นิพนธ์ต้นฉบับ • Original Article

ผลของเมลาโทนินต่อภาวะความจำบกพร่องที่ถูกเหนี่ยวนำโดยยาเคมีบำบัด 5-fluorouracil ในหนูแรทโตเต็มวัย

กรรวี สุวรรณโคตร[1], สุชาดา ครุฑศรี[1], อนุสรา อารณะโรจน์[1], ภรณ์ทิพย์ ชัยสว่าง[1], วนัสนันท์ แป้นนางรอง[1,2], วรรณี ชัยจรูญคณารักษ์[1], จริยา อำคา เวลบาท[1,2,3*]

[1]ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

[2]ศูนย์วิจัยและพัฒนาผลิตภัณฑ์สุขภาพจากสมุนไพร มหาวิทยาลัยขอนแก่น

[3]กลุ่มวิจัยและพัฒนาด้านประสาทวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น

The Effect of Melatonin on Memory Deficits Induced by 5-Fluorouracil Chemotherapy in Adult Rats

Kornrawee Suwannakot[1], Suchada Krutsri[1], Anusara Aranarochana[1], Pornthip Chaisawang[1], Wanassanun Pannangrong[1,2], Wunnee Chaijaroonkhanarak[1], Jariya Umka Welbat[1,2,3*]

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

[2]Center for Research and Development of Herbal Health Products, Khon Kaen University, Khon Kaen 40002, Thailand

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

หลักการและวัตถุประสงค์: เมลาโทนิน หรือ N-acetyl-5-methoxytryptamine มีคุณสมบัติเป็นสารต้านอนุมูลอิสระ (antioxidant) และ free radical scavenger เมลาโทนินควบคุมการสร้างเซลล์ประสาทใหม่ (neurogenesis) และส่งผลดีต่อความจำในหนูแรทโตเต็มวัยจากการศึกษาก่อนหน้านี้พบว่า 5-fluorouracil (5-FU) ซึ่งเป็นยาเคมีบำบัดที่ใช้ในการรักษาโรคมะเร็ง เป็นสาเหตุทำให้เกิดภาวะความจำบกพร่อง การศึกษานี้จึงได้ศึกษาผลของเมลาโทนินต่อภาวะความจำบกพร่องที่เกิดจากการถูกเหนี่ยวนำด้วยยาเคมีบำบัด 5-FU

วิธีการศึกษา: หนูแรทเพศผู้สายพันธุ์ Sprague Dawley ถูกแบ่งออกเป็น 6 กลุ่ม ได้แก่ กลุ่ม control, กลุ่ม melatonin, กลุ่ม 5-FU, กลุ่ม preventive, กลุ่ม recovery และกลุ่ม throughout โดยที่หนูได้รับเมลาโทนิน (8 มิลลิกรัม/กิโลกรัม/วัน) โดยการฉีดทางหน้าท้อง วันละ 1 ครั้ง เวลา 19.00 น. เป็นเวลา 21 วัน และได้รับยาเคมีบำบัด 5-FU (25 มิลลิกรัม/กิโลกรัม/วัน) ทางหลอดเลือดดำ 5 ครั้ง ทุกๆ 3 วัน โดยเริ่มให้ในวันที่ 9 ของการทดลอง หนูกลุ่มที่ได้รับยาเคมีบำบัด 5-FU ร่วมกับเมลาโทนินได้รับเมลาโทนิน วันละ 1 ครั้ง เวลา 19.00 น.

Background and Objective: Melatonin (N-acetyl-5-methoxytryptamine) has antioxidant properties and functions as a free radical scavenger. Interestingly, melatonin modulates neurogenesis and has positive effects on memories in adult rats. 5-fluorouracil (5-FU) chemotherapy is widely used to treat cancer and causes memory deficits. The present study investigated the effects of melatonin on memory deficits induced by 5-FU.

Methods: Male Sprague Dawley rats were divided into 6 groups; control, melatonin, 5-FU, preventive, recovery and throughout groups. Melatonin (8 mg/kg/day) was administered by intraperitoneal injection once a day at 7.00 pm. for 21 days. Rats received 5-FU (25 mg/kg/day) by intravenous injection 5 times every 3 days starting on day 9. In co-treatment groups, 5-FU-treated rats received melatonin once a day at 7.00 pm. for 21 days during treatment (day 1 to day 21, preventive group) or after treatment (day 22 to day 42, recovery group) or both time periods (day 1 to day 42, throughout group). After that, the memories were determined using novel object location (NOL) and novel object recognition (NOR) tests.

