Biochemical and Biophysical Research Communications 436 (2013) 104–109

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Compound K is able to ameliorate the impaired cognitive function and hippocampal neurogenesis following chemotherapy treatment

Jin-gang Hou [a], Jian-jie Xue [a], Mi-ra Lee [a], Meng-qi Sun [b], Xing-hua Zhao [c], Yi-nan Zheng [d], Chang-keun Sung [a,*]

[a] Department of Food Science and Technology, College of Agriculture and Biotechnology, Chungnam National University, Taejon, Republic of Korea
[b] Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China
[c] Cangzhou Medical College, Hebei, China
[d] College of Chinese Medicinal Material, Jilin Agricultural University, Changchun, Jilin, China

ARTICLE INFO

Article history:
Received 16 May 2013
Available online 31 May 2013

Keywords:
Compound K
Chemotherapy agent
Neurogenesis
Amelioration

ABSTRACT

Chemotherapy frequently results in neurocognitive deficits that include impaired learning and memory. Thus, it is important to prevent or ameliorate the persistence of cognitive impairment. Compound K was employed to examine the ameliorating effect on chronic treatment with cyclophosphamide. Eight week-old ICR mice were given 80 mg/kg cyclophosphamide, cyclophosphamide combined with compound K (2.5, 5 and 10 mg/kg) or saline injections once per week for 4 weeks. Passive avoidance test and *Y* maze were used to evaluate memory and learning ability. Immunohistochemical staining for progenitor cell and immature neurons was used to assess changes in neurogenesis. Compound K (10 mg/kg) is able to ameliorate the decrease of neurogenesis in the hippocampus caused by cyclophosphamide. These results suggest that compound K might be a potential strategy to ameliorate or repair the disrupted hippocampal neurogenesis induced by the side effect of chemotherapy agent.

1. Introduction

Chemotherapy is a frequently used treatment strategy for cancer. Cyclophosphamide (CTX) is one of the alkylating agents, with its metabolites causing alkylating crosslinks within and between DNA strands of dividing cells, causing apoptosis [1]. The brain is effectively protected against potentially harmful compounds by the blood–brain barrier (BBB) and the CTX is able to cross it [2]. Since CTX is aimed at the inhibition of the process of cell division, it will likely affect the cell proliferation in the brain in view of capacity for crossing the BBB. Hippocampal neurogenesis occurs throughout life in the granule cell layer of dentate gyrus [3] and its correlates with memory and learning have been widely reported [4–6]. Moreover, of the alkylating agents, the effect of CTX on cognitive performance has most frequently been described in the literature and explored in a number of animal studies [7,8]. In these studies, there were no effects of CTX on anxiety behavior [9] and cue-fear or acquisition of fear response [10]. In rats, CTX in combination with doxorubicin treatment for 4 weeks significantly impaired performance on the novel place recognition and contextual fear conditioning task [11]. In addition, methotrexate and 5-Fluorouracil combination with CTX can induce transient impairment in the performance of simple learning and memory task but persistent cognitive dysfunction in the more complex test [12]. Interestingly, in one study of female Fischer-344 rats with CTX treatment, despite the toxic effects of CTX, the rats exhibited better maze performance and following 7–9 weeks of recovery evidence of improved learning and LTP were noted [13]. These evidences suggest that impaired cognition and hippocampal neurogenesis are involved in the development of a chemotherapy (CTX) treatment. A number of studies suggest that ginseng can be effective in the attenuation of learning deficits due to brain damage and aging in rodent [14,15]. Compound K is the intestinal metabolite of major ginsenoside Rb1 [16] and widely exhibits various anti-tumor activities [17,18].

In present study, in order to investigate the effect of compound K on chemotherapy-induced cognitive impairment, the passive avoidance task and *Y* maze task were used to test hippocampal-dependent memory after the final CTX injection. In addition, Bromodeoxyuridine (BrdU), a thymidine analog incorporated into cells during DNA synthesis, was used to examine the dividing cells. Then cell-specific marker doublecortin (DCX) was employed to examine the expression levels of immature neuron in the dentate gyrus of the hippocampus of adult mice.

