นิพนธ์ต้นฉบับ . Original Article

ผลของไครซินต่อยาเคมีบำบัดเมโธเทรกเซทเหนี่ยวนำให้ความจำและการสร้างเซลล์ประสาทในสมองส่วนฮิปโปแคมปัสลดลงในหนูแรทโตเต็มวัย

ธนพร อะโนศรี[1], โสรยา แก้วงาม[1], นัตยา ศรีตะวัน[1], ราม ประจิตต์[1], กรรวี สุวรรณโคตร[1], อภิวัฒน์ ศิริโชติ[1], อนุสรา อารณะโรจน์[1], วนัสนันท์ แป้นนางรอง[1], จริยา อำคา เวลบาท[1,2*]

[1]*ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น*

[2]*กลุ่มวิจัยและพัฒนาด้านประสาทวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น*

Effect of Chrysin on Methotrexate Chemotherapy Induced Reductions of Memory and Hippocampal Neurogenesis in Adult Rats

Tanaporn Anosri[1], Soraya Kaewngam[1], Nataya Sritawan[1], Ram Prajit[1], Kornrawee Suwannakot[1], Anusara Aranarochana[1], Apiwat Sirichoat[1], Wanassanan 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*

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หลักการและเหตุผล: เมโธเทรกเซทเป็นยาเคมีบำบัดที่ใช้อย่างแพร่หลายในการรักษามะเร็งหลายชนิด มีการศึกษาพบว่าการรักษาด้วยเมโธเทรกเซทสามารถเหนี่ยวนำให้เกิดภาวะความจำบกพร่อง ไครซินเป็นสารจำพวกฟลาโวนอยด์ที่พบได้ในธรรมชาติ การศึกษาที่ผ่านมาพบว่าไครซินมีคุณสมบัติในการป้องกันการเสื่อมของระบบประสาทและฟื้นฟูความจำ ดังนั้นการศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของไครซินต่อความจำบกพร่องที่เกิดจากการเหนี่ยวนำด้วยเมโธเทรกเซท

วิธีการศึกษา: หนูแรท เพศผู้ สายพันธุ์ Sprague Dawley จำนวน 72 ตัว ถูกแบ่งออกเป็น 6 กลุ่ม ได้แก่ control, MTX, chrysin 10, chrysin 30, chrysin 10+MTX และ chrysin 30+MTX โดยกลุ่ม control ได้รับน้ำเกลือและโพรพิลีนไกลคอล ไครซิน (10 และ 30 มิลลิกรัม/กิโลกรัม) ให้โดยการป้อนเป็นเวลา 15 วัน เมโธเทรกเซท (75 มิลลิกรัม/กิโลกรัม) ให้โดยการฉีดเข้าหลอดเลือดดำในวันที่ 8 และ 15 ของการทดลอง หลังสิ้นสุดการให้สาร 3 วัน น้ำหนักและการเคลื่อนไหวของหนูถูกนำมาวิเคราะห์ และความจำถูกทดสอบด้วยการทดสอบ novel object location (NOL) และ novel object recognition (NOR)

ผลการศึกษา: ข้อมูลของทุกกลุ่มพบว่า ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติของระยะเวลาในการสำรวจวัตถุ ในการทดสอบ NOL และ NOR พบว่ากลุ่ม control, chrysin 10,

Background and objective: Methotrexate (MTX) is a chemotherapy drug that is the most widely used to protect against many malignancies. Several reports have shown that MTX can induce cognitive impairment. Chrysin is a natural flavonoid that is found in natural products. Previous studies have found that chrysin has neuroprotective and cognitive improving properties. Therefore, this study was designed to determine the protective effect of chrysin against MTX-induced memory impairments.