*Corresponding Author: Jariya Umka Welbat, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand Email: jariya@kku.ac.th Tel: (+66) 846676105

โดยหนูกลุ่ม preventive ได้รับเมลาโทนินในช่วงวันที่ 1-21 ของการทดลอง หนูกลุ่ม recovery ได้รับเมลาโทนินในช่วงวันที่ 22-42 ของการทดลอง ส่วนหนูกลุ่ม throughout ได้รับเมลาโทนินตลอดการทดลองจากวันที่ 1-42 จากนั้นหนูได้ถูกทดสอบความจำโดยวิธี novel object location (NOL) และ novel object recognition (NOR)

ผลการศึกษา: พบว่าหนูกลุ่มที่ได้รับยาเคมีบำบัด 5-FU ร่วมกับเมลาโทนินทุกกลุ่ม (กลุ่ม preventive, กลุ่ม recovery และ กลุ่ม throughout) มีการเพิ่มของน้ำหนักน้อยกว่ากลุ่ม control อย่างมีนัยสำคัญทางสถิติการทดสอบความจำด้วยการทดสอบ NOL และ NOR พบว่าหนูทุกกลุ่มใช้เวลารวมในการสำรวจวัตถุไม่แตกต่างกัน จากการทดสอบ NOL พบว่าหนูที่ได้รับยาเคมีบำบัด 5-FU ร่วมกับเมลาโทนินทุกกลุ่มสามารถแยกวัตถุในตำแหน่งใหม่ออกจากตำแหน่งเก่าแตกต่างอย่างมีนัยสำคัญทางสถิติ ในทางกลับกันการทดสอบ NOR พบว่าหนูไม่สามารถแยกวัตถุใหม่ออกจากวัตถุเก่าได้ในหนูทุกกลุ่มที่ได้รับยาเคมีบำบัด 5-FU ร่วมกับเมลาโทนิน

สรุป: จากการศึกษาพบว่าการได้รับเมลาโทนินทั้งในช่วงระหว่างที่ได้รับยาเคมีบำบัด 5-FU ช่วงหลังได้รับยาเคมีบำบัด 5-FU หรือได้รับตลอดการทดลองมีผลทำให้การบกพร่องของความจำชนิด spatial ดีขึ้นได้ ในขณะที่การได้รับเมลาโทนินร่วมกับยาเคมีบำบัด 5-FU ไม่ทำให้ความจำชนิด declarative ดีขึ้นดังนั้นการศึกษานี้จึงแสดงให้เห็นว่าความจำบกพร่องชนิด spatial ที่เกิดจากยาเคมีบำบัด 5-FU สามารถดีขึ้นได้เมื่อได้รับการรักษาร่วมกับเมลาโทนิน

คำสำคัญ: ไฟฟ์-ฟลูออโรยูราซิล, เมลาโทนิน, ความจำ

Results: The results showed that weight gain of co-treated with melatonin groups was significantly less than control group. All groups were not significantly different in the total exploration time in both NOL and NOR tests. Rats in co-treated groups could significantly discriminate the object between novel and familiar locations in NOL test. On the other hand, co-treatment with melatonin failed to discriminate novel and familiar objects in NOR test.

Conclusion: This study demonstrated that co-treatment with melatonin during or after or both time periods ameliorated spatial memory deficits but co-administration with melatonin did not relieve declarative memory deficits. So, this study demonstrates that spatial memory deficits caused by 5-FU could be attenuated by melatonin administration.

Keywords: 5-fluorouracil, melatonin, memory

ศรีนครินทร์เวชสาร 2561; 33(2): 183-9. • Srinagarind Med J 2018; 33(2): 183-9.