* Corresponding author. Fax: +82 42 822 2287.
E-mail address: sungchangkeun@126.com (C.-k. Sung).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.05.087

2. Materials and methods

2.1. Animals

Male ICR mice (8 weeks old) were used in present research. They were housed in a room maintained at 24 ± 2 °C and artificial lighting from 7:00 to 19:00 h as well as free access to food and water. All the experimental procedures were complied with the Guide for the Care and Use of Laboratory Animal issued by the National Institutes of Committee of Animal Experiments at the Chungnam National University.

2.2. Drug treatment

CTX (Sigma–Aldrich, St. Louis, MO) was dissolved in sterile 0.9% saline. Mice were injected intraperitoneally (i.p.) with 80 mg/kg of CTX and orally administrated with 2.5 mg/kg, 5 mg/kg and 10 mg/kg of compound K (Fu song county nature biotechnology, Co. Ltd.). The vehicle group was injected i.p. with 0.9% saline or 80 mg/kg of CTX. Behavioral dysfunction in mice was evaluated by passive avoidance test (8 mice per group) and *Y* maze test (8 mice per group) at 24 h after the last injection.

To assess the impact of chemotherapy on hippocampal neurogenesis, 5-bromo-2′-deoxyuridine (BrdU; 100 mg/kg intraperitoneally; Sigma–Aldrich) was administrated for 3 consecutive days after the final drug injection.

The diagram of time course was shown in Fig. 1.

2.3. Passive avoidance test

Passive avoidance test was performed in identical compartments. The illuminated compartment (20 × 20 × 20 cm) contained a 100 W bulb, and the floor of the non-illuminated compartment (20 × 20 × 20 cm) was composed of 2 mm stainless steel rods with 1 cm interval. These two compartments were separated by guillotine door (5 × 5 cm). For acquisition trials, mice were initially placed in the illuminated compartment and the door was opened 15 s later. When the mouse entered the non-illuminated compartment, the door was closed and an electrical foot shock (0.5 mA) of 5 s durations was delivered through the stainless steel rods. Twenty-four hours after acquisition trial, the mice were again placed in the illuminated compartment for the retention trials. The time taken for a mouse to enter the non-illuminated compartment after door opening was termed as step-through latency times in retention trials. If a mouse did not enter non-illuminated compartment within 300 s, it was assumed that the mouse had remembered the single training trial.

2.4. Y maze

The *Y*-maze is a three-arm horizontal maze (40 × 4 × 12 cm) in which the arms are symmetrically arranged at 120° angles from each other (start arm, other arm and novel arm). The maze was constructed from dark opaque polyvinyl plastic. Mice were initially placed within one arm, and then the sequence and number of arm entries were recorded for each mouse for 10 min free exploration. An actual alternation was defined as entries into all three arms in consecutive sequences. Maze arms were thoroughly cleaned between tasks to remove the residual odors. 24 h after the last injection mice were gently placed in the maze. The percentage of alternations was defined according to the following equation: % = [(number of alternation)/(total arm entries −2)] × 100. The number of arm entries served as indicator of locomotors activity.

2.5. Tissue sampling

The effect of compound K on the cognitive impairment induced by CTX was observed after mice received administration of both compound K and CTX. The mice were sacrificed, and then hippocampus were dissected from each group ($n = 6$ mice per group). These samples were subjected to the processes for embedding in paraffin wax after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4).

2.6. Immunohistochemistry

Coronal sections (8 μm) were cut by microtome and deparaffinized through regular protocols. For BrdU-immunostaining, the sections were hydrolyzed with 2 N HCl in PBS (pH 7.4) at 37 °C for 15 min, and then stained by the Invitrogen kit (Invitrogen, CA, USA). The sections were incubated in serum blocking solution, in a 1:50 dilution of a mouse monoclonal antibody against BrdU (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and overnight at 4 °C, in biotinylated secondary antibody at room temperature for 30 min, and finally in streptavidin-peroxidase conjugate at room temperature for 20 min. After each step, the sections were rinsed with PBS. The sections were then incubated in 3, 3′-diaminobenzidine (DAB) solution. After that, sections were incubated in 1% ferric

