body weight of rats in valproic acid group had significantly less than control group after valproic acid treatments ($p<0.001$). Similarly, rats in preventive, recovery and throughout groups showed a weight loss during drug administration ($p<0.001$). However, they showed improved weight gain after the end of the study but remained significantly lower than in control group.

Total exploration time and the discrimination index before drug administration

Before drug administration, the rats in each group were determined the memory using the novel object location and novel object recognition tests. In novel object location test, the total exploration time showed no significant difference among the groups ($p>0.05$, Fig. 3A), indicating that all rats had the same baseline of locomotor ability. The discrimination index was significantly above zero in all groups ($p<0.05$, Fig. 3B), indicating that rats had normal memory to discriminate between novel and familiar object locations. Likewise, the rats showed their normal locomotor activity in novel object recognition test. There was no significant difference among the groups ($p>0.05$, Fig. 4A). The rats showed their normal memory to discriminate between novel and familiar objects shown by the discrimination index that was significantly higher above zero in all groups ($p<0.05$, Fig. 4B).

Melatonin improves spatial memory impairments caused by valproic acid.

The novel object location test was used to determine spatial memory. There was no significant difference among the groups in terms of total exploration time ($p>0.05$, Fig. 5A). The discrimination index was significantly above zero in control, melatonin, preventive and throughout groups ($p<0.05$, Fig. 5B) but was not significantly different from zero in the valproic acid -treated and recovery groups ($p>0.05$). These findings show that rats in control, melatonin, preventive, and throughout groups could discriminate between novel and familiar object location, indicating that melatonin can prevent and improve the spatial memory impairments caused by valproic acid.

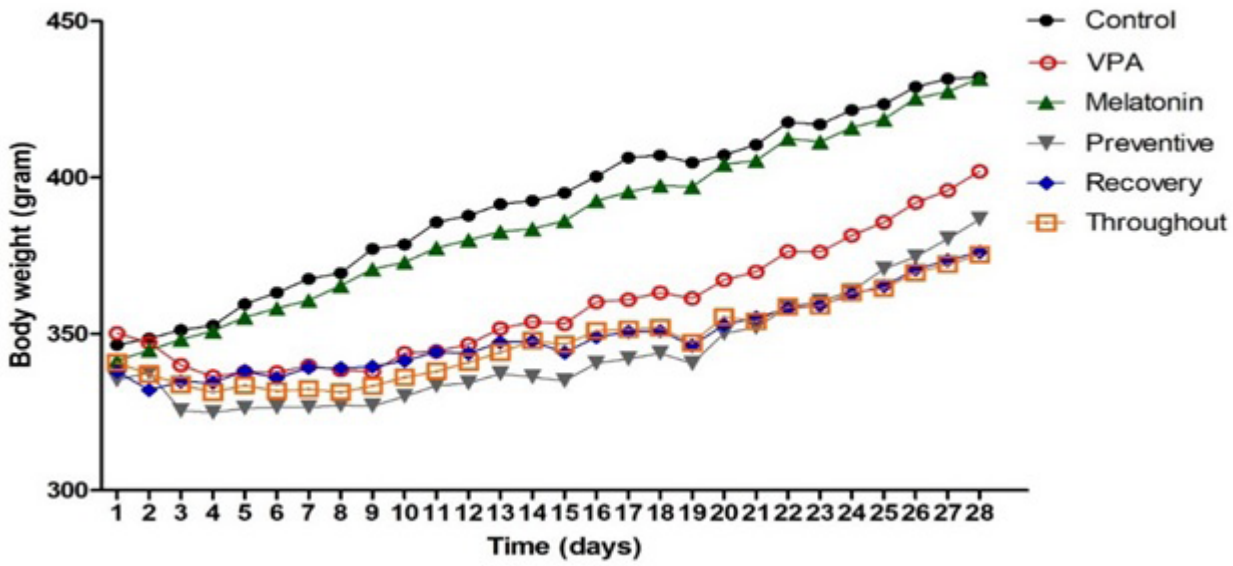

Figure 2 Body weight of rats in control, valproic acid, melatonin, preventive, recovery and throughout groups during day 1 to day 28.

