Protective role of *Cordyceps militaris* in A β _{1–42}-induced Alzheimer's disease in vivo

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Received: 4 April 2018 / Revised: 8 November 2018 / Accepted: 20 November 2018 / Published online: 4 December 2018
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Abstract According to the “amyloid cascade hypothesis”, amyloid-beta (A β) protein occupied one of the risk factors of Alzheimer's disease (AD). *Cordyceps militaris* (CM) has been reported to exert anti-inflammatory, anti-oxidant, and neuroprotective activities; however, its activity against cognitive dysfunction has not been studied yet. In this study, the CM ethanol extract was administered with a dose of 100 or 200 mg/kg for 2 weeks, and behavioral assessments were performed for learning and memory function in A β _{1–42}-induced AD mice models. Supplementation with CM extract enhanced new route consciousness and novel object recognition, and in the Morris water maze test, CM-administered groups showed less time to reach to the hidden platform compared with the control group. Moreover, the CM extract inhibited nitric oxide production and lipid peroxidation in the brain, liver, and kidney. The present study indicated that CM could have the protective role from cognitive impairment and progression of AD.

Keywords Alzheimer's disease · Amyloid beta · Cognition · *Cordyceps militaris* · Memory

Introduction

The occurrence of dysfunctions in neurodegenerative disease such as Alzheimer's disease (AD) and Parkinson's disease increases with age (Markesbery, 1997). AD is believed to be the most common cause of dementia presents with symptoms of memory loss and cognitive disorder. Although almost 1 million new AD cases per year are expected to occur by 2050 (Alzheimer's Association, 2016), the cause of AD has not been clearly elucidated yet. According to the amyloid cascade hypothesis, deposition of amyloid-beta (A β) protein might lead to AD (Hardy and Higgins, 1992). Excessive accumulation of A β creates senile plaques (SP), which are found in AD patients' brains, and are derived from the cleavage of amyloid precursor protein (Hardy and Higgins, 1992). It was believed that A β _{1–42} could be the main component of SP that results in AD (Zheng et al., 2013). Furthermore, accumulation of A β can also produce neurotoxic effects, leading to the appearance of oxidant stress and promoting microglial activation (Yan et al., 1996). Oxidative stress involves an imbalance between reactive oxygen species (ROS) and the defense system against ROS, which results in AD. Therefore, identification of protective agents against A β -induced oxidative stress has been in focus for treatment of AD patients. Previous studies have demonstrated that accumulation of A β in the brain of AD patients is the main reason for the disease, which suggested that the large amount of A β deposition leads to A β plaques are related to AD progression (Alzheimer's Association, 2016). Since A β is closely connected with synaptotoxicity and cognitive dysfunction, Zheng et al. (2013) have reported that A β _{1–42}-injected mice showed a wide range of neurodegenerative features in hippocampus and decreased both cytokines interferon- γ and nuclear factor-kappa B levels in the

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hippocampus, resulting in neurogenesis reduction. Likewise, excessive $A\beta_{1-42}$ levels in the brain lead to oxidative stress and neurotoxicity, inflammation, and eventually cognitive disorder. The evidence has shown an association between $A\beta$ and oxidative stress, which can induce an increase in ROS, excess lipid peroxidation, and formation of NO (Markesbery, 1997).

Cordyceps militaris (CM), a medicinal mushroom, was used as a folk tonic food in Asian countries since ancient times and is now widely used in pharmaceutical industries (Ma et al., 2015). Several studies have demonstrated that CM attenuates oxidative stress and protects against cancer progression and diabetes (Rao et al., 2010). CM is an entomogenous fungus with beneficial effects such as the hyperglycemia prevention, free radical scavenging activity, and anti-inflammatory effects (Hwang et al., 2008; Ma et al., 2015). In addition, CM exerts anti-inflammatory activity by nitric oxide (NO) inhibition in cellular system (Choi et al., 2012). The improvement effect of CM against memory deficits in rats treated with scopolamine was also demonstrated (Lee et al., 2011). On the basis of these reports, we proposed that CM might play a protective role against nervous system impairment in $A\beta_{1-42}$ -induced AD mice. Thus, in the present study, we investigated the protective effect of CM in $A\beta_{1-42}$ -induced AD mice models.