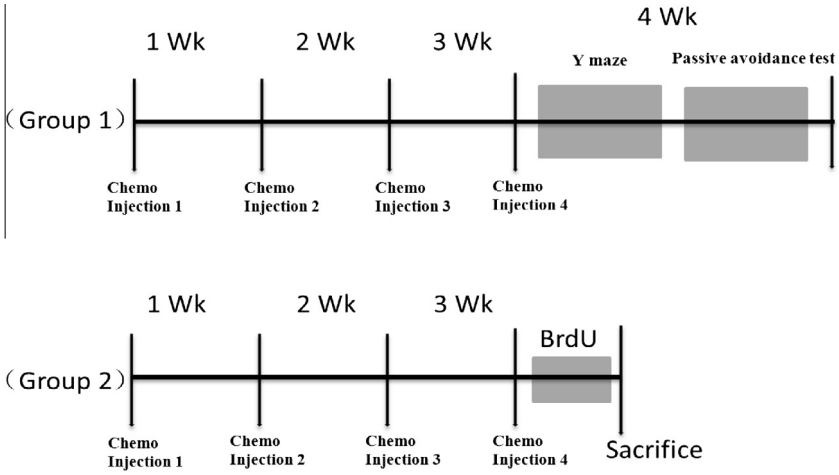

Fig. 1. Schematic time line. Group one: mice received 1 injection per week for 4 consecutive weeks, followed by *Y* maze and passive avoidance test. Group two: mice treated with compound K by daily oral administration received 1 injection per week, followed by 3 consecutive BrdU injections, and then sacrificed 2 h after the last injection.

chloride solution at room temperature for 5 min. BrdU-positive nuclei exhibited deposits of dark brown or black-colored precipitates. DCX staining was carried out to identify immature neurons. Sections were disposed in citrate buffer (0.01 M, pH 6.0) and kept at 80 °C for 30 min. The sections were washed with PBS, blocked with serum blocking solution for 30 min, and then followed by primary anti-body incubation (goat-anti-DCX, 1:200; Santa Cruz) overnight at 4 °C. Subsequently, biotinylated donkey anti-goat IgG (1 h, 1:200; Santa Cruz) was used to the color development. The sections were counterstained with hematoxylin and cover slipped under histomount.

2.7. Microscopy and cell counting

The sections were analyzed using an Olympus microscope equipped with digital camera (Olympus). Cells were counted under the condition of unknown experimental conditions. Every ten sections throughout the hippocampus were processed for counting (5 or 6 sections per animal). Data for DCX are represented as the mean number of DCX^+ neurons in granule cell layer-subgranular zone (GCL-SGZ) region per section. $BrdU^+$ cells were quantified across hippocampal subfields (dentate gyrus, GCL-SGZ layers) and are represented as the mean number of $BrdU^+$ per hippocampal section.

2.8. Statistical analysis

All statistical analyses were conducted using Statistics 20 (SPSS, IBM Corporation). A value of $P \leqslant 0.05$ was considered statistically significant. When a statistically significant overall group effect was found, multiple comparison were made using Fisher protected least significant different (FPLSD) *post hoc* tests to compare the individual groups.

Weight, in grams, was analyzed in 5 time points (i.e., baseline and 1 week after each injection) by repeated measures ANOVA with group as the between-subjects factor.

3. Results

3.1. Chemotherapy-induced weight changes

The mice gained weight over the course of study given that the starting age was 2 months. As seen in Fig. 2, the chemotherapy-treated animals tended to weigh less than control group (saline). Repeated measures ANOVA revealed a significant time × group effect [$F(2, 12) = 3.64$; $P = 0.04$]. The group with 10 mg/kg of compound K did not differ significantly from control (saline) at 1-week post injection 4 [$F(1, 4) = 33.6$; $P = 0.586$]. CTX-treated (control) animals weighed about 5.7 g (or 14%) less than control group (saline) at 1-week post-injection 4 and CTX combined with compound K-treated animals weighed about 4.1 g (or 10%, 2.5 mg/kg) and 2 g (or 5%, 5 mg/kg) less.