Introduction

Memory deficits and dementia are universal problems. There are many factors that cause memory deficits such as stress[1], depression[2], brain trauma[3] and aging[4]. Especially, treating cancer patients with chemotherapy drugs leads to memory deficits and decreasing neurogenesis[5]. 5-FU is a chemotherapy drug used in the treatment of cancers such as breast, colorectal and skin cancers. 5-FU inhibits DNA synthesis and causes RNA damage by its active metabolite such as fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). The drug easily crosses the blood-brain barrier and then decreases neurogenesis[6,7]. A previous study has shown that 5-FU reduces cell proliferation and survival in the subgranular zone (SGZ) of the dentate gyrus which is associated with memory deficits[8]. Melatonin has antioxidant properties that enhance neurogenesis[4]. A previous study has suggested that melaton in promotes precursor cell survival *in vitro*[9]. In animal models, melatonin can improve learning and spatial memory impairments induced by hyperhomocysteinemia[10] and D-galactose[4], respectively. There are no evidences about the effects of melatonin on memory deficits caused by 5-FU. The hypothesis of this research was to investigate

the possibility of melatonin to improve memory deficits caused by 5-FU.

Materials and Methods

Animals and treatment

Male Sprague Dawley rats (National Laboratory Animal Center, Mahidol University, Bangkok, Thailand) weighing 180-200 grams were used for all experiments. The experimental protocols were approved by the Khon Kaen University Ethics Committee in Animal Research (project number. ACUC-KKU-45/2559). Rats were group-housed in a 12 h light/dark cycle with ad libitum food and water. Seventy two rats were randomly divided into 6 groups and allowed to habituate for 7 days before drug administration. 5-fluorouracil (5-FU) and melatonin were purchased from Boryung pharmaceutical co., Ltd., Korea and MP Biomedical, LLC, France, respectively.

On day 9 of the experiment, rats were administered with 5-FU (25 mg/kg, 5 i.v. doses every 3 days) in 5-FU, preventive, recovery and throughout groups. Rats were administered with saline solution (5 i.v. doses, every 3 days) and 10% ethanol (i.p. 1 time/day from day 1 to day 21) in control group. In addition, rats were treated with melatonin (8 mg/kg, i.p.1 time/day) for 21 consecutive days at 7.00 p.m. in melatonin (day 1 to day 21), preventive (day 1 to day 21) and recovery (day 22 to day 42) groups. Finally, rats were treated with melatonin for 42 consecutive days in throughout group (day 1 to day 42) (Fig. 1).

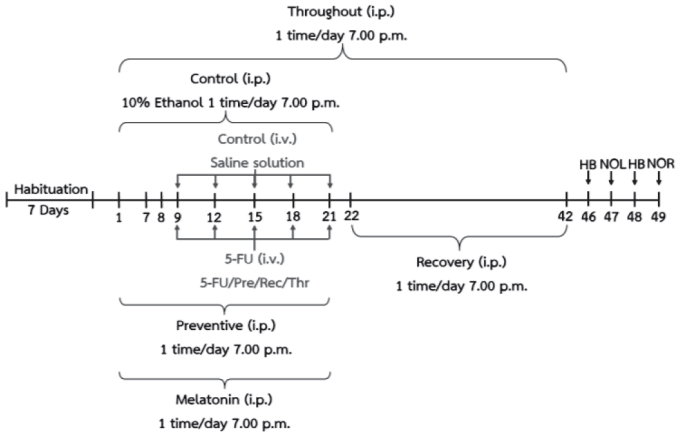

Figure 1 Timeline of drug administration and behavioral tests.

Behavioral testing

Novel object location (NOL)

The NOL test were used to determine spatial working memory. The method consisted of an arena (open quadrate box, dimensions 50 cm wide x 50 cm long x 50 cm high) and plastic bottles filled with water to weigh them down. One day before NOL test, animals were habituated by allowing them to freely explore an open-field arena in the absence of objects for 30 minutes. The next day, animals were habituated again for 3 minutes. In the familiarization trial, two similar objects were placed in separate locations in the arena and each animal was allowed to explore the objects for 3 minutes. Then, the animals were rested to their home cages for 15 minutes (inter-trial interval) while the objects and the arenas were cleaned with 20% alcohol to remove olfactory cues. In the choice trial, the animals were returned to the arena for 3 minutes in which one object was remained in the familiar location whereas one object was moved to a novel location[11].