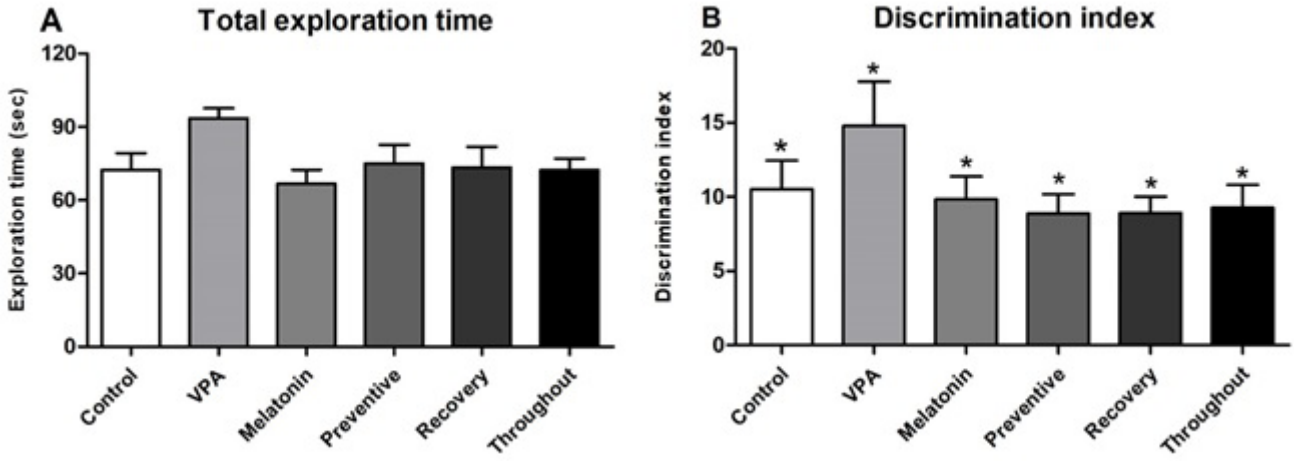

Figure 3 Novel object location test before treatment. Total exploration time was not significantly different among groups ($p>0.05$, A). Discrimination index was significantly different from zero in all groups (*$p<0.05$, B).

Melatonin improves non-spatial memory impairments caused by valproic acid.

The novel object recognition test was used to determine non-spatial memory. There was no significant difference among the groups in terms of total exploration time ($p>0.05$, Fig. 6A). This result shows that discrimination index of control, melatonin, preventive and throughout groups was significantly above zero ($p<0.05$, Fig. 6B) but not significantly different from zero in the valproic acid -treated and recovery groups ($p>0.05$). These findings show that rats in control, melatonin, preventive, and throughout groups could discriminate between familiar and novel object, indicating that melatonin can prevent and improve the non-spatial memory impairments caused by VPA.

Discussion

In the present study, we found that valproic acid can cause memory impairment in valproic acid treated rats. Nevertheless, this impairment was improved by melatonin co-administration.

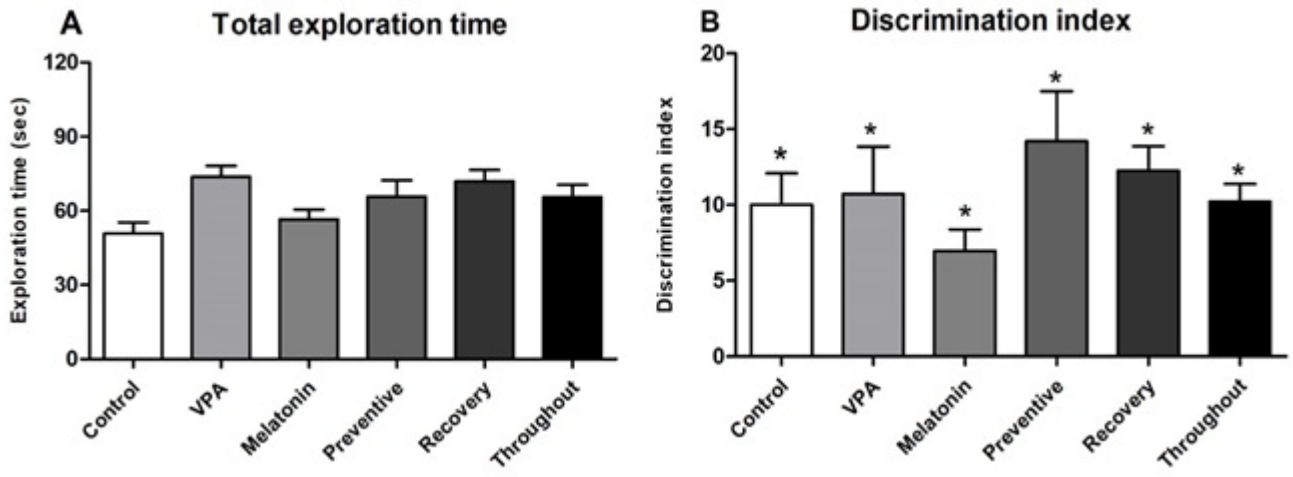

Figure 4 Novel object recognition test before treatment. Total exploration time was not significantly different among groups ($p>0.05$, A). Discrimination index was significantly different from zero in all groups (*$p<0.05$, B).