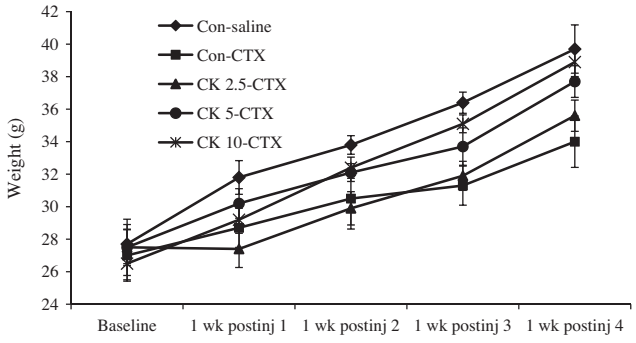

Fig. 2. Compound K treatment ameliorates chemotherapy-induced bodyweight loss over the course of study compared to control animals. Body weight was measured at baseline and 1 week after each injection (post-inj). Data are represented as means ± SEM.

3.2. Compound K ameliorates the impairment on passive avoidance and Y maze performance

Significant overall group effects were found both in passive avoidance test [$F(2.10) = 61.43$, $P = 0.015$] and *Y* maze [$F(2.6) = 15.54$, $P = 0.004$] after four-week treatment. As can be seen in Fig. 3A, in passive avoidance test, although CTX treated mice significantly decreased the step-through latency ($P = 0.007$) compared with control group (saline), CTX combined with compound K (5 mg/kg, $P = 0.585$; 10 mg/kg, $P = 0.163$) did not differ from control group (saline). In *Y* maze test (Fig. 3B), CTX treated mice showed significant decrease in alternation percentage ($P = 0.017$). CTX combined with compound K (5 mg/kg, $P = 0.158$; 10 mg/kg, $P = 0.067$) did not significant affect the alternation percentage compared with control group (saline). In addition, CTX treated mice revealed significantly decreased locomotion during the acquisition phage ($P < 0.005$) compared with control animals and compound K treated groups (2.5 mg/kg, $P < 0.001$; 5 mg/kg, $P < 0.038$; 10 mg/kg, $P < 0.047$).

3.3. Effects of compound K on hippocampal neurogenesis of CTX-treated mouse

The impact of chemotherapy on neurogenesis was assessed using immature (DCX) neuronal marker. DCX is widely used as a marker of immature neurons in the dentate gyrus. Newly born cells in the SGZ and GCL express DCX within 3 h after commitment to a neuronal lineage and remain stable from approximately 12 to 14 days[19]. The number of DCX^+ neurons per hippocampus was assessed in each group. ANOVA revealed an overall significant effect of group on the number of DCX^+ cells [$F(2,18) = 50.97$; $P = 0.005$], and *post hoc* tests showed that CTX group had significantly fewer DCX^+ cells than saline-treated controls ($P < 0.004$). Importantly, the DCX^+ cells was reduced by 38% in CTX group, but 19%, 11% and 6% in CTX and Compound K (2.5 mg/kg, 5 mg/kg and 10 mg/kg) respectively (Fig. 4A). These reductions indicate the marked toxicity of the chemotherapeutic agents on neurogenic cell populations in the hippocampus and the potential function of compound K on amelioration of this reduction.

To further analyze the effects of the drugs on neurogenesis, we also quantified the number of neuron progenitor by BrdU. ANOVA revealed an overall significant effect of group for the number of $BrdU^+$ cells [$F(2,12) = 6.57$, $P = 0.01$], and *post host* tests showed that CTX group had significantly fewer $BrdU^+$ cells than saline-treated controls ($P < 0.05$). This represents a 33% drop in CTX-treated group, 26% drop in Compound K (2.5 mg/kg), 19% drop in Compound K (5 mg/kg) and 8% drop in Compound K (10 mg/kg), which suggests that neuron progenitor was adversely affected by chronic exposure to CTX and compound K is able to ameliorate this impact (Fig. 4B).