Materials and methods

Plant materials

The ethanol extract of CM was obtained from National Institute of Horticultural and Herbal Science (RDA, Jeollabuk-do, Korea) and was dissolved in water before use.

Reagents

$A\beta_{1-42}$, donepezil, griess reagent, and malondialdehyde (MDA) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were acquired from Lancaster Synthesis (Ward Hill, MA, USA) and Kanto Chemical Co., Inc. (Tokyo, Japan), respectively. Phosphoric acid and butanol were obtained from Samchun Pure Chemical Co. Ltd (Seoul, Korea).

Experimental designs

Five-week-old male ICR mice were purchased from Orient Inc., (Gyeonggi-do, Korea), weighing 25–28 g and provided diet and water under a 12-h shift of light–dark cycle at temperature of 20 ± 2 °C and humidity of $50 \pm 10\%$. Total 40 mice were randomized by weight and divided into

the following groups ($n = 8$ per group) with a similar average weight at the initial stage: (1) normal group; (2) $A\beta_{1-42}$ -injected control group; (3) $A\beta_{1-42}$ injection + CM (100 mg/kg/day) group; (4) $A\beta_{1-42}$ injection + CM (200 mg/kg/day) group; (5) $A\beta_{1-42}$ injection + donepezil (5 mg/kg/day) group. The CM extract and donepezil water solution were administered at doses of 100 or 200 mg/kg/day and 5 mg/kg/day, respectively. Mice in the normal group and $A\beta_{1-42}$ -injected control group were orally administered to water, while other groups were orally administered CM extract or donepezil for 14 days. All studies were implemented under the guidelines of the Pusan National University Institutional Animal Care and Use Committee (PNU-IACUC, approval number: PNU-2016-1383). The experimental timetable was designed as illustrated in Fig. 1.

$A\beta_{1-42}$ -infused mouse model

$A\beta_{1-42}$ protein was dissolved in 0.9% NaCl (1 $\mu\text{g}/\mu\text{L}$) and incubated at 37 °C for aggregation over 3-days before use (Li et al., 2010). Before intracerebroventricular (i.c.v.) injection, mice were anaesthetized by a mixture of Zoletil 50® (30 mg/kg) and Rompun (10 mg/kg). According to the procedure established by Paxinos and Franklin (2004), $A\beta_{1-42}$ protein (3 $\mu\text{L}/3$ min) was injected into the right lateral ventricle at stereotaxic coordinates (anteroposterior, -0.2 mm; mediolateral, -1.0 mm; dorsoventral, -2.5 mm) taken from the atlas of the mouse brain (Park et al., 2012). The animals in the normal group received 0.9% NaCl injections and those in the other groups were injected with aggregated $A\beta_{1-42}$ protein.

T-maze test

The T-maze test was implemented in accordance with the method established by Montgomery (1952). The T-formed equipment was made of black-painted boards. The maze was composed of a start box, and left arm and right arm (50 cm in length at the start and goal stems; 13 cm in width; 20 cm in height) with a clapboard to separate the two sides. In the training session, the left arm was blocked, mice were put in the start box, and a 10 min of the time required to explore the right arm exploration was recorded. After training, the mice were put back in the cages. Then, 24 h later, in the test session, the blocked left arm was opened to allow the mice to explore both left and right sides of the T-maze during 10 min. The space perceptive ability (%) was calculated as the ratio of the entry frequency in the left or right arms to the total number of arm entries multiplied by 100.

Fig. 1 Behavioral experimental schedule for mice injected with Aβ₁₋₄₂

Novel object recognition test

The object recognition test (Bevins and Besheer, 2006) was completed in an open-field square apparatus (40 × 40 × 40 cm), which was black painted. For the training session, two identical objects (A, A') were placed at fixed distances in the square apparatus. The number of touches of each object was counted 10 min after the mice were placed in the center of the square field. After a 24 h test session, the mice were placed back into the same apparatus and allowed to touch objects, with the shape of one of the objects different from what it was previously (A, B). Cognitive function was measured as object cognitive ability (%), describing the ratio of the number of touches with the familiar object (training section) or the novel object (test section) throughout the entire exploration period.

