เมลาโทนินช่วยรักษาความจำบกพร่องหลังจากได้รับยาเคมีบำบัดเมโทเทรกเซท ในหนูแรทโตเต็มวัย

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Melatonin Can Ameliorate Memory Deficits Induced by Methotrexate Chemotherapy in Adult Rats

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หลักการและวัตถุประสงค์: เมโทเทรกเซท (methotrexate) เป็นยาเคมีบำบัดที่นิยมใช้ในการรักษาผู้ป่วยโรคมะเร็ง จากการศึกษาที่ผ่านมาพบว่า methotrexate มีผลในการลดการสร้างและการอยู่รอดของเซลล์ประสาทในหนูแรท เมลาโทนิน (melatonin) เป็นฮอร์โมนที่สร้างจากต่อมไพเนียล ซึ่งมีฤทธิ์ในการต้านสารอนุมูลอิสระ อีกทั้งมีผลต่อกระบวนการสร้างเซลล์ประสาทใหม่อีกด้วย ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของ melatonin ในการป้องกันและฟื้นฟูความจำบกพร่องหลังจากได้รับยาเคมีบำบัด methotrexate ในหนูแรทโตเต็มวัย

วิธีการศึกษา: การศึกษานี้ใช้หนูแรทสายพันธุ์ Sprague dawley เพศผู้ โดยแบ่งออกเป็น 6 กลุ่ม ประกอบด้วย กลุ่ม control, melatonin, methotrexate, preventive, recovery และ throughout หนูแรทที่ถูกชักนำให้เกิดความจำเสื่อมด้วย methotrexate (75 มิลลิกรัม/กิโลกรัม) ในวันที่ 8 และ 15 ของการทดลอง (กลุ่ม methotrexate) หรือได้รับร่วมกับ melatonin (8 มิลลิกรัม/กิโลกรัม/วัน) เป็นระยะเวลา 15 วัน (กลุ่ม melatonin, preventive, recovery) และ 30 วัน (กลุ่ม throughout) หลังจากนั้นทำการทดสอบความจำชนิดสเปเชียลและนอนสเปเชียล (spatial, non-spatial) โดยใช้ novel object location และ novel object recognition ตามลำดับ และนำมาคำนวณเป็นค่า discrimination index

Background and Objectives: Methotrexate is commonly used as chemotherapy for cancer patients. Previous studies reported that methotrexate reduces cell proliferation and survival in rats. Melatonin is a neuro-hormone and produced by the pineal gland. Melatonin is a free radical scavenger and induces neurogenic process. Therefore, this study aimed to investigate effects of melatonin that prevents and recovers on memory deficits caused by methotrexate chemotherapy in adult rats.

Methods: Male Sprague dawley rats were divided into 6 groups, including control, melatonin, methotrexate, preventive, recovery and throughout groups. Rats were treated with methotrexate (75 milligram/kilogram) on day 8 and day 15 or together with melatonin (8 milligram/kilogram/day) for 15 days (melatonin, preventive and recovery groups) and 30 days (throughout group). Novel object location and novel object recognition tests were used to determine 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, these studies found that total exploration time were not significant in different among groups (p>0.05). Moreover, discrimination index in

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ผลการศึกษา: จากการศึกษานี้ในการทดสอบ novel object location และ novel object recognition พบว่าค่า total exploration time เมื่อเปรียบเทียบกันในหนูแรทแต่ละกลุ่มไม่แตกต่างกัน (p>0.05) และหนูแรทในกลุ่ม control, melatonin, preventive, recovery และ throughout มีค่าdiscrimination index สูงกว่ากลุ่ม methotrexate อย่างมีนัยสำคัญทางสถิติ (p<0.05)

สรุป: melatonin สามารถป้องกันและฟื้นฟูความจำชนิด spatial และ non-spatial ที่บกพร่องหลังจากได้รับยาเคมีบำบัด methotrexate ในหนูแรทโตเต็มวัย

คำสำคัญ: เมลาโทนิน, เมโทเทรกเซท, ความจำบกพร่อง, ความจำชนิดสเปเชียล, ความจำชนิดนอนสเปเชียล

control, melatonin, preventive, recovery and throughout groups were significantly different as compared to the methotrexate group (p<0.05).

Conclusion: Melatonin can prevent and recover spatial and non-spatial memory deficits caused by methotrexate chemotherapy in adult rats.