4. Discussion

In the present study, chronic exposure to CTX impairs cognition of mouse tested by *Y* maze and passive avoidance test. Both of these tasks are well known to involve hippocampus, and consistent with the deficits, disruptions in hippocampal neurogenesis in treated animals were observed. Meanwhile, in order to ameliorate this adversely effect, we evaluated compound K with three dosages

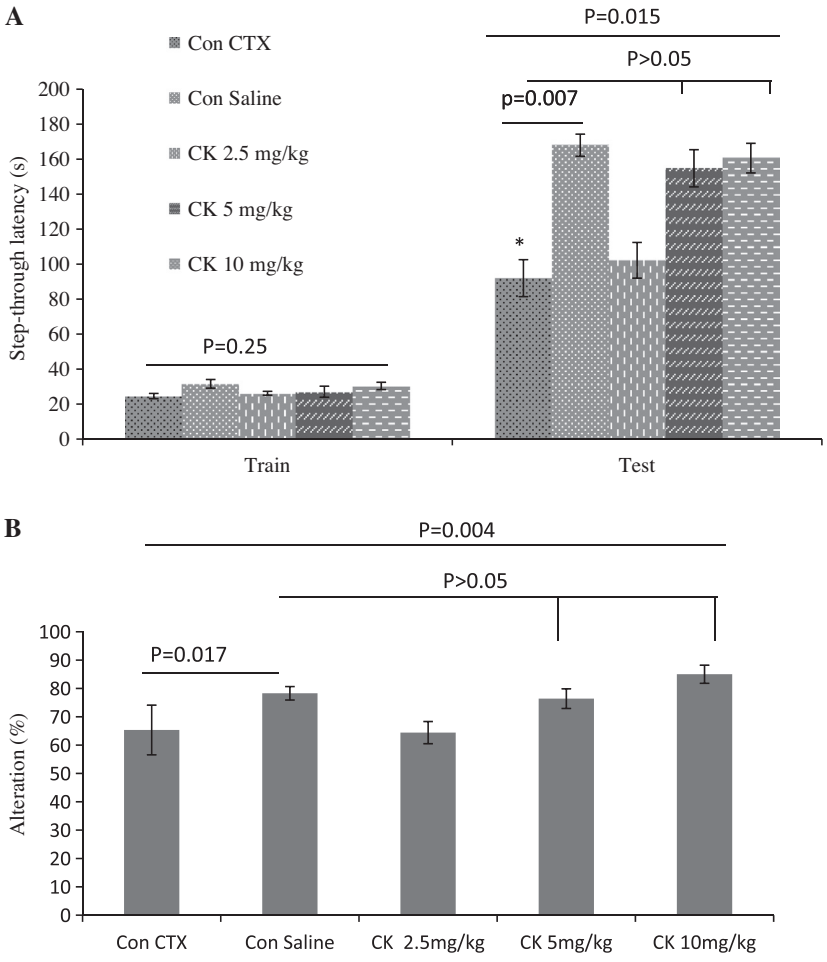

Fig. 3. Compound K treatment ameliorates chemotherapy-induced cognition impairment in passive avoidance test (A) and *Y* maze test (B). Data are represented as means ± SEM and *P* values are derived from *post hoc* comparisons. *A significant lower proportion of step-through latency in CTX treated animals than in saline treated animals.

(2.5, 5 and 10 mg/kg) on the represented models. The effect of chronic exposure to CTX on learning and memory test was significant (Fig. 3A). This suggests that spatial memory in *Y* maze was disrupted by drug treatment (CTX). Meanwhile, a specific impairment in the passive avoidance was detected (Fig. 3B). This finding suggests that CTX treatment disrupted memory for avoiding aversive environment which has been shown to rely on hippocampal function.

To explain these functional disruptions, we hypothesized that CTX treatment would have adverse effect on hippocampal neurogenesis and neuron progenitor. Significant reductions in the number of immature neurons (DCX) were found in CTX groups compared with controls. Furthermore, neuron progenitor also differed between control and CTX groups. Taken together, CTX treatment severely affects the cell genesis and morphologic development of newly born neurons was suggested in present result. These results are consistent with previous studies conducted in mice in which acute CTX treatment resulted in impaired performance on passive avoidance task [8], but not consistent with a report in rats that a chronic CTX treatment shows transient improvement in cognitive function after post treatment of 7 weeks [13]. Differences in cognitive results between studies emphasize the need for more studies with the control of dosing schedules and post treatment assessment time points. Further studies should employ more comprehensive tasks to evaluate the cognitive abilities.

