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Effects of cordycepin on Y-maze learning task in mice

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ABSTRACT

Cordycepin (3′-deoxyadenosine) is the major bioactive component of *Cordyceps militaris* that has been widely used in oriental countries as a Traditional Chinese Medicine and healthy food for preventing early aging, improving physical performance and increasing lifespan. *Cordyceps militaris* extracts other than cordycepin have been reported to improve cognitive function. Although cordycepin is one of the most utilized *Cordyceps militaris* components, it remains unknown whether cordycepin could improve learning and memory. Here we investigated effects of cordycepin on learning and memory in healthy and ischemic mice using Y-maze test. We found that oral cordycepin administration at dose of 10 mg/kg significantly improved Y-maze learning performance both in healthy and ischemic mice. However, cordycepin at dose of 5 mg/kg enhanced Y-maze learning only in ischemic mice but not healthy mice. In this study, simultaneously, we found that orally administrated cordycepin significantly decreased the neuronal loss induced by ischemia in hippocampal CA1 and CA3 regions. Collectively, our results can provide valuable evidence that cordycepin may act as a nootropic product or potential clinical application in improving cognitive function of patients with ischemic stroke in the future.

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1. Introduction

Cordycepin, an adenosine analog 3′-deoxyadenosine, is the major bioactive component of *Cordyceps militaris* that has been widely used in oriental countries as a Traditional Chinese Medicine and healthy food for preventing early aging, improving physical performance and increasing lifespan. Recent researchers have found that cordycepin can preserve multiple biological functions, such as anti-inflammatory, antioxidation and anticancer (Shin et al., 2009; Liu et al., 2010; Ramesh et al., 2012; Kim et al., 2011; Wu et al., 2007). In terms of cognitive enhancement, extracts from *Cordyceps militaris* rather than cordycepin have been reported to prevent scopolamine-induced memory impairment (Gong et al., 2011), ameliorate A β-induced memory defect (Jin et al., 2004), and improve D-galactose or ethanol-induced cognitive dysfunctions (Ji et al., 2009; Li et al., 2012b; Cho et al., 2003). This preventing or ameliorating the impairment in learning and memory is possibly related to increasing antioxidative enzyme activity (Ji et al., 2009) and preventing neuronal cell death (Jin et al., 2004). Although cordycepin is putatively believed as the major component of *Cordyceps militaris*, there is no evidence for effects of cordycepin on learning and memory so far.

It is well known that learning and memory impairment is a frequent complication of brain ischemia (Hong et al., 2000). In the central nervous system, ischemia often causes irreversible brain damage due to the cascade of events leading to neuronal injury and subsequent neuronal cell death. These events include the release of cytokines and free radicals, and the induction of inflammation, apoptosis and excitotoxicity (Fawcett and Asher, 1999; Kuroda and Siesjö, 1997). Previous studies have shown that cordycepin may protect against ischemic injury by decreasing the extracellular glutamate level, increasing the activity of superoxide dismutase (Cheng et al., 2011). However, it remains unknown whether cordycepin could attenuate the deficit in learning and memory induced by ischemia.

By using Y-maze test to assess the cordycepin on learning and memory in healthy and ischemic mice, the data in our study can provide valuable evidence that cordycepin may act as a nootropic product or potential clinical application in improving cognitive function of patients with ischemic stroke in the future.

2. Materials and methods

2.1. Drugs

Cordycepin with 98% purification was obtained following the extraction and separation using a column chromatographic method (Ni et al., 2009), and the other chemicals were purchased from Sigma (St. Louis, USA).

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2.2. Animals, groups and treatments

Six-to eight-week-old male Kunming mice (20–25 g) were housed at 22 ± 3 °C, 55 ± 5% humidity, and 12 h light/dark cycle from 08:00 to 20:00 with free access water and food. Experimental procedures in this study were conducted under National Institutes of Health guidelines (Guide for the Care and Use of Laboratory Animals, NIH publication 93 23, revised 1985). The mice were allowed to adapt to these conditions for 3 days before experimentations.

2.2.1. Effects of cordycepin on survival rates in mice

Since cordycepin has been documented to induce cell death (Chen et al., 2008) and slow down cell growth (Chang et al., 2008), in the present study, we thus first investigated the safe-dosage using three doses at 5, 10 and 20 mg/kg, respectively. Cordycepin was dissolved in distilled water and administrated orally twice daily using a feeding needle for 21 days, and control group received double distilled water instead of cordycepin. The number of surviving mice was analyzed at days of 7, 14 and 21 after orally treated cordycepin.