Morris water maze test

The Morris water maze test was performed using the method described by Morris (1984), with a slight modification. The laboratory apparatus consisted of a circular pool (80 cm in diameter; 40 cm in height), which was divided into four quadrants filled with water by using water-soluble non-toxic white color poster to make it invisible. Water temperature was maintained at 22 ± 2 °C. A small platform (8 cm in diameter) was set approximately 1 cm below the water surface in the center of one target quadrant at a fixed position during the training trial. Four different posters on the wall of the equipment provided visual cues for navigation. Three training trials per day were carried out for 4 days. At each training trial, mice were placed in the water facing the pool wall and allowed to swim for a latency time within 1 min to find the hidden platform. If a mouse was unable to reach the platform, it was guided smoothly to the platform and allowed to rest on the platform for few seconds. In addition, the latency time

that each mouse spent in the target quadrant without the hidden platform was calculated. For the last test, the setup was filled with transparent pool water and the platform was put back into the target quadrant below 1 cm the water. The time for each mouse to arrive at the platform was recorded.

Measurement of lipid peroxidation

Lipid peroxidation measurement by assessment of MDA levels was conducted according to the method from Ohkawa et al. (1979). Soon after finishing the behavioral experiment, mice were dissected under Zoletil 50[®] and Rompun anaesthetization. Organs including the brain, liver, and kidney were removed immediately and stored with ice. The dissected organs were homogenized with 0.9% NaCl. The homogenate was mixed with thiobarbituric acid reacting substances (TBARS) solution, which contains 20% phosphoric acid, 46 mM TBA solution and 920 mM TCA solution. After boiling at 100 °C for 20 min, the mixed solution was ice-cooled, then 7 mL of butanol was added, and all the mixtures were centrifuged at 3000 rpm for 10 min. The absorbance values of the supernatant were measured at 540 nm while the lipid peroxidation level was calculated through the MDA standard curve.

NO scavenging activity

The method of NO production measurement was referred to Bryan and Grisham (2007). In this assessment, 150 μL of tissue homogenate was mixed with 130 μL of distilled water and 100 μL of the mixture was added with the same volume of Griess reagent. The absorbance value of the mixture was measured at 540 nm and the production of NO was substituted into the sodium nitrite (NaNO₂) standard curve.

Statistical analysis

All the results were emphasized as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA), followed by Duncan's multiple tests. The data from both the T-maze test and the novel object recognition test were assessed using Student's *t* test. Statistical significance was considered at $p < 0.05$.

Results and discussion

A β is known as a key component of senile plaques found in brain; and deposits in the brain region where serving memory and cognition, especially in hippocampus. The i.c.v injection of A β induced neuronal death in hippocampus (Li et al., 2010). The cerebrospinal fluid is formed in lateral ventricles, so that when injecting A β i.c.v, it will irrigate through all brain parts (Simon and Iliff, 2016). Haughey et al. (2002b) also have reported that the lateral ventricle i.c.v with A β can induce spatial learning impairment. In addition, Park et al. (2012) showed increased level of A β in the hippocampus by i.c.v injection compared with the non-A β -treated group, indicating that i.c.v injection with A β could be activated in the hippocampus.

The T-maze test has been used to estimate the cognition of rodents for decades (Montgomery, 1952). In our experiment, the A β_{1-42} -induced control group showed no significant differences between the old and new routes, indicating cognitive impairment by A β_{1-42} injection. In contrast, mice administered 100 or 200 mg/kg/day CM showed a higher percentage of entries in the new route than the old route, compared with the control group (Fig. 2). The data suggests that CM could improve spatial memory against A β_{1-42} -induced cognitive disorder.