Keywords: melatonin, methotrexate, memory deficit, spatial memory, non-spatial memory

Introduction

Methotrexate is a folate antagonist[1]. High-dose methotrexate is commonly used in chemotherapy for treatment of various neoplastic diseases, such as leukemia, osteogenic sarcoma, non-Hodgkin lymphoma and breast cancers[2]. Recent studies have demonstrated that methotrexate inhibits deoxyribonucleic acid (purine and pyrimidine base) and ribonucleic acid synthesis and induces oxidative stress[2-4]. Methotrexate can disrupt hippocampal neurogenesis impairment, such as cell proliferation, survival and differentiation which is associated with memory deficits[4-6]. Methotrexate is not only interfere cell division but also decreases blood supply into hippocampus[5]. Ischemic of the hippocampus decreases neurogenesis that generates oxidative stress that involves memory deficit[4, 5]. In addition, methotrexate can destroy memory process, including encoding, storage, consolidation and retrieval[5, 6]. Methotrexate can also impair short term and long-term memory[6, 7].

Melatonin is a neuro-hormone and produced from the pineal gland[8, 9]. Previous studies have shown that melatonin (8 milligram/kilogram) up-regulates neurogenesis (cell proliferation, cell survival and neuronal differentiation)[10]. In addition, chronic administration of melatonin (14 days) increases volume of granular cell layer, cell survival, dendrites and complexity in maturation[11].

Recently, there is no evidence of effects of melatonin on memory deficits caused by methotrexate.

Therefore, the aim of this study was to investigate the effect of methotrexate and melatonin co-administration on a reduction of memory using novel object location and novel object recognition tests.

Materials and methods

Animals

Seventy-two male Sprague dawley rats (age: 4-5 weeks, weight: 180-220 gram) were used in this study. Rats were obtained from the national laboratory animal center, Mahidol University, Salaya Nakornpatom. The experimental protocol was approved by the Khon Kean University Ethics Committee in Animal Research (project number: ACUC-KKU-46/2559). Rats were controlled under standard laboratory condition with 25-30 °C and 12 hours, dark/lights. Rats were weighed daily to observe that they were in a normal condition. Rats were divided into6 groups (12 rats in each group): control, melatonin, methotrexate, preventive, recovery and throughout groups.

Melatonin preparation

Melatonin (sigma Aldrich, Inc., St. Louis, USA) was prepared freshly every day.Melatonin was dissolved in minimum volume of 100% ethanol and diluted in 0.9% normal saline solution (0.9% NaCl) and final concentration of ethanol was less than 1%. Melatonin was produced to generate the final concentration of 8 milligram/kilogram/day during drug treatment. Melatonin

was wrapped in aluminum foil to prevent light to induce melatonin degradation.

Drug treatment and protocol

The vehicle group received saline solution (0.9% Nacl, Ajax Finechem Pty Ltd., Australia) 0.5 milliliter/kilogram by intravenous injection on day 8 and day 15 and ethanol (final concentration was less than 1%) by intraperitoneal injection at 7.00 post meridiem for 15 days from day 1 to day 15 (Fig. 1). For melatonin administration, rats received melatonin 8 milligram/ kilogram by intravenous injection, 7.00 post meridiem for 15 days during methotrexate treatment in preventive group, 15 days after treatment in recovery group and 30 days during and after treatment in throughout group (Fig. 1). The methotrexate group received methotrexate 75 milligram/kilogram intravenous injection on day 8 and day 15 (Fig. 1). After methotrexate and saline solution administration, rats received leucovor in 6 milligram/ kilogram (1 milliliter/kilogram) at hour 18,26,42,50 by intravenous injection in control, methotrexate, preventive, recovery and throughout groups.
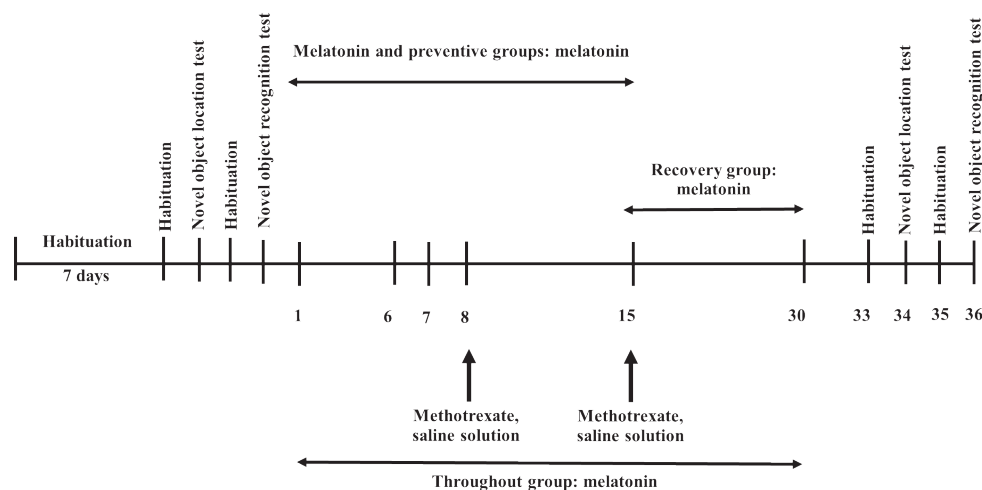
Figure 1 Timeline of drug administration and behavioral testing in control, melatonin, methotrexate, preventive, recovery and throughout groups.