Novel object recognition (NOR)

The NOR test were used to determine declarative memory. Similarly, animals were habituated in an open-field arena in the absence of objects for 30 minutes one day prior to NOR testing. In the familiarization trial, animals were habituated again for 3 minutes, two similar objects were placed in separate locations in the arena and each animal was allowed to explore the objects

for 3 minutes. Then, the animals were returned to their home cages for 15 minutes while the objects and the arenas were cleaned. In the choice trial, the animals were returned to the arena for 3 minutes in which familiar and novel object was placed in the same location. Exploration time of both NOL and NOR tests was recorded blind two times and averaged using a stopwatch. The discrimination index (DI) were used to evaluate both tests. The experiments were recorded by VDO camcorder (Version-052, OKER, Crown computer Co., Ltd, Bangkok, Thailand).

Statistical Analysis

Two-way ANOVA were used to determine animal weight among groups. One-way ANOVA was used to compare total exploration time of NOL and NOR tests. One sample *t* test was used to compare DI of NOL and NOR tests by using Graph Pad Prism 5.

Results

Effect of melatonin on weight gain

Weight gain of the animals in melatonin group showed no significant difference compared with control group ($p>0.05$, Fig. 2). While, weight gain of the animals in 5-FU group was significantly lower than control group ($p<0.05$, Fig. 2). Weight gain of the animals in co-treated groups was significantly less than control group ($p<0.05$, Fig. 2). These results indicate that 5-FU disrupted weight gain.

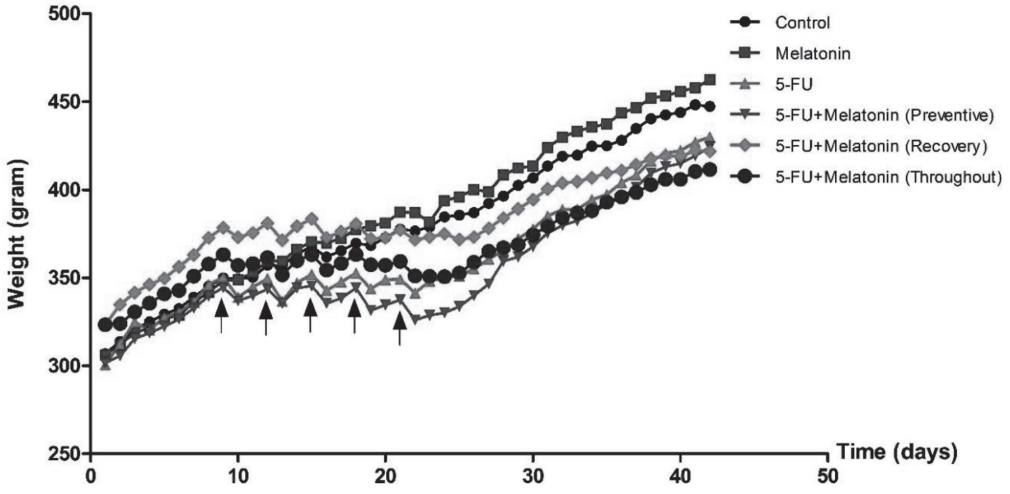

Figure 2 The weight gain of animals throughout the experiment. Arrows indicate 5-FU (25 mg/kg/day)/saline injection.

Effect of melatonin on memories

There were no significant differences in the total exploration time in both behavioral tests ($p>0.05$, Fig. 3A and 3B), indicating that none of the groups were impaired in their activity. The DI is defined as the ability to discriminate between two objects of the animals. DI was determined by calculating the difference between the exploration time of the novel and familiar locations or objects. In NOL test, animals in control, melatonin and co-treated groups could significantly discriminate the object between novel and familiar locations ($p<0.05$, Fig. 4A) but did not found in 5-FU group ($p>0.05$, Fig. 4A). In NOR test, the animals in control and melatonin groups significantly discriminated between the novel and familiar objects ($p<0.05$, Fig. 4B) but not in preventive and recovery groups ($p>0.05$, Fig. 4B). Moreover, animals were failed to discriminate the objects in throughout group ($p<0.05$, Fig. 4B). The results indicate that co-treatment with melatonin improved spatial memory deficits in all groups whereas co-treatment with melatonin did not relieve declarative memory deficits.