Methods: Seventy-two male Sprague Dawley rats were divided into 6 groups; control, MTX, chrysin 10, chrysin 30, chrysin 10+MTX and chrysin 30+MTX groups. The control group received saline and propylene glycol. Chrysin (10 and 30 mg/kg) was administered by oral gavage for 15 days. A single dose of MTX (75 mg/kg) was administered by intravenous injection on days 8 and 15. Three days after the end of drug administration, the body weight and locomotor activity were determined. The memories were evaluated 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. E-mail: jariya@kku.ac.th

chrysin 30, chrysin 10+MTX และ chrysin 30+MTX สามารถจำแนกตำแหน่งและวัตถุเก่าใหม่ได้อย่างมีนัยสำคัญทางสถิติยกเว้นกลุ่ม MTX
สรุป: การศึกษาในครั้งนี้ชี้ให้เห็นว่า เมโธเทรกเซทสามารถเหนี่ยวนำให้เกิดภาวะความจำบกพร่อง ในทางตรงกันข้ามไครซินสามารถฟื้นฟูภาวะความจำบกพร่องในหนูแรทที่ได้รับเมโธเทรกเซท

คำสำคัญ: ไครซิน, เมโธเทรกเซท, ความจำบกพร่อง

Results: The data of all groups showed no significant differences in term of total exploration times. In both the NOL and NOR tests, the control, chrysin 10, chrysin 30, chrysin 10+MTX and chrysin 30+MTX groups could significantly discriminate between the familiar and novel location or object except the MTX group.
Conclusion: This study demonstrates that MTX induces memory impairment. In contrast, chrysin could improve memory impairment in rats received MTX.

Keywords: Chrysin, Methotrexate, Memory impairment

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Introduction

Methotrexate (MTX), a folic acid antagonist, is a chemotherapy drug that is the most widely used to protect against many kinds of cancers[1]. MTX inhibits dihydrofolate reductase (DHFR), which is an enzyme that changes folic acid into tetrahydrofolate (FH_4). When the level of FH_4 is decreased, synthesis of purine and thymidylate needed for synthesis DNA / RNA is also blocked and consequently inhibits DNA and RNA synthesis[2]. Several reports have shown that treatment with chemotherapy can induce cognitive impairment, changes of structures and functions of the brain. These impacts are associated with learning and memory deficits[3] and decreases of cell division, cell survival, and numbers of immature neurons in the subgranular zone (SGZ) in the hippocampus[4-7].

Chrysin (5,7-dihydroxyflavone) is a natural flavonoid that is present in natural products; for example, honey, bee propolis, blue passion flower and many plant extracts[8]. Previous studies have found that chrysin has a wide range of biological effects including anti-inflammatory, neuroprotective, anti-apoptotic, cognitive improving effects and antioxidant activities[9]. Moreover, a previous study has found that chrysin at the dose of 10 and 30 mg/kg can improve recognition and spatial memory in D-galactose-induced aging rats[10].

Therefore, this study was designed to determine the protective effect of chrysin against MTX-induced impairments of memory and neurogenesis in the SGZ of the hippocampal dentate gyrus (DG) in adult rats.

Materials and Methods

Animals

This experiment was conducted using seventy-two adult male Sprague-Dawley rats, age 4-6 weeks old, body weight 180-200 g from Nomura Siam International Co., Ltd. Pathumwan, Bangkok. The animals were controlled in the environment with a 12:12 h light: dark cycle, which temperature is maintained at 23-25 °C. Food and water were always available for animals during the experiment. The experimental protocol and all handling procedures were approved by the Khon Kaen University Ethics Committee in Animal Research (AEKKU 56/62).

Drug administration

The animals were divided into 6 groups (12 animals per group) and allowed to acclimatize to the facility for 7 days prior to the experimentation. The control group: rats received normal saline solution 1 ml/kg and propylene glycol (Ajax Finechem Pty Ltd., Australia) 1 ml/kg. The MTX group: the animals received 75 mg/kg of MTX (pharmachemie BV, harsblem, Netherland) dissolved in 0.9% saline solution (Ajax Finechem Pty Ltd., Australia) for intravenous (i.v.) injection to the tail vein on day 8 and day 15. The chrysin 10 and 30 groups: the animals received chrysin (Sigma Aldrich, Inc., St. Louis, USA) 10 or 30 mg/kg dissolved in propylene glycol by oral gavage for 15 days. The chrysin 10 and 30 + MTX groups: the animals received MTX and chrysin the same dose as the chrysin 10, chrysin 30 and MTX groups.

