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20(S)-protopanaxadiol (PPD) alleviates scopolamine-induced memory impairment via regulation of cholinergic and antioxidant systems, and expression of Egr-1, c-Fos and c-Jun in mice

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ABSTRACT

20(*S*)-protopanaxadiol (PPD) possesses various biological properties, including anti-inflammatory, antitumor and anti-fatigue properties. Recent studies found that PPD functioned as a neurotrophic agent to ameliorate the sensory deficit caused by glutamate-induced excitotoxicity through its antioxidant effects and exhibited strong antidepressant-like effects *in vivo*. The objective of the present study was first to investigate the effect of PPD in scopolamine (SCOP)-induced memory deficit in mice and the potential mechanisms involved. In this study, mice were pretreated with PPD (20 and 40 μmol/kg) and donepezil (1.6 mg/kg) intraperitoneally (i.p) for 14 days. Then, open field test was used to assess the effect of PPD on the locomotor activity and mice were daily injected with SCOP (0.75 mg/kg) to induce cognitive deficits and then subjected to behavioral tests by object location recognition (OLR) experiment and Morris water maze (MWM) task. The cholinergic system function, oxidative stress biomarkers and protein expression of Egr-1, c-Fos, and c-Jun in mouse hippocampus were examined. PPD was found to significantly improve the performance of amnesia mice in OLR and MWM tests. PPD regulated cholinergic function by inhibiting SCOP-induced elevation of acetylcholinesterase (AChE) activity, decline of choline acetyltransferase (ChAT) activity and decrease of acetylcholine (Ach) level. PPD suppressed oxidative stress by increasing activities of antioxidant enzymes such as superoxide dismutase (SOD) and lowering maleic diadehyde (MDA) level. Additionally, PPD significantly elevated the expression of Egr-1, c-Fos, and c-Jun in hippocampus at protein level. Taken together, all these results suggested that 20(S)-protopanaxadiol (PPD) may be a candidate compound for the prevention against memory loss in some neurodegenerative diseases such as Alzheimer's disease (AD).

1. Introduction

Alzheimer's disease (AD), the most common type of dementia, is characterized by loss of neurons especially in brain regions associated with learning and memory [1] and is a complex disease of multiple pathologies associated with degeneration of several neuronal populations, especially central cholinergic pathways [2,3]. The incidence of AD increases with age and has been predicted to affect 1 in 85 people globally by the year 2050 [1]. Dysfunction of cholinergic system, including loss of cholinergic cells in the basal forebrain and hippocampus, appears to play a critical role in the pathogenesis of dementia [4]. A decrease in brain acetylcholine (Ach) level appears to be a critical element in producing dementia in AD patients [5]. Ach is synthesized by choline acetyltransferase (ChAT) in cholinergic neurons and hydrolyzed by acetylcholinesterase (AChE) after its release. Thus, overactivity of AChE can further decrease the Ach level in the brains of patients with AD [6]. On the other hand, inhibition of AChE can increase the availability of Ach in the synaptic cleft and is currently the most common treatment strategy for the symptoms of AD [7]. Various AChE inhibitors, including donepezil, tacrine, rivastigmine, and galamantine, have been used for the treatment of AD for many years; however, the effectiveness of these agents is limited because they lose

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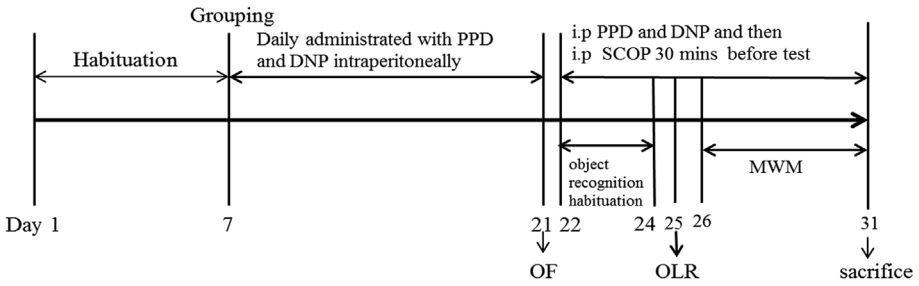

Fig. 1. The experimental procedure. After 7 days of habituation period, the mice were randomly assigned to five groups (12 animals in each group): Control group (Con); Scopolamine model group (SCOP, 0.75 mg/kg); SCOP + PPD (min group, 20 μmol/kg; max group, 40 μmol/kg) groups and SCOP + donepezil group (DNP, 1.6 mg/kg). Then, mice received pretreatment with PPD and DNP for 14 days before inducing amnesia by SCOP. After pretreatment, the open field (OF) test was firstly used to evaluate the effect of PPD on the locomotor activity without SCOP administration. Thereafter, memory impairment was induced by intraperitoneal administration of SCOP (0.75 mg/kg) once daily and object location recognition (OLR) experiment and Morris water maze (MWM) task were conducted 30 min after SCOP administration. All drugs including PPD, DNP and SCOP were administrated by intraperitoneal (i.p) injection (20 mL/kg). Mice in each group totally received PPD (20 and 40 μmol/kg), DNP (1.6 mg/kg) for 25 days and SCOP (0.75 mg/kg) for 10 days until the end of the behavioral test. Following the behavioral tests, mice were sacrificed and their hippocampus were isolated for further biochemical analysis.

effectiveness as the disease progresses [8]. Additionally, it is generally accepted that oxidative stress plays a major role in the cognitive deficits observed in AD. Oxidative stress causes metabolic activity reduced when the damage appears in the mitochondrial components, even causes neuronal death and neurodegeneration, especially in those cognitive related areas including hippocampus [9]. Therefore, there is a need for more drug candidates having neuroprotective potential and/or disease-modifying ability.