Generally, a novel object is more attractive than the familiar one in animals, especially rodents; hence the novel object recognition test is used to investigate the level of cognitive function (Bevins and Besheer, 2006). To study spatial object memory, we conducted the novel object recognition task in A β_{1-42} -induced AD mice. The present experiment revealed that in the training session (A, A'), no significant differences were showed in object exploration among all the groups. However, after 24 h in the test section using two different objects (A, B), the normal group showed a higher significant recognition ability for the novel object than the familiar object, but the A β_{1-42} -treated control group did not show differences in recognition ability. Meanwhile, mice administered CM at 100 or 200 mg/kg/day showed a significantly higher novel object

Fig. 2 Effect of extracts from *Cordyceps militaris* (CM) on T-maze test. Normal = 0.9% NaCl injection + administration of water; Control = A β_{1-42} injection + administration of water; CM100 = A β_{1-42} injection + administration of CM (100 mg/kg/day); CM200 = A β_{1-42} injection + administration of CM (200 mg/kg/day); PO = A β_{1-42} injection + administration of donepezil (5 mg/kg/day). Values are mean $\pm$ SD (n = 8 per group). The letters (a, b) among groups represent significant differences ($p < 0.05$) by Duncan's multiple range test and the asterisk (*) represent the significantly different of space perceptive abilities with old and new routes by Student's *t* test ($p < 0.05$)

Fig. 3 Effect of extracts from *Cordyceps militaris* (CM) on the results of the novel object recognition test. Normal = 0.9% NaCl injection + administration of water; Control = A β_{1-42} injection + administration of water; CM100 = A β_{1-42} injection + administration of CM (100 mg/kg/day); CM200 = A β_{1-42} injection + administration of CM (200 mg/kg/day); PO = A β_{1-42} injection + administration of donepezil (5 mg/kg/day). Values are mean $\pm$ SD (n = 8 per group). The letters (a-c) among groups represent significant differences ($p < 0.05$) by Duncan's multiple range test and the asterisk (*) represent the significantly different of object recognition abilities with familiar and novel objects by Student's *t* test ($p < 0.05$)

cognitive ability (Fig. 3). This suggests that CM has a protective effect against object recognition impairment.

The Morris water maze test has been established as a reliable test for analyzing memory deficit and cognitive ability (Morris, 1984). As demonstrated in Fig. 4(A), all the groups showed a shorter time to reach the hidden platform during the learning section. After 4 days of training, both CM100- and CM200-administered groups reached the hidden platform in shorter time than the $A\beta_{1-42}$ -induced control group. In addition, in order to monitor the CM-related memory improvement that could be attributable to the visual and swimming ability, the latency in reaching to the exposed platform was also

measured [Fig. 4(B, C)]. The results showed no significant differences in all the experimental groups, indicating that the effect of memory improvement on CM treated $A\beta_{1-42}$ -induced mice models was not involved in the swimming and visual abilities but the improvement of cognitive function.

Several studies have evidenced that $A\beta$ causes the oxidative stress in the liver, kidney, and skeletal muscle (Roher et al., 2009). Over-generation of ROS and NO is used to be a marker of oxidative stress. Excessive ROS levels in the brain imply apoptotic cell death, which leads to neurodegenerative diseases (Markesbery, 1997). The oxidative stress in lipid bilayers forms aldehydes that

Fig. 4 Effect of extracts from *Cordyceps militaris* (CM) in the Morris water maze test. (A) Mice were allowed to look for the hidden platform that was invisible under water within 60 s. (B) The time spent in the hidden platform was calculated on the final day of the water maze test. (C) The time spent in the exposed platform in transparent water was calculated in the water maze test. Normal = 0.9% NaCl injection + administration of water;

Control = $A\beta_{1-42}$ injection + administration of water; CM100 = $A\beta_{1-42}$ injection + administration of CM (100 mg/kg/day); CM200 = $A\beta_{1-42}$ injection + administration of CM (200 mg/kg/day); PO = $A\beta_{1-42}$ injection + administration of donepezil (5 mg/kg/day). Values are mean $\pm$ SD (n = 8 per group). The letters (a-c) among groups represent significant differences ($p < 0.05$) by Duncan's multiple range test. NS: Non-significant