It is also possible that ginsenoside would ameliorate this functional deficits caused by CTX treatment given that ginsenoside have been proved to enhance memory and learning [14,20]. To address this possibility, we conducted a rare ginsenoside of protopanaxadiol saponin, compound K. Interestingly; we found compound K (10 mg/kg) reversed the functional decrements in both behavioral test and stem cell depletion. Cognitive impairment induced by chemotherapy might due to indirect chemical toxicity, oxidative damage, direct injury to the neurons or immune system [21]. CTX is one of the alkylating agents that react with DNA strand prevents the cell from replicating but did not induce apoptosis in adult dentate gyrus of mouse [8]. In addition, compound K arrest the G1 phase of the cell cycle in U937 human monocytic leukemia [22] and is able to suppress ultraviolet radiation-induced apoptosis by inducing DNA repair in human keratinocytes [23]. This synergistic cytotoxic effect and ability of DNA repair might be the possible explanation for the amelioration of compound K on the inhibition of hippocampal neurogenesis caused by CTX treatment. In addition, microenvironmental factor such as inflammation affect the regulation of endogenous neurogenesis [11]. Anti-inflammatory mechanism of compound K in activated microglia and its neuroprotective effect were proved both in vivo and vitro with mice

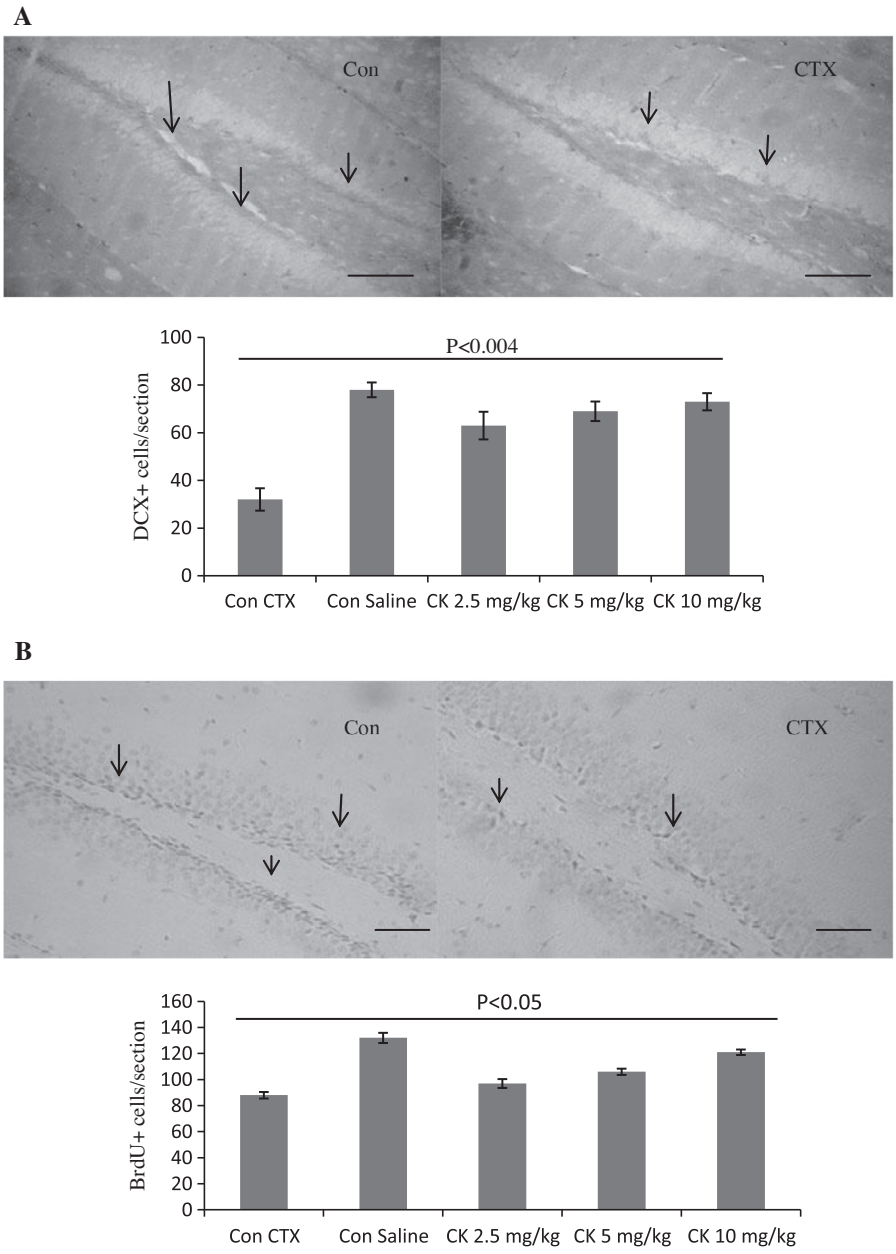

Fig. 4. Treatment with CTX decreases the number of immature neurons (A) and progenitor cell genesis (B) in the dentate gyrus of the hippocampus. Quantification is represented in column diagram. Quantification is represented in column diagram. (A) Scale bar, 100 μm. (B) Scale bar, 50 μm.

model [24,25]. Those data suggested another possible mechanism for compound K.