After MTX injection, animals received leucovorin (LCV) by intraperitoneal (i.p.) injection at a dose of 6 mg/kg after 18 hours and at a dose of 3 mg/kg after

26, 42, and 50 hours to decrease toxicity of MTX. Three days after drug administration, novel object location (NOL) and novel object recognition (NOR) tests were performed for 4 days.

Behavioral tests

Before and after drug administration, behavioral tests were performed using the NOL and NOR tests for 4 days. Both tasks consist 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).

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

The NOL test consists of habituation, familiarization and choice trials. In the habituation, the animals were placed in an empty arena for 30 minutes. The next day, the animals were placed in an empty arena for habituation again for 3 minutes. In the familiarization trial, the animals were returned to the arena in the presence of two identical objects that were placed in different locations for 3 minutes and then the animals were moved back to their cages for 15 minutes. In the choice trial, one of the objects was placed in the same location (familiar location; FL) and the other one was placed in a novel location (NL). The animals were allowed to explore the objects in the arena for 3 minutes.

The NOR test, the habituation and familiarization trials are the same as those of the protocol of the NOL test. In the choice trial, the animals were returned to the arena that was present one of the familiar objects (FO) and a novel object (NO). The animals were allowed to explore the objects in the arena for 3 minutes. The exploration time was recorded using EthoVision® XT software and used to calculate total exploration time for determining locomotor activity of animals and the exploration time was also used to evaluate discrimination index (DI) that is defined as the ability to discriminate between novel and familiar locations or objects. Normally, animals will spend more time investigating an object in a novel location than a familiar location in the NOL test. Likewise, animals will explore a novel object more than that of a familiar object in the NOR test. Therefore, the DIs should be significantly greater than zero.

Statistical analysis

All statistical analysis was conducted using GraphPad Prism (Version 6.0; 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. One-way ANOVA was used to determine total exploration time. Discrimination index was compared using one sample t-test.

Results

Effects of chrysin and MTX on body weight

The body weight data showed no significant differences among the chrysin 10, chrysin 30 and control groups ($p>0.05$, two-way ANOVA). After MTX injection, there was a significant difference of body weight in the MTX, chrysin 10+MTX and chrysin

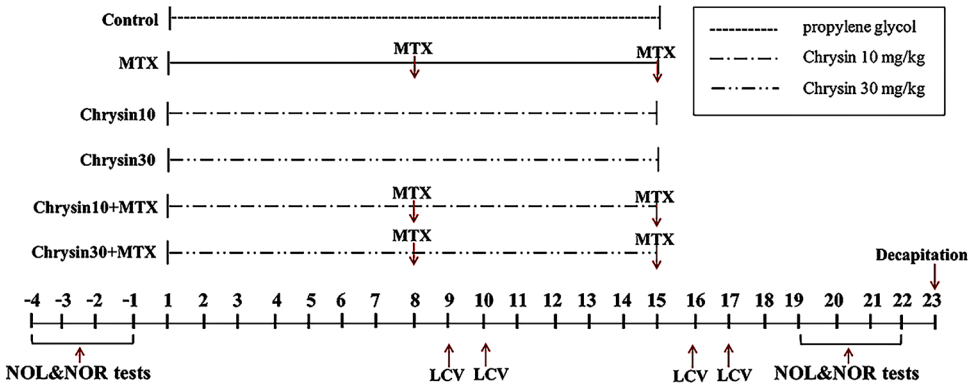

Figure 1 Timeline of drug administration and behavioral tests.