2.2.2. Effects of cordycepin on learning and memory in healthy mice

Healthy mice were randomly divided into three groups: control, 5 and 10 mg/kg cordycepin-treated groups. Cordycepin was administrated orally twice daily for 21 days. The animal of control group treated with an equal volume of double distilled water. At the end of cordycepin-treated, the learning and memory in mice was evaluated using the Y-maze learning task.

2.2.3. Effects of cordycepin on learning and memory in ischemic mice

The ischemia model was described previously (Marosi et al., 2009). In brief, mice were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Transient cerebral ischemia was induced by occluding both common carotid arteries with aneurysm clips for 15 min, and circulation was restored by removing these clips. Mice were randomly divided into sham-operated, ischemia, 5 and 10 mg/kg cordycepin-treated groups. The same operation was carried out on the sham-operated mice except ligatures. Cordycepin was applied for two weeks before operation and one week after operation, and the same methods of administration and dosages (5 and 10 mg/kg) were used as healthy animals. Animals were allowed 7 days to recover from surgery before Y-maze learning task.

2.3. Y-maze test

The behavioral test was conducted after 21 days of drugs treatment. As described previously (Li et al., 2012a; Huang et al., 2013), Y-maze (90 cm long × 90 cm wide × 76 cm high), which consists of three arms, was placed in a darken room. Animals placed in the intersection of three arms were trained to choose entering the randomly bright arm, which was illuminated by a 15 W lamp suspended in the end of each arm. During the behavioral training period, each animal was trained to choose entering the randomly bright arm in the first 10 s, and the choice of the darken arm was count as a 'discrimination error'. Whenever the animal made an error, it received a brief electric foot shock (35 V AC, 4.5 mA; continued 1 s and started at 5 s after lamp-bright), until it entered the bright arm. The training procedure was performed during an 8-day period in healthy animals and a 10-day period in ischemia animals, and mice received 20 trials at 25–35 s random intervals every 24 h. Ninety percent correct rate served as learning criterion.

2.4. Preparation of paraffin-embedded tissues and HE staining

At the end of Y-maze learning task, sham and ischemic mice were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and killed by rapid decapitation. The brains were removed and immersed in 4% paraformaldehyde and kept at 4 °C (Cheng et al., 2012). Brains were then dehydrated in graded ethanol, embedded in paraffin and cut with a slice thickness of 4 μm and paraffin sections were baked and de-waxed. Hematoxylin staining was carried out, followed by 1% hydrochloric acid alcohol differentiation and Eosin staining was performed. Five slices were taken from each mouse brain. Three or four non-over-lapping view fields were selected randomly in each slice to count the number of positive cells. Each paraffin section was placed under a framed-box of BX51T microscope (Olympus, Japan). After adjustment to the intermediate pyramidal stratum of hippocampal CA1 and CA3 respectively, the number of survival neuronal somata was counted in the middle of hippocampal CA1 and CA3 subregions under the 20 × objective.

2.5. Statistics

The survival rate of mice was expressed as a percentage and evaluated by chi-square test, and other data was expressed as mean ± standard deviation (S.D.). Correct response rates were statistically analyzed using two-way ANOVA with factors "treat" and "time". Numbers of neurons, total trials and training days to reach learning criterion were analyzed using one-way ANOVA. Differences between the groups were considered to be statistically significant when P value < 0.05.

3. Results

3.1. Effects of cordycepin on survival rates in mice

Effects of cordycepin on the survival rates in mice were shown as Table 1. As compared to control group, oral administration of cordycepin at dosages of 5 and 10 mg/kg showed no obvious differences in survival rates of mice at days of 7, 14 and 21 ($P > 0.05$). However, survival rates of mice in 20 mg/kg cordycepin-treated group were significantly lower than of control group at days of 14 and 21 after application of cordycepin ($P < 0.05$, $P < 0.01$), suggesting that there is a toxicity of cordycepin at dose of 20 mg/kg by oral treatment twice daily for 21 days we tested on animals. Therefore, the effect of cordycepin on Y-maze learning task was carried out at 5 and 10 mg/kg as safe dosages.

3.2. Cordycepin enhances Y-maze learning task at a dose of 10 mg/kg but not 5 mg/kg in healthy mice

As shown in Fig. 1A, correct response rates in three group animals were gradually increased during the behavioral training

Table 1
Effect of *cordycepin* on surviving rate in mice ($n = 20$).

Groups	Surviving rate (%)			
	0 day	7 days	14 days	21 days
Control	100	100	95	95
5 mg/kg	100	95	95	95
10 mg/kg	100	100	95	90
20 mg/kg	100	95	85*	75**

* $p < 0.05$ compared with control group.
** $p < 0.01$ compared with control group.

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