Novel object location test

Novel object location test was performed 3 days after drug administration. One day prior to novel object location testing, rats were habituated in an arena for 30 minutes. The novel object location test consisted of familiarization and choice trials. In familiarization trial, rats were put in an arena for 3 minutes and explored two similar objects in two corners. After that, rats were returned to their cages for 15 minutes (inter-trial interval). During this period, 20% ethanol was used to clean the objects and the arena to get rid of the olfactory clue. After that, one object was placed in the same place or familiar location. The other object was moved to a new location or novel location. In choice trial, rats were placed back to the arena and explored objects for 3 minutes. Time spent exploring in novel object location test was recorded by video camera version 0-52, OKER (Crown computer co., Ltd, Bangkok Thailand). Exploration time of novel object location test was calculated and converted to discrimination index. The discrimination index is defined as the ability of rats to discriminate between novel location and familiar location.

Novel object recognition test

Before the novel object recognition test, rats were habituated in an arena for 30 minutes. Next day, in familiarization trial, rats were put into the arena and explored two duplicate objects in two corners for 3 minutes. After that, rats were moved to cage for 15 minutes.During this period, 20% ethanol was used to

clean the objects and the arena to get rid of the olfactory clue.Then, one of the old or familiar objects and a new object or novel object were placed in the positions. In choice trial, rats were put into an arena and explored different objects for 3 minutes. The time spent exploring in novel object recognition test was recorded by video camera version 0-52, OKER (Crown computer co., Ltd, Bangkok Thailand). Exploration time of novel object recognition test was calculated and converted to discrimination index. The discrimination index is defined as the ability of rats to discriminate between the novel object and familiar object.

Discrimination index 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)[12].

Statistical analysis

All parameters were calculated using GraphPad Prism 5.0 software (GraphPad software Inc., sar Diego, CA, USA) and p<0.05was considered as a significant level. Two-way analysis of variance and one-way analysis of variance were used to analyze weight of rats and total exploration time and discrimination index, respectively.

Results

Weight

Body weight of rats in melatonin group was not significantly different from control group (p>0.05, two-way analysis of variance) (Fig 2). After methotrexate injections, methotrexate and preventive groups had significantly less body weight compared to control group from day 17 to day 22 and day 16 to day 21, respectively (p<0.05, two-way analysis of variance) (Fig 2). At the end of the study, rats in both groups increased weight gain to the same level as the control group (p>0.05, two-way analysis of variance) (Fig 2). Similarly, body weight of rats in recovery and throughout groups was significantly less than control group from day 18 to day 30 (p<0.05, two-way analysis of variance) (Fig 2) and then the body weight tended to increase.
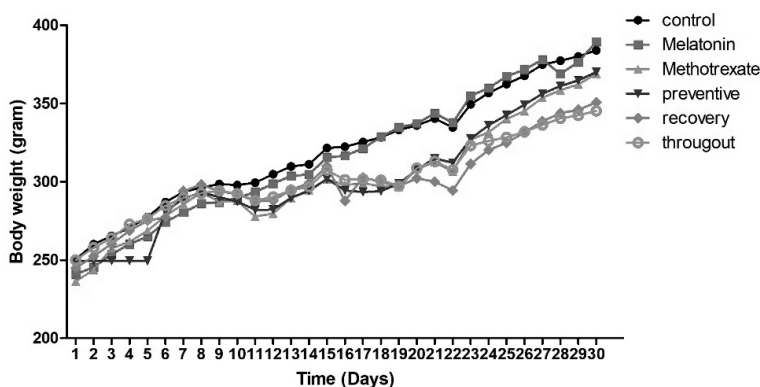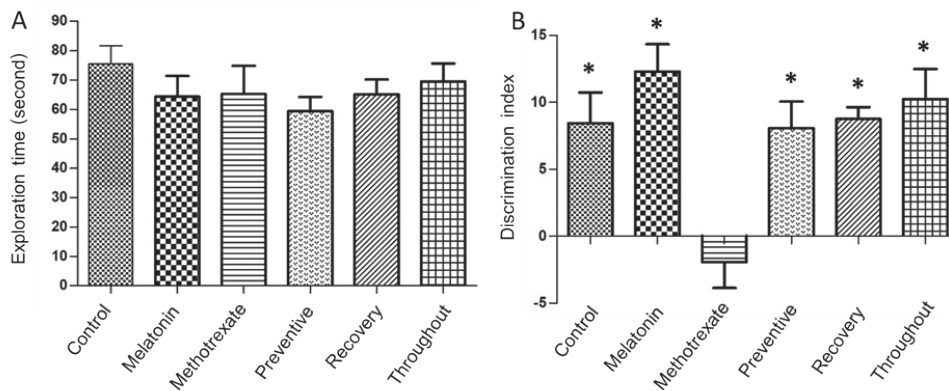