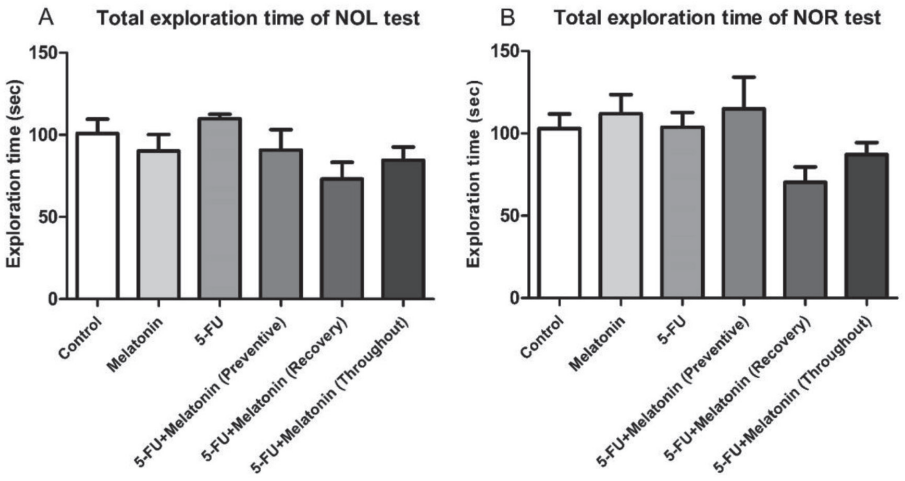

Figure 3 The total exploration time of the animals exploring all objects in NOL (A) and NOR tests (B).

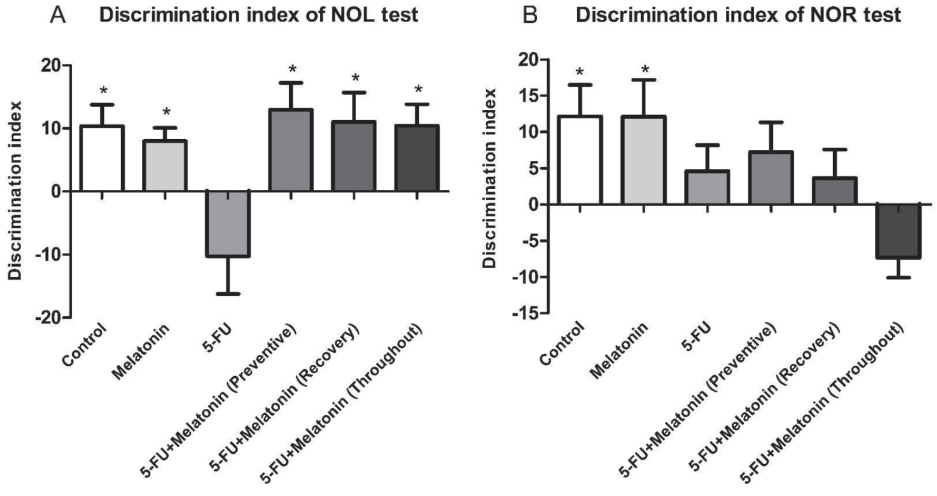

Figure 4 The discrimination index of NOL (A) and NOR tests (B). (*significant difference compared to zero, $p<0.05$).

Discussion

In the present study, weight gain of 5-FU and co-treatment groups was lower than control group. This is consistent with a previous study, which has shown that 5-FU treated rats decrease weight gain[6]. It has been reported that 5-FU disrupts weight gain by decreasing intestinal absorption and inducing intestinal injury which causes mucositis[12]. These damages reduce mucosal DNA, RNA and protein content[13] and increase mucosal permeability[14], a factor of bacterial translocation from intestinal lumen to the blood stream[15]. These result in clinical syndrome of diarrhea, dehydration and weight loss[16]. In the present study, weight gain of rats in melatonin group was not significantly different when compared to control group throughout the experiment, indicating that melatonin does not interfere weight gain of rats.