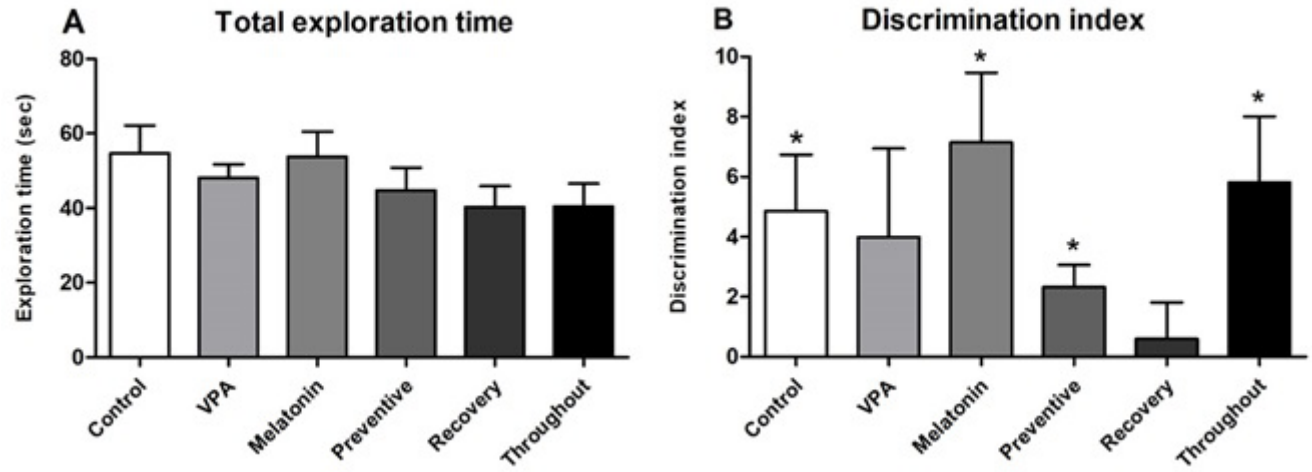

Figure 5 Novel object location test after treatment. Total exploration time was not significantly different among groups ($p>0.05$, A). Discrimination index of control, melatonin, preventive and throughout groups was significantly different from zero (*$p<0.05$, B).

The body weight of rats in melatonin group was not significantly different compared with control group. This indicates that melatonin did not affect the body weight. The effect of valproic acid on body weight demonstrated that valproic acid caused the weight loss in preventive, recovery and throughout groups. Body weight of rats in these three groups were tended to increase but significantly lower than valproic acid group. Therefore, the results in this study indicated that valproic acid treatment could decrease body weight. Previous studies have reported that valproic acid decreases body weight[19-21]. This result coincides with a study that rats treated with valproic acid and fluoxetine, however this mechanism has not yet been elucidated[22]. Future studies should be needed to investigate the mechanism of melatonin co-administration and valproic acid regarding to body weight.

The results of novel object location and novel object recognition tests demonstrated that valproic acid induced spatial and non-spatial memory impairments which are consistent with previous studies[13,23]. Valproic acid has been shown to impair memory processes both of spatial and non-spatial memory. Valproic acid-induced behavioral abnormalities including social interaction impairment,