Figure 2 Body weight of rats in control, melatonin, methotrexate, preventive, recovery and throughout groups during day 1 to day 30.

The effect of melatonin improves spatial memory deficits caused by methotrexate.

The novel object location test was used to determine spatial memory. In the novel object location test, there was no significant difference in total exploration time among groups(p>0.05, one-way analysis of variance) (Fig 3A). The data show that discrimination index of control, melatonin, preventive, recovery and throughout groups was significantly different when compared to methotrexate group (p<0.05, one-way analysis of variance) (Fig 3B), indicating that rats in control, melatonin, preventive, recovery and throughout groups could discriminate between novel location and familiar location. This result shows that melatonin can prevent and recover from spatial memory deficits caused by methotrexate.
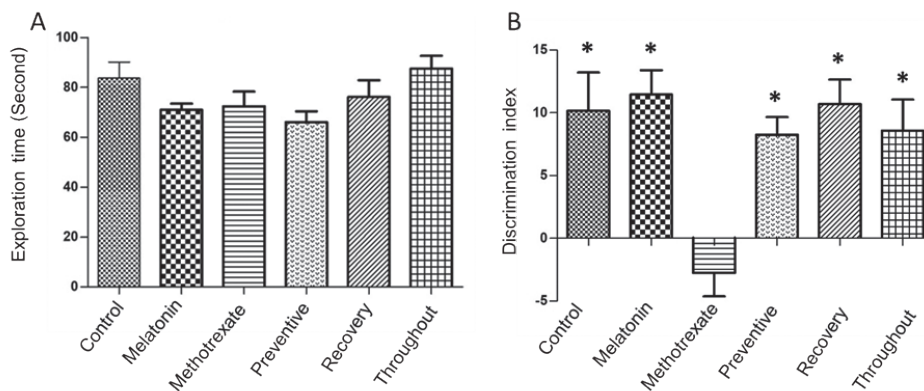
Figure 3 Novel object location test. Total exploration time was not significantly different among groups (p>0.05, A). Discrimination index of control, melatonin, preventive, recovery and throughout groups was significantly different when compared with methotrexate group (*p<0.05, B).

The effect of melatonin improves non-spatial memory deficits caused by methotrexate.

The novel object recognition test was used to determine non-spatial memory. Total exploration time was not significantly different among groups (p>0.05, one-way analysis of variance) (Fig 4A). This result shows that discrimination index of control, melatonin, preventive, recovery and throughout groups was significantly higher than methotrexate group (p<0.05, one-way analysis of variance) (Fig 4B), indicating an ability to discriminate between novel object and familiar object of rats in these groups. This result demonstrates that melatonin can prevent and improve impairment of non-spatial memory caused by methotrexate.

Figure 4 Novel object recognition test. There was no significant difference among groups (p>0.05, A). Discrimination index of control, melatonin, preventive, recovery and throughout groups was significantly different from methotrexate group (*p<0.05, B).

Discussion

The present study found that methotrexate treated rats showed memory deficits. However, this was ameliorated by co-treatment with melatonin. Body weight of rats in recovery and throughout groups was increased but less than control group.