The novel object location (NOL) and novel object recognition (NOR) are used as behavioral index by using the natural preference for novelty of rats[11,17,18]. The NOL test was chosen as a test of spatial memory which depends on hippocampus[19]. While, the NOR test was chosen as a test of declarative memory[20] that is associated to medial temporal lobe. This lobe is a set of structures that is special for the hippocampus and adjacent cortical areas including entorhinal, perirhinal and parahippocampal cortex[21]. In this study, 5-FU group could not discriminate the objects placed in

different locations as demonstrated in discrimination index analysis of NOL test. This result is consistent with the previous study which has shown that 5-FU treated rats had a lower preference index than vehicle rats using object location recognition (OLR) test. It indicates that rats had memory deficit in spatial memory[7]. Additionally, 5-FU chemotherapy affects newborn neurons by disruption of hippocampal neurogenesis[5,7]. Recently, it has been reported that 5-FU reduces cell proliferation and survival in the subgranular zone of dentate gyrus which is associated with memory deficits[8]. In addition, the present study demonstrated that 5-FU decreased the discrimination index in NOR test, suggesting that 5-FU chemotherapy impaired declarative memory[20]. This result is in line with a previous study that showed significantly reduced preference of novel object in 5-FU treated rats compared to control rats. It indicates that 5-FU chemotherapy affected perirhinal cortex function[22]. 5-FU treated rats had a difficulty to receive incoming sensory information from the visual, olfactory and somatosensory[23]. It was reported that the perirhinal cortex plays an important role in object recognition memory[24] because the perirhinal cortex is involved in object recognition. It is necessary to information about familiarity or novelty of an object[18].

Several studies reported positive effects of melatonin in term of memory. The present study showed results of discrimination index (DI) which co-treatment with melatonin rats (preventive, recovery and throughout groups) had the ability to discriminate between two objects located in familiar and novel locations using NOL test. These results demonstrated that melatonin ameliorated spatial memory deficits in 5-FU-treated rats received co-treatment with melatonin during (preventive group) or after (recovery group) or both (throughout group) time periods. Similarly, it has been reported that melatonin improved memory impairments in mice using Morris water maze (MWM) test which is a spatial memory test[4]. Melatonin increases pCREB (Phosphorylated cyclic AMP response element binding protein) in positive nuclei of the dentate gyrus. Immature neurons express CREB which is associated with hippocampal neurogenesis in adult mice[25]. Moreover, melatonin stimulates dendrite maturation[26] and increases synaptic connectivity, Dendrite maturation and synaptic connectivity are important to hippocampal neuronal circuitry[27]. In the present study, animals could not discriminate novel and familiar objects in co-treatment of 5-FU and melatonin groups (preventive, recovery and throughout groups) using NOR test. Moreover, animals failed to discriminate the objects in throughout group. These results demonstrated that melatonin did not prevent and improve declarative memory deficits in any time periods of co-treatment groups. This suggests that melatonin improved spatial memory deficits in term of protective and recovery effects from 5-FU chemotherapy, but it did not show in declarative memory test.

Conclusion

This research showed that co-treatment with melatonin improved spatial memory deficits induced by 5-FU. While, co-administration with melatonin did not relieve declarative memory deficits. So, this study suggests that spatial memory deficits caused by 5-FU are attenuated by melatonin administration. The present study will be beneficial for cancer patients who are suffered from 5-FU chemotherapy treatment.

Acknowledgement

The authors would like to thank invitation research from Faculty of Medicine (IN60220), Khon Kaen university for financially support.

References

1. Zhao TT, Shin KS, Park HJ, Kim KS, Lee KE, Cho YJ, et al. Effects of (-)-sesamin on chronic stress-induced memory deficits in mice. Neurosci Lett 2016; 634: 114-8.
2. Darcet F, Gardier AM, David DJ, Guilloux J-P. Chronic 5-HT4 receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression. Neurosci Lett 2016; 616: 197-203.
3. Lesniak A, Leszczynski P, Bujalska-Zadrozny M, Pick CG, Sacharczuk M. Naloxone exacerbates memory impairments and depressive-like behavior after mild traumatic brain injury (mTBI) in mice with upregulated opioid system activity. Behav Brain Res 2017; 326: 209-16.