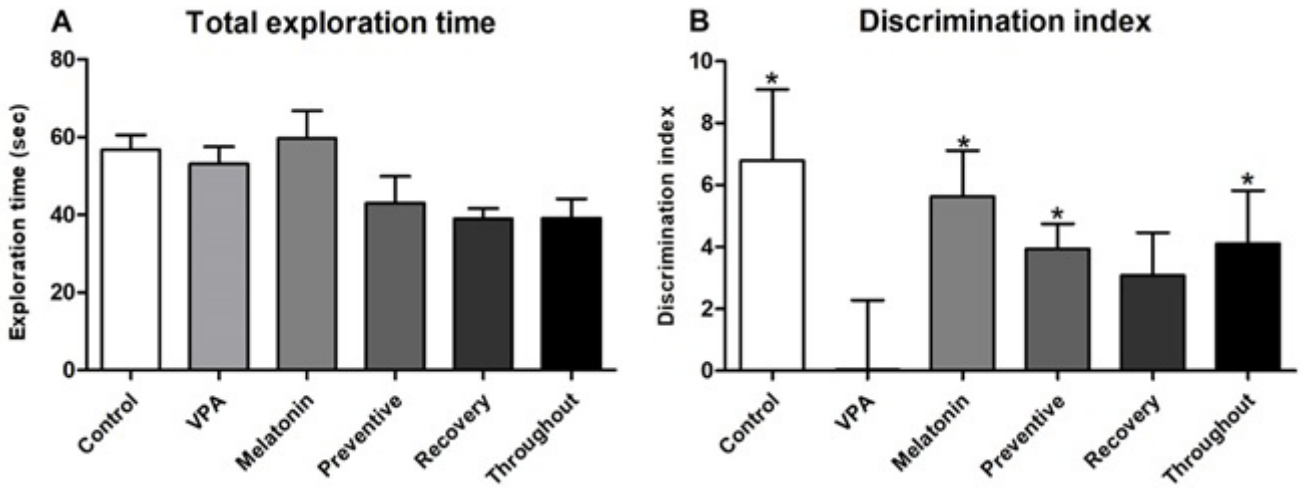

Figure 6 Novel object recognition test after treatment. Total exploration time was not significantly different among groups ($p>0.05$, A). Discrimination index of control, melatonin, preventive and throughout groups was significantly different from zero (*$p<0.05$, B).

anxiety-like behavior and spatial learning disability[13,23-25]. Valproic acid treatment can induce cognitive impairments, which could be associated with a decrement in hippocampal neurogenesis, particularly cell proliferation, survival and differentiation[13,22,26]. Our results show that rats receiving valproic acid were ineffective to discriminate between two identical objects in familiar and novel locations, indicating an impairment in spatial memory. The discrimination index of VPA-treated and preventive groups are likely different but there was no significant difference. In addition, they showed an inability to discriminate between the novel object and familiar object, indicating an impairment in non-spatial memory. The subgranular zone of the dentate gyrus is one of neurogenic regions which is known to be involved in hippocampal-dependent learning and memory. This discrimination is performed with an intact hippocampal formation and is lost when hippocampal neurogenesis is reduced[11,13.] In this study, the impairments were improved by melatonin co-treatment. The rats which had received melatonin co-administration showed the ability to discriminate between an object in a novel location and one in a familiar location. Furthermore, they were able to discriminate between novel object and familiar object in novel object location test. Therefore, melatonin can prevent and improve spatial and non-spatial memory impairments induced by VPA. Previous studies have demonstrated that melatonin treatment reverses cognitive impairment by improving spatial and non-spatial memory[27,28]. Adverse effects during valproic acid treatment in patients[29] and in rats[30] have been associated with overproduction of reactive oxygen species (ROS) and reductions in antioxidant enzymes including glutathione peroxidase, superoxide dismutase, and catalase[31]. Melatonin and its metabolites have been suggested as a potent free radical scavenger mostly all reactive oxygen and nitrogen species. There are also effective in protecting biomolecules from oxidative damage such as nuclear DNA, membrane lipids, and cytosolic proteins. Additionally, melatonin also increases the activities of antioxidant enzymes, including glutathione peroxidase, superoxide dismutase, and catalase[15,16]. The increasing of antioxidant enzyme by melatonin can reduce oxidative stress-induced dysfunctions of hippocampal neurogenesis and memory formation. The neuroprotective effect due to antioxidant properties of melatonin have been proven in the rat hippocampus.[17,32,33]. Melatonin enhances cell proliferation in the dentate gyrus of rats34 and exerts the potential effects on neuronal differentiation[35]. Melatonin promotes cell survival by increasing the number of new neurons derived from adult

hippocampal neural precursor cells. It is believed that melatonin has a potential role in controlling adult hippocampal neurogenesis[17,36]. Oxidative stress is reported to be associated with the onset of cognitive impairments. Melatonin could attenuate cellular death in the hippocampal dentate gyrus by the protect role against radiation-induced impairment of neurogenesis and cognitive functions[18].

A recent report has shown that exposure to methamphetamine (METH) induces a reduction of the cell proliferation of adult rat hippocampus, resulting in the impairment of learning and memory. Therefore, pretreatment with melatonin ameliorates this effect. Low cell proliferation and differentiation capacities of hippocampal stem cells are related with learning and memory impairments[37]. Melatonin also prevents the decreases in neurogenesis that caused by METH, suggesting that melatonin might prevent the METH-induced learning and memory impairments[37,38]. For these studies, the effects of melatonin on memory impairments caused by VPA might be due to increasing of neurogenesis that is associated with memory.

Conclusion

The present study showed that valproic acid induced a cognitive impairment which was displayed as a reduction in spatial and non-spatial memory. Valproic acid treated rats were impaired in discrimination between the objects in familiar and novel locations or novel object and familiar object. These behavioral changes caused by valproic acid were prevented and improved by melatonin co-administration. Melatonin co-administration in treated rats shows the efficacies to discriminate between the objects in familiar and novel locations in novel object location test. In addition, melatonin co-administration improves the abilities of rats to discriminate between familiar object and novel object in novel object recognition test. These results indicate that melatonin may be able to prevent and improve the spatial and non-spatial memory impairments caused by VPA in adult rats.

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