30+MTX groups when compared with the control group on day 11 and 12 ($p<0.05$, two-way ANOVA). However, the body weight gain was found during the treatment to the beginning of the behavioral tests as showed in figure 2.

Effects of chrysin and MTX in the behavioral tests

- Before drug administration

Total exploration time was measured to determine locomotor activity of animals. The data showed no significant differences in term 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).

In both the NOL and NOR tests, the discrimination index (DIs) of animals in all groups showed significant differences from 0 ($p<0.05$, one-way ANOVA, fig. 4A and 4B).

- After drug administration

The data of all groups showed no significant differences in term of total exploration time in both the NOL and NOR tests ($p>0.05$, one-way ANOVA, fig. 5A and 5B), which is similar to the results before drug administration.

In both the NOL and NOR tests, the DIs of the control, chrysin 10, chrysin 30, chysin10+MTX and chrysin 30+MTX groups were significantly higher than 0 ($p<0.05$, one-way ANOVA, Fig. 6A and 6B) except the MTX group.

Discussion

In the present study, we found that MTX could induce memory impairment in MTX treated rats. In contrast, chrysin 10 and 30 mg/kg combined with MTX could improve this impairment.

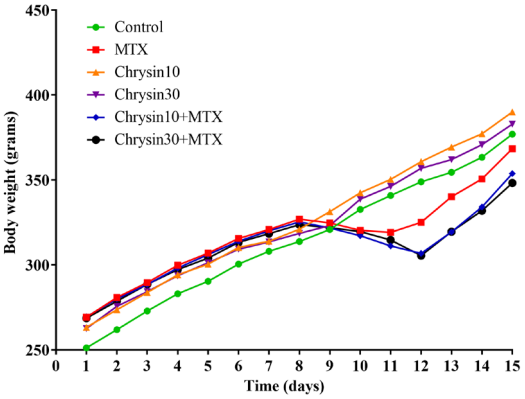

Figure 2 Body weight of animals in the control, MTX, chrysin 10 and chrysin 30, chrysin 10+MTX and chrysin 30+MTX groups throughout the experiment

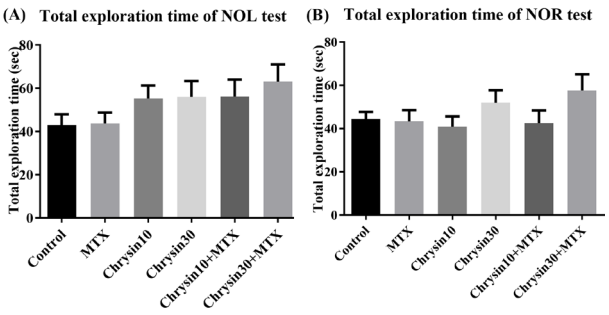

Figure 3 Total exploration time of all animals in the NOL (A) and NOR (B) tests before drug administration.

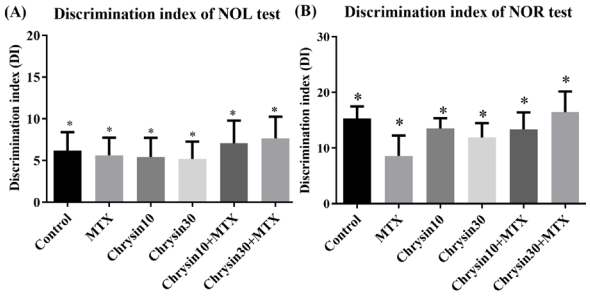

Figure 4 The DIs of the animals in the NOL (A) and NOR (B) tests before drug administration (*$p<0.05$ significant difference compared to zero).