associate with lipid peroxidation. It has been elucidated that high levels of aldehydes are a toxic factor of lipid peroxidation (Butterfield et al., 2002). Previous studies have demonstrated that A β induced lipid peroxidation in organs (Butterfield et al., 2002). In addition, the NO-production level in the brain is also another target to indicate neuronal apoptosis (Combs et al., 2001). The AD-induced oxidative damage was attenuated by the use of natural materials such as green tea, *Schisandra chinensis*, and *Eriobotrya japonica* (Jeong et al., 2013; Kim et al., 2011). Similarly, our study also showed an increase of oxidative stress in the A β_{1-42} -induced AD mouse brain, liver, and kidney. Firstly, the inhibitory effects of CM against lipid peroxidation induced by A β_{1-42} are illustrated in Table 1. MDA levels in the brain, liver, and kidney were significantly elevated by A β injection. Particularly, the MDA level in the brain in the normal group was 35.60 nmol/mg protein, but that in A β_{1-42} -treated control group was 62.29 nmol/mg protein. However, oral administration of CM100 and CM200 attenuated MDA levels by 47.86 nmol/mg protein and 38.07 nmol/mg protein, respectively. Secondly, the effect of CM on NO generation in the brain, liver, and kidney in mice injected with A β_{1-42} was described in Table 2. NO production in brains in the normal group was 45.74 μ mol/L/mg protein, while that in that A β_{1-42} -induced control group was 53.61 μ mol/L/mg protein. In addition, NO production reduced to 45.40 μ mol/L/mg protein and 32.17 μ mol/L/mg protein after administration of CM100 and CM200, respectively. The NO generation levels in mice livers also showed significant differences in the CM-administered groups. CM treatment might attenuate the NO production induced by A β_{1-42} in tissues. These results indicated that the increased level of

Table 1 Effect of extracts from *Cordyceps militaris* (CM) on A β_{1-42} -induced lipid peroxidation

Group	MDA (nmol/mg protein)		
	Brain	Liver	Kidney
Normal	35.60 $\pm$ 14.61 ^b	27.28 $\pm$ 5.32 ^{ab}	57.05 $\pm$ 5.88 ^{ab}
Control	62.29 $\pm$ 11.04 ^a	30.85 $\pm$ 4.21 ^a	63.71 $\pm$ 5.03 ^a
CM100	47.86 $\pm$ 9.37 ^{ab}	19.45 $\pm$ 8.57 ^{ab}	60.30 $\pm$ 11.31 ^{ab}
CM200	38.07 $\pm$ 10.08 ^b	22.32 $\pm$ 9.53 ^{ab}	62.29 $\pm$ 6.45 ^{ab}
PO	38.19 $\pm$ 10.68 ^b	18.29 $\pm$ 8.32 ^b	51.05 $\pm$ 7.53 ^b

Normal = 0.9% NaCl injection + administration of water; Control = A β_{1-42} injection + administration of water; CM100 = A β_{1-42} injection + administration of CM (100 mg/kg/day); CM200 = A β_{1-42} injection + administration of CM (200 mg/kg/day); PO = A β_{1-42} injection + administration of donepezil (5 mg/kg/day). Values are presented as the mean $\pm$ SD (n = 8 per group). The different letters (a, b) indicate significant differences ($p < 0.05$) by Duncan's multiple range test

Table 2 Effect of extracts from *Cordyceps militaris* (CM) on A β_{1-42} -induced nitric oxide formation

Group	NaNO ₂ (μ mol/mg protein)		
	Brain	Liver	Kidney
Normal	45.74 $\pm$ 5.90 ^b	75.33 $\pm$ 23.92 ^b	47.82 $\pm$ 13.08 ^{NS}
Control	53.61 $\pm$ 6.60 ^a	109.89 $\pm$ 29.27 ^a	55.56 $\pm$ 7.93
CM100	45.40 $\pm$ 4.05 ^b	71.91 $\pm$ 21.47 ^b	39.63 $\pm$ 7.97
CM200	32.17 $\pm$ 3.43 ^c	72.59 $\pm$ 18.71 ^b	44.03 $\pm$ 15.94
PO	27.96 $\pm$ 1.67 ^c	55.73 $\pm$ 9.62 ^b	51.22 $\pm$ 17.65