Panax ginseng C.A. Mayer, an ancient and well-known herbal drug in traditional Chinese medicine, possesses a number of beneficial effects in the central nervous system (CNS) [10,11]. Ginsenosides are responsible for the enhancing effects on learning observed following treatment with *Panax ginseng*. The ginsenosides are classified as protopanaxadiols or protopanaxatriols. Orally administered protopanaxadiol-type ginsenosides are finally metabolized to 20(*S*)-protopanaxadiol (PPD) by gut microbiota [12–15]. PPD has been reported to possess many biological properties including anti-inflammatory, antitumor [16,17] and anti-fatigue [18] properties. Recent publications found that PPD functioned as a neurotrophic agent to ameliorate the sensory deficit caused by glutamate-induced excitotoxicity through its antioxidant effects and enhances mitochondrial function and exhibited strong antidepressant-like effects *in vivo* [19,20]. However, there has been no research on the memory enhancing effect of PPD in animal experiment. Thus, this present study was carried out to investigate the effects of PPD in scopolamine-induced memory deficit in mice and the potential mechanisms involved.

2. Materials and methods

2.1. Apparatus and reagents

The open field computer-aided controlling system, object recognition test system and Morris water maze system were all developed jointly by China astronaut research and training center and Chinese Academy of Medical Sciences (CAMS) Institute of Medicinal Plant Development (IMPLAD). 20(S)-protopanaxadiol (PPD) (purify > 98% by HPLC) was purchased from Ruifensi Biological Technology Co. Ltd. (Chengdu, Sichuan, China). Scopolamine was purchased from Sigma (USA), Donepezil hydrochloride (Aricept) was purchased from Eisai (Ibaraki, Japan), AChE, ChAT, Ach, SOD and MDA commercial kits were obtained from Jiancheng Biological Technology Co. Ltd. (Nanjing, Jiangsu, China).

2.2. Animals

Sixty ICR male mice (weighing 20-22 g, Vital river, Beijing, China) were housed at (23 ± 2)°C under 12-h light and dark cycles, and allowed access to food and water *ad libitum*. Mice were allowed to acclimatize for one week before the beginning of the experiment. All experimental procedures were carried out under the approval and supervision of the Academy of Experimental Animal Center of the Institute of Medicinal Plant Development and in accordance with the NIH Guide for the Care and Use of Laboratory Animals. And all efforts were made to minimize the suffering of the animals.

2.3. Experimental design

After 7 days of acclimatization period, the animals were randomly assigned to five groups (12 animals in each group): Control group (Con); Scopolamine model group (SCOP, 0.75 mg/kg); SCOP + PPD (min group, 20 μmol/kg; max group, 40 μmol/kg) groups and SCOP + donepezil group (DNP, 1.6 mg/kg). Then, mice received pretreatment with PPD and DNP for 14 days before inducing amnesia by SCOP. After pretreatment, the open field (OF) test was firstly used to evaluate the effect of PPD on the locomotor activity without SCOP administration. Thereafter, memory impairment was induced by intraperitoneal administration of SCOP (0.75 mg/kg) once daily and object location recognition (OLR) experiment and Morris water maze (MWM) task were conducted 30 min after SCOP administration. All drugs including PPD, DNP and SCOP were administrated by intraperitoneal (i.p.) injection (20 mL/kg). Mice in each group totally received PPD (20 and 40 μmol/kg), DNP (1.6 mg/kg) for 25 days and SCOP (0.75 mg/kg) for 10 days until the end of the behavioral test. Following the behavioral tests, animals were sacrificed and their hippocampus were isolated for further biochemical analysis. The experiment procedure was illustrated in Fig. 1.

2.4. SCOP and drug treatment

After pretreatment and OF test, all animals except the control group were injected with SCOP 0.75 mg/kg (i.p.) to induce cognitive deficits in mice, 30min before each behavioral task. PPD and DNP treatments were carried out 30 min before SCOP administration in the respective groups. The drugs (PPD and DNP) were administered intraperitoneally (i.p.) at a volume corresponding to 0.2 ml/10 g body weight. SCOP, PPD and DNP were dissolved in saline (0.9%, Nacl). Based on the fact that DNP is an cholinesterase inhibitor, the scopolamine-induced amnesia model mechanism involves increase in the AChE activity in the brain leading to spatial learning deficits. Thus, DNP was used as a positive control in this study.

2.5. Behavioral studies

2.5.1. Open field (OF) test

After 14 days of pretreatment, the open field test was firstly used to assess the effects of PPD on the locomotor activity for eliminating its interference in cognitive function using an open field computer-aided controlling system. The apparatus consists of four metal tanks (30 cm × 28 cm × 35 cm) with a 120 Lux light source on the ceiling and a video camera fixed at the top of each tank. 30 mins after drug dosing, each animal was placed into the center of the tank and allowed to explore freely for 3 min. The total distances and average speeding in