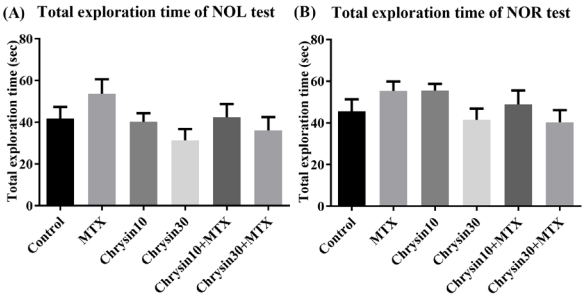

Figure 5 Total exploration time of the animals in the NOL (A) and NOR (B) tests after drug administration.

Body weight of chrysin treated rats was not significantly different when compared with the control group. This indicates that chrysin did not affect to body weight[9, 10]. In contrast, the animals in the MTX and MTX combined with chrysin treated groups showed significance different when compared with the control group. This indicates that MTX induced decreases of body weight. A previous study has suggested that MTX induces both apoptosis and crypt hypoplasia that lead to small intestinal mucositis[11] and show some gastrointestinal symptoms such as abdominal pain, bloating, and diarrhea[12]. These would

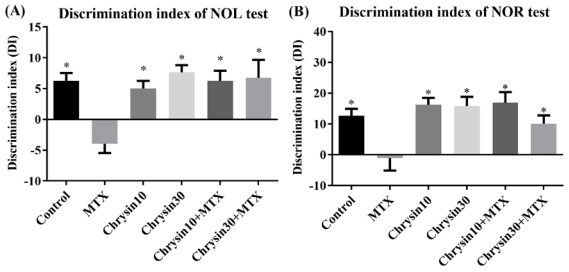

Figure 6 The DIs of the animals in the NOL (A) and NOR (B) tests after drug administration (*p<0.05 significant differences compared to zero).

be the causes of decreases in body weight after MTX administration.

Locomotor activity is a spontaneous activity and an important movement in rodents[13]. The present study, total exploration times were measured to determine locomotor activity of animals before and after drug administration. We found chrysin and MTX had no effect on locomotor activity that was consistent with previous studies[9, 14, 15]. Therefore, these findings reveal that the animals had the ability to explore the object in both NOL and NOR tests without negative reinforcement.

The SGZ of the DG in the hippocampus is an important area for the neurogenesis process and associated with spatial and recognition memory[16, 17]. Previous studies have suggested that new neuronal generation in the DG is related to improving of spatial memory[4, 7]. The NOL and NOR tests were used to measure hippocampus spatial memory and recognition impairment respectively[18]. Results from the present study show that the DI in the MTX group was not significantly higher than 0 in both the NOL and NOR tests. This data indicates that animals receiving MTX showed memory impairments[15, 19]. Similarly, Lyons et al. have suggested that MTX dose 75 mg/kg induces hippocampal dysfunction and cognitive impairment using the novel location recognition (NLR) test[14].

Previous studies have shown that treatment with MTX inhibits dihydrofolate reductase (DHFR) that causes an inhibition of DNA and RNA synthesis[19]. Furthermore, MTX can induce toxicity in neural stem cells, which causes abnormality of cell division and increases apoptosis within the SGZ of the DG in the hippocampus and leads to an impairment of the memory system[20]. Moreover, MTX can activate generation of oxidative stress by up-regulating reactive oxygen species (ROS) formation[21].

The animals in combination with the MTX and chrysin groups were able to discriminate the objects in the NL or NO and the FL or FO, according to the positive DIs value, suggesting that chrysin attenuated memory deficit in these animals[9, 22]. Previous study has supported that chrysin can improve memory by eliminating free radicals that contribute to the peroxide of lipid peroxidation[10] and inhibits the NF-kB signaling pathway that causes reduction of inflammatory responses in the cells[8]. Furthermore, chrysin has been known as a natural ligand for peroxisome proliferator activated receptor gamma (PPAR-γ), which regulates oxidative stress and inflammation in the central nervous system (CNS)[23].

Conclusion

This study demonstrates that chrysin improved memory impairments in adult rats induced by MTX. This study suggests that chrysin has a beneficial effect to improve spatial and recognition memory impairment.

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