Normal = 0.9% NaCl injection + administration of water; Control = A β_{1-42} injection + administration of water; CM100 = A β_{1-42} injection + administration of CM (100 mg/kg/day); CM200 = A β_{1-42} injection + administration of CM (200 mg/kg/day); PO = A β_{1-42} injection + administration of donepezil (5 mg/kg/day). Values are presented as the mean $\pm$ SD (n = 8 per group). The different letters (a-c) indicate significant differences ($p < 0.05$) by Duncan's multiple range test. NS: Non-significance

MDA and NO production in A β_{1-42} mouse model led to oxidative stress associated with AD. Furthermore, the memory deficits and cognitive disorder induced by A β_{1-42} might be improved with CM treatment by attenuation of oxidative damage through lowering the amount of MDA and NO in tissues.

AD is the most common type of dementia, still remaining unclear fundamental cause. According to Markesbery (1997), a hypothesis of oxidative stress that can cause AD has been proposed; indicated that free radical-induced oxidative stress accelerate the process of neuron death, then to the onset of AD. In previous study, the anti-oxidative activity of CM was observed in vitro test. In addition, He et al. (2013) indicated that cordycepin, the major component of CM, has DPPH and hydroxyl radical scavenging property, which is similar to that activity of vitamin C. In addition, previous study showed that CM-treated groups exerted high cell survival rate in hippocampus under ischemic mouse model, indicating that CM has beneficial effect against neuronal cell apoptosis from oxidative damage (Hwang et al., 2008). Cordycepin, which has been known as an important bioactive compound of CM, suppressed the levels of pro-inflammatory cytokines in lipopolysaccharide-induced microglial cells (Kim et al., 2006). Also, cordycepin exerted the neuro-protective effect on oxidative damage in A β -induced hippocampal neurons (Song et al., 2018). We have observed the protective effects of CM against A β -induced neuroinflammation and apoptosis in neuronal cells and brain of mouse. In A β -treated C6 glial cells, CM inhibited ROS production and protected neuroinflammatory responses via the regulation of inducible nitric oxide synthase and cyclooxygenase-2 protein expression. Furthermore, the

protein expression in mitogen-activated protein kinase signaling including phospho-p38 and phospho-JNK pathway indicated the reduced neuronal cell apoptosis level in neuronal cells and the A β -injected mouse brain tissue with CM treatment. Based on the evidences, we suggest that CM showed the protective effect on oxidative stress and neuroinflammation by regulation of inflammation- and apoptosis-related pathway.

It is unclear whether extract or compounds from CM can penetrate across blood brain barrier (BBB). However, they can affect the BBB permeability. The BBB is a defense gate to restrict the harmful and toxicity substances that invades into the brain parenchyma. However, when injury occurs, the BBB can be opened; thus, the harmful and toxicity substances move in, inducing inflammatory responses and neuronal damage (Saraiva et al., 2016). CM is known as an edible medicine for the diseases treatment. Ma et al. (2010) have identified kurarinone, a kind of flavonoids from CM, with antioxidant activity and anticancer effect. It can cross the BBB to influence the brain function (Hanrahan et al., 2015). In addition, cordycepin has protective effect on traumatic brain injury-induced BBB integrity, suggesting that it probably has the capacity of BBB penetration (Yuan et al., 2016).

In the neuron development, the neural stem cells presented in the subventricular zone (SVZ), are related to the neurogenesis (Zhang et al., 2007). The subgranular zone (SGZ) of the hippocampal dentate gyrus was also an important region linking to the memory formation. And a previous study showed that the activity of neural progenitor cells in the SGZ of the dentate gyrus were disrupted by A β deposition under A β -overexpressed amyloid precursor protein (APP) mice, which might cause memory impairment and an early onset of Alzheimer's disease (Haughey et al., 2002a). Zhang et al. (2007) also showed the decreased neurogenesis in SVZ under A β -deposited APP transgenic mice. Therefore, overexpression of A β affects SVZ and SGZ region in the brain, which probably lead to memory impairment. In addition, cordycepin, a major component from CM, has reported to perform a preventive function in neuronal degeneration in hippocampal CA1 area and dentate gyrus region of oxygen-glucose deprivation-injured ischemia mouse, implicating the neuroprotective effect of cordycepin (Cheng et al., 2011). Therefore, based on the evidences, CM might also provide the neurogenesis effect both in the SVZ and the SGZ of the dentate gyrus in the A β -injected mice model.