In summary, hippocampus-dependent memory was impaired by CTX treatment is consistent with the decrease in neurogenesis. Compound K is able to ameliorate this dysfunction. These results suggest that compound K might be a strategy to prevent or repair this unintended side effect of chemotherapy treatment. Further studies are needed to assess how compound K ameliorate the disruption of hippocampal neurogenesis.

References

[1] S.T. Matalon, A. Ornoy, M. Lishner, Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (Cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta), Reprod. Toxicol. 18 (2004) 219–230.

[2] M.C. Janelsins, J.A. Roscoe, M.J. Berg, B.D. Thompson, M.J. Gallagher, G.R. Morrow, C.E. Heckler, P. Jean-Pierre, L.A. Opanashuk, R.A. Gross, IGF-1 partially restores chemotherapy-induced reductions in neural cell proliferation in adult C57BL/6 mice, Cancer Invest. 28 (2010) 544–553.

[3] J. Altman, S.A. Bayer, Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods, J. Comp. Neurol. 301 (1990) 365–381.

[4] E. Gould, A. Beylin, P. Tanapat, A. Reeves, T.J. Shors, Learning enhances adult neurogenesis in the hippocampal formation, Nat. Neurosci. 2 (1999) 260–265.

[5] E. Gould, P. Tanapat, Stress and hippocampal neurogenesis, Biol. Psychiatry 46 (1999) 1472–1479.

[6] C.G. Gross, Neurogenesis in the adult brain: death of a dogma, Nat. Rev. Neurosci. 1 (2000) 67–73.

[7] A.B. Reiriz, G.K. Reolon, T. Preissler, J.O. Rosado, J.A. Henriques, R. Roesler, G. Schwartsmann, Cancer chemotherapy and cognitive function in rodent models: memory impairment induced by cyclophosphamide in mice, Clin. Cancer Res. 12 (2006) 5000 (author reply 5000–5001.).

[8] M. Yang, J.S. Kim, M.S. Song, S.H. Kim, S.S. Kang, C.S. Bae, J.C. Kim, H. Wang, T. Shin, C. Moon, Cyclophosphamide impairs hippocampus-dependent learning and memory in adult mice: possible involvement of hippocampal neurogenesis in chemotherapy-induced memory deficits, Neurobiol. Learn. Mem. 93 (2010) 487–494.