Results of weight gain in our study indicate that methotrexate can decrease rate of body weight gain in preventive, recovery and throughout groups. Weight gain of rats in melatonin group was not significantly different compared with control group. This indicates that melatonin per se did not affect weight gain. However,

weight gain of recovery and throughout groups was significantly lower than methotrexate group. This result is in line with a study that rats treated with methotrexate and fluoxetine, however this mechanism is not clear[13]. Future studies should be needed to investigate the mechanism of co-treatment of melatonin and methotrexate that is involved in weight gain. Previous studies have reported that methotrexate destroys gastrointestinal tract by damaging lining of mouth, stomach and reducing absorption of small intestine[14]. Additionally, methotrexate induces diarrhea in patients and decreases body weight in both rats and patients[14]. Therefore, methotrexate can decrease body weight in this study.

The results of novel object location and novel object recognition tests demonstrated that methotrexate induces spatial and non-spatial memory deficits which is consistent with previous studies[6, 14]. Both novel object location and novel object recognition tests are hippocampal dependent and do not need any positive and negative reinforcements[15, 16]. Methotrexate had been shown to impair memory process, for instance recall, retrieval, spatial and non-spatial memory[14]. Methotrexate causes deficits in the abilities of rats to perform task and cognitive impairment which is associated with hippocampal neurogenesis, especially cell proliferation and differentiation[5, 7, 14]. Methotrexate inhibits deoxyribonucleic acid synthesis by inhibiting dyhydrofolate reductase, thymidine synthase and aminoimidazole carboxamide ribonucleotide transferase enzymes in folate metabolism[4]. Inhibition of these enzymes interferes cell division that is related to hippocampal neurogenesis and memory deficits[4, 5, 7, 14]. Moreover, methotrexate reduces blood supply into hippocampus by inducing ischemic of the hippocampus in rats[5, 7]. Ischemia of the hippocampus activates microglia activity that releases cytokine kinase[5, 6]. The cytokine induces neuroinflamation and oxidative stress[5, 6]. Moreover, oxidative stress reduces neurogenesis and reduces memory[5, 6, 9, 17].

In the present study, administration of both melatonin and methotrexate shows that rats can discriminate between novel location and familiar location in novel object location test.In addition, preventive, recovery and throughout groups can discriminate between novel object and familiar object. Therefore, melatonin can prevent and recover spatial and non-spatial memory deficits induced by methotrexate. Previous studies have demonstrated that melatonin can improve spatial, non-spatial memory[17, 18]. The subgranular zone of the hippocampus is one of neurogenic regions where adult hippocampal neurogenesis occurs[10]. Hippocampal neurogenesis generates new neurons into hippocampal circuit that involves in memory formation[10]. Recent studies have found that melatonin (8 milligram/kilogram) increases neurogenic process, for instance cell proliferation, survival and differentiation[10, 19]. In addition, administration of melatonin (8 milligram/kilogram) for 14 days increases dendrite complexity of new neurons which is involved in hippocampal synaptic system and related in different types of learning and memory, such as trace eye blink condition, fear condition, spatial and episodic memory[19]. Melatonin is a free radical scavenger and induces antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and glutathione reductase[4, 5]. Increasing of antioxidant enzyme can reduce oxidative stress that destroys hippocampal neurogenesis and memory formation[9, 17]. Melatonin can decrease cytokine released by microglia activity that is involved in hippocampal neurogenesis impairment[5]. Moreover, melatonin activates M1 and M2 receptor and activates tropomyosin receptor kinase B receptors[9, 17, 20]. Tropomyosin receptor kinase B receptors are important for cell survival, neuronal plasticity and brain derived neurotropic factor level[17, 21]. These factors increase neurogenic process which is associated with memory formation[17, 21]. Taken together,the preventive and recovery effects of melatonin on memory deficits caused by methotrexate might be due to increasing of neurogenesis that is associated with memory.

Conclusion

In this study, methotrexate treated rats cannot discriminate between novel location and familiar location or novel object and familiar object. Accordingly, administration of both melatonin and methotrexate

increases the abilities of rats to discriminate between novel location and familiar location in novel location test. Besides novel object location test, rats receiving both melatonin and methotrexate can discriminate between novel object and familiar object in novel object recognition test. These results demonstrate that melatonin can prevent and improve spatial and non-spatial memory deficits caused by methotrexate in adult rats.

Acknowledgments

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