Regulatory Toxicology and Pharmacology 78 (2016) 53–58

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Cereboost™, an American ginseng extract, improves cognitive function via up-regulation of choline acetyltransferase expression and neuroprotection

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ARTICLE INFO

Article history:
Received 19 January 2016
Received in revised form
1 April 2016
Accepted 20 April 2016
Available online 22 April 2016

Keywords:
Amyloid-beta peptide
Cognitive function
Cereboost™
American ginseng (*Panax quinquefolius*)
Choline acetyltransferase
Neuroprotection

ABSTRACT

In Alzheimer disease (AD), amyloid-beta (Aβ) peptides induce the degeneration of presynaptic cholinergic system, in which decreased activity of enzyme choline acetyltransferase (ChAT) responsible for acetylcholine synthesis is observed. Cereboost™, an extract of American ginseng extract, contains a high concentration of Rb1 ginsenoside which is a well-known ingredient improving human cognitive function. We investigated the effects of Cereboost™ on learning and memory function of mice challenged with an $A\beta_{1-42}$ peptide and the underlying mechanisms *in vitro*. Cereboost™ protected against $A\beta_{1-42}$-induced cytotoxicity in F3.ChAT stem cells, and enhanced the ChAT gene expression. $A\beta_{1-42}$ injection into the mouse brain impaired the cognitive function, which was recovered by oral administration of Cereboost™. In addition, Cereboost™ restored brain microtubule-associated protein 2 and synaptophysin as well as acetylcholine concentration. The results demonstrate that Cereboost™ administration recovered the cognitive function of AD model animals by enhancing acetylcholine level via ChAT gene expression and neuroprotection.

1. Introduction

Alzheimer's disease (AD) is a progressively degenerative brain disease, which is clinically characterized by cognitive impairment and behavioral disorders. Several molecules, especially amyloid-beta (Aβ) peptides, were identified as potential risk factors underlying the development of AD, as accumulation of Aβ peptides was found in postmortem brain samples