In conclusion, we confirmed that administration of CM at doses of 100 and 200 mg/kg prevented A β -induced oxidative damage in the AD mouse model by obtaining evidence through behavioral experiments, inhibition of lipid peroxidation, and NO formation. As a result, CM could play a role in preventing memory deficits and

learning disorder in AD. We expect that CM would be useful for prevention and treatment for the progression of memory loss and improve cognitive impairment.

Acknowledgements This work was supported by a 2-Year Research Grant of Pusan National University.

References

- Alzheimer's Association. 2016 Alzheimer's disease facts and figures. *Alzheimers Dement.* 12: 459–509 (2016)
- Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nat. Protoc.* 1: 1306–1311 (2006)
- Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. *Free Radic. Biol. Med.* 43: 645–657 (2007)
- Butterfield DA, Castegna A, Lauderback CM, Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol. Aging* 23: 655–664 (2002)
- Cheng Z, He W, Zhou X, Lv Q, Xu X, Yang S, Zhao C, Guo L. Cordycepin protects against cerebral ischemia/reperfusion injury in vivo and in vitro. *Eur. J. Pharmacol.* 664: 20–28 (2011)
- Choi JH, Kim GS, Lee SE, Cho JH, Sung GH, Lee DY, Kim SY, Lee TH, Noh HJ. Anti-inflammatory effects of *Cordyceps militaris* extracts. *J. Mushroom* 10: 249–253 (2012)
- Combs CK, Karlo JC, Kao SC, Landreth GE. β -Amyloid stimulation of microglia and monocytes results in TNF α -dependent expression of inducible nitric oxide synthase and neuronal apoptosis. *J. Neurosci.* 21: 1179–1188 (2001)
- Hanrahan JR, Chebib M, Johnston GA. Interactions of flavonoids with ionotropic GABA receptors. *Adv. Pharmacol.* 72: 189–200 (2015)
- Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256: 184–185 (1992)
- Haughey NJ, Liu D, Nath A, Borchard AC, Mattson MP. Disruption of neurogenesis in the subventricular zone of adult mice, and in human cortical neuronal precursor cells in culture, by amyloid β -peptide. *Neuromol. Med.* 1: 125–135 (2002a)
- Haughey NJ, Nath A, Chan SL, Borchard AC, Rao MS, Mattson MP. Disruption of neurogenesis by amyloid β -peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. *J. Neurochem.* 83: 1509–1524 (2002b)
- He YT, Zhang XL, Xie YM, Xu YX, Li JR. Extraction and antioxidant property in vitro of cordycepin in artificially cultivated *Cordyceps militaris*. *Adv. Mat. Res.* 750: 1593–1596 (2013)
- Hwang IK, Lim SS, Yoo KY, Lee YS, Kim HG, Kang IJ, Kwon HJ, Park J, Choi SY, Won MH. A phytochemically characterized extract of *Cordyceps militaris* and cordycepin protect hippocampal neurons from ischemic injury in gerbils. *Planta. Med.* 74: 114–119 (2008)
- Jeong EJ, Lee HK, Lee KY, Jeon BJ, Kim DH, Park JH, Song JH, Huh J, Lee JH, Sung SH. The effects of lignan-riched extract of *Shisandra chinensis* on amyloid- β -induced cognitive impairment and neurotoxicity in the cortex and hippocampus of mouse. *J. Ethnopharmacol.* 146: 347–354 (2013)
- Kim MJ, Lee J, Seong AR, Lee YH, Kim YJ, Baek HY, Kim YJ, Jun WJ, Yoon HG. Neuroprotective effects of *Eriobotrya japonica* against β -amyloid-induced oxidative stress and memory impairment. *Food Chem. Toxicol.* 49: 780–784 (2011)

- Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin DJ, Han SK, Park SM, Park JH, Park HI, Sung JM. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF- κ B through Akt and p38 inhibition in RAW 264.7 macrophage cells. *Eur. J. Pharmacol.* 545: 192–199 (2006)
- Lee B, Park J, Park J, Shin HJ, Kwon S, Yeom M, Sur B, Kim S, Kim M, Lee H, Yoon SH. *Cordyceps militaris* improves neurite outgrowth in Neuro2A cells and reverses memory impairment in rats. *Food Sci. Biotechnol.* 20: 1599–1608 (2011)
- Li J, Wang C, Zhang JH, Cai JM, Cao YP, Sun XJ. Hydrogen-rich saline improves memory function in a rat model of amyloid-beta-induced Alzheimer's disease by reduction of oxidative stress. *Brain Res.* 1328: 152–161 (2010)
- Ma XC, Sun C, Huang SS, Wang JK, Zhang BJ, Li FY, Wang G, Deng S, Cui J. Preparative isolation and purification of four prenylflavanones from microbial biotransformation of kurarinone by high-speed counter-current chromatography. *Sep. Purif. Technol.* 76: 140–145 (2010)
- Ma L, Zhang S, Du M. Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice. *Nutr. Res.* 35: 431–439 (2015)
- Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic. Biol. Med.* 23: 134–147 (1997)
- Montgomery KC. A test of two explanations of spontaneous alternation. *J. Comp. Physiol. Psychol.* 45: 287–293 (1952)
- Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11: 47–60 (1984)
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351–358 (1979)
- Park SJ, Jung JM, Lee HE, Lee YW, Kim DH, Kim JM, Hong JG, Lee CH, Jung IH, Cho YB, Jang DS. The memory ameliorating effects of INM-176, an ethanolic extract of *Angelica gigas*, against scopolamine- or A β 1–42-induced cognitive dysfunction in mice. *J. Ethnopharmacol.* 143: 611–620 (2012)
- Paxinos G, Franklin KB. The mouse brain in stereotaxic coordinates. Gulf professional publishing, Houston, TX (2004)
- Rao YK, Fang SH, Wu WS, Tzeng YM. Constituents isolated from *Cordyceps militaris* suppress enhanced inflammatory mediator's production and human cancer cell proliferation. *J. Ethnopharmacol.* 131: 363–367 (2010)
- Roher AE, Esh CL, Kokjohn TA, Castaño EM, Van Vickle GD, Kalback WM, Patton RL, Luehrs DC, Dausgs ID, Kuo YM, Emmerling MR. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement.* 5: 18–29 (2009)
- Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: overcoming blood-brain barrier to treat neurodegenerative diseases. *J. Control. Release* 235: 34–47 (2016)
- Simon MJ, Iliff JJ. Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. *Biochim. Biophys. Acta. Mol. Basis Dis.* 1862: 442–451 (2016)
- Song H, Huang LP, Li Y, Liu C, Wang S, Meng W, Wei S, Liu XP, Gong Y, Yao LH. Neuroprotective effects of cordycepin inhibit A β -induced apoptosis in hippocampal neurons. *Neurotoxicology* 68: 73–80 (2018)
- Yan SD, Chen X, Fu J, Chen M. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* 382: 685–691 (1996)
- Yuan J, Wang A, He Y, Si Z, Xu S, Zhang S, Wang K, Wang D, Liu Y. Cordycepin attenuates traumatic brain injury-induced impairments of blood-brain barrier integrity in rats. *Brain Res. Bull.* 127: 171–176 (2016)
- Zhang C, McNeil E, Dressler L, Siman R. Long-lasting impairment in hippocampal neurogenesis associated with amyloid deposition in a knock-in mouse model of familial Alzheimer's disease. *Exp. Neurol.* 204: 77–87 (2007)
- Zheng M, Liu J, Ruan Z, Tian S, Ma Y, Zhu J, Li G. Intrahippocampal injection of A β 1–42 inhibits neurogenesis and down-regulates IFN- γ and NF- κ B expression in hippocampus of adult mouse brain. *Amyloid* 20: 13–20 (2013)