4. Yoo DY, Kim W, Lee CH, Shin BN, Nam SM, Choi JH, et al. Melatonin improves D-galactose-induced aging effects on behavior, neurogenesis, and lipid peroxidation in the mouse dentate gyrus via increasing pCREB expression. J Pineal Res 2012; 52: 21-8.
5. ElBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Chur-yoe GT, et al. Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. Behav Brain Res 2010; 208: 112-7.
6. Lyons L, ElBeltagy M, Bennett G, Wigmore P. Fluoxetine counteracts the cognitive and cellular effects of 5-fluorouracil in the rat hippocampus by a mechanism of prevention rather than recovery. PLoS One 2012; 7: e30010.
7. Mustafa S, Walker A, Bennett G, Wigmore PM. 5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus. Eur J Neurosci 2008; 28: 323-30.
8. Chaisawang P, Sirichoat A, Chaijaroonkhanarak W, Pannangrong W, Sripanidkulchai B, Wigmore P, et al. Asiatic acid protects against cognitive deficits and reductions in cell proliferation and survival in the rat hippocampus caused by 5-fluorouracil chemotherapy. PLoS One 2017; 12: e0180650.
9. Sotthibundhu A, Phansuwan-Pujito P, Govitrapong P. Melatonin increases proliferation of cultured neural stem cells obtained from adult mouse subventricular zone. J Pineal Res 2010; 49: 291-300.
10. Baydas G, Ozer M, Yasar A, Tuzcu M, Koz ST. Melatonin improves learning and memory performances impaired by hyperhomocysteinemia in rats. Brain research. 2005; 1046: 187-94.
11. Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behav Brain Res 1999; 99: 191-200.
12. Huang FS, Kemp CJ, Williams JL, Erwin CR, Warner BW. Role of epidermal growth factor and its receptor in chemotherapy-induced intestinal injury. Am J Physiol-Gastr L2002; 282: 432-42.
13. Mao Y, Kasravi B, Nobaek S, Wang L, Adawi D, Roos G, et al. Pectin-supplemented enteral diet reduces the severity of methotrexate-induced enterocolitis in rats. Scand J Gastroenterol 1996; 31: 558-67.
14. Forsgård RA, Korpela R, Holma R, Lindén J, Frias R, Spillmann T, et al. Intestinal permeability to iohexol as an in vivo marker of chemotherapy-induced gastrointestinal toxicity in Sprague-Dawley rats. Cancer Chemoth Pharm 2016; 78: 863-74.
15. Berg RD. Bacterial translocation from the gastrointestinal tracts of mice receiving immunosuppressive chemotherapeutic agents. Current Microbiology 1983; 8: 285-92.
16. Farrell CL, Bready JV, Rex KL, Chen JN, DiPalma CR, Whitcomb KL, et al. Keratinocyte growth factor protects mice from chemotherapy and radiation-induced gastrointestinal injury and mortality. Cancer Res 1998; 58: 933-9.
17. Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp Brain Res 1997; 113: 509-19.
18. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 2012; 13: 93-110.
19. Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H. Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learning &memory 2002; 9: 49-57.
20. Winters BD, Saksida LM, Bussey TJ. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. Neurosci Biobehav Rev 2008; 32: 1055-70.
21. Baxter MG. "I've seen it all before": explaining age-related impairments in object recognition. Behav Neurosci 2010; 124: 706-9.
22. Fardell JE, Vardy J, Shah JD, Johnston IN. Cognitive impairments caused by oxaliplatin and 5-fluorouracil chemotherapy are ameliorated by physical activity. Psychopharmacology 2012; 220: 183-93.
23. Clarke JR, Cammarota M, Gruart A, Izquierdo I, Delgado-García JM. Plastic modifications induced by object recognition memory processing. Proc Natl Acad Sci 2010; 107: 2652-7.
24. Aggleton JP, Albasser MM, Aggleton DJ, Poirier GL. Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. Behav Neurosci 2010; 124: 55-68.
25. Nakagawa S, Kim JE, Lee R, Chen J, Fujioka T, Malberg J, et al. Localization of phosphorylated cAMP response element-binding protein in immature neurons of adult hippocampus. J Neurosci 2002; 22: 9868-76.
26. Ramirez-Rodriguez G, Ortiz-Lopez L, Dominguez-Alonso A, Benitez-King GA, Kempermann G. Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 2011; 50: 29-37.
27. Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 2007; 54: 559-66.