[9] G.W. Konat, M. Kraszpulski, I. James, H.T. Zhang, J. Abraham, Cognitive dysfunction induced by chronic administration of common cancer chemotherapeutics in rats, Metab. Brain Dis. 23 (2008) 325–333.
[10] J.E. MacLeod, J.A. DeLeo, W.F. Hickey, T.A. Ahles, A.J. Saykin, D.J. Bucci, Cancer chemotherapy impairs contextual but not cue-specific fear memory, Behav. Brain Res. 181 (2007) 168–172.
[11] L.A. Christie, M.M. Acharya, V.K. Parihar, A. Nguyen, V. Martirosian, C.L. Limoli, Impaired cognitive function and hippocampal neurogenesis following cancer chemotherapy, Clin. Cancer Res. 18 (2012) 1954–1965.
[12] T.L. Briones, J. Woods, Chemotherapy-induced cognitive impairment is associated with decreases in cell proliferation and histone modifications, BMC Neuroscience 12 (2011).
[13] G.D. Lee, D.L. Longo, Y. Wang, J.M. Rifkind, L. Abdul-Raman, J.A. Mamczarz, K.B. Duffy, E.L. Spangler, D.D. Taub, M.P. Mattson, D.K. Ingram, Transient improvement in cognitive function and synaptic plasticity in rats following cancer chemotherapy, Clin. Cancer Res. 12 (2006) 198–205.
[14] G. Zhang, A. Liu, Y. Zhou, X. San, T. Jin, Y. Jin, Panax ginseng ginsenoside-Rg2 protects memory impairment via anti-apoptosis in a rat model with vascular dementia, J. Ethnopharmacol. 115 (2008) 441–448.
[15] J.H. Yang, S.J. Han, J.H. Ryu, I.S. Jang, D.H. Kim, Ginsenoside Rh2 ameliorates scopolamine-induced learning deficit in mice, Biol. Pharm. Bull. 32 (2009) 1710–1715.
[16] D.Y. Kim, H.D. Yuan, I.K. Chung, S.H. Chung, Compound K, intestinal metabolite of ginsenoside, attenuates hepatic lipid accumulation via ampk activation in human hepatoma cells, J. Agric. Food Chem. 57 (2009) 1532–1537.
[17] S. Chae, K.A. Kang, W.Y. Chang, M.J. Kim, S.J. Lee, Y.S. Lee, H.S. Kim, D.H. Kim, J.W. Hyun, Effect of Compound K, a metabolite of ginseng saponin, combined with gamma-ray radiation in human lung cancer cells *in Vitro* and *in Vivo*, J. Agric. Food Chem. 57 (2009) 5777–5782.
[18] Z. Zhang, G.J. Du, C.Z. Wang, X.D. Wen, T. Calway, Z. Li, T.C. He, W. Du, M. Bissonnette, M.W. Musch, E.B. Chang, C.S. Yuan, Compound K, a ginsenoside metabolite, inhibits colon cancer growth via multiple pathways including p53–p21 interactions, Int. J. Mol. Sci. 14 (2013) 2980–2995.
[19] G. Kempermann, D. Gast, G. Kronenberg, M. Yamaguchi, F.H. Gage, Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice, Development 130 (2003) 391–399.
[20] H. Bao, J. Zhang, S. Yeo, C.-S. Myung, H. Kim, J. Kim, J. Park, J. Cho, J. Kang, Memory enhancing and neuroprotective effects of selected ginsenosides, Arch. Pharmacal Res. 28 (2005) 335–342.
[21] D. Barton, C. Loprinzi, Novel approaches to preventing chemotherapy-induced cognitive dysfunction in breast cancer: the art of the possible, Clin. Breast Cancer 3 (2002) S121–S127.
[22] K. Kang, Y. Kim, S. Kim, S. Chae, Y. Koh, H. Kim, M. Choo, D. Kim, J. Hyun, G1 phase arrest of the cell cycle by a ginseng metabolite, compound K, in U937 human monocytic leukamia cells, Arch. Pharmacal Res. 28 (2005) 685–690.
[23] B.-X. Cai, D. Luo, X.-F. Lin, J. Gao, Compound K suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair in human keratinocytes, Arch. Pharmacal Res. 31 (2008) 1483–1488.
[24] J.-S. Park, J.A. Shin, J.-S. Jung, J.-W. Hyun, T.K. Van Le, D.-H. Kim, E.-M. Park, H.-S. Kim, Anti-inflammatory mechanism of compound K in activated microglia and its neuroprotective effect on experimental stroke in mice, J. Pharmacol. Exp. Ther. 341 (2012) 59–67.
[25] E.-H. Joh, I.-A. Lee, I.-H. Jung, D.-H. Kim, Ginsenoside Rb1 and its metabolite compound K inhibit IRAK-1 activation—the key step of inflammation, Biochem. Pharmacol. 82 (2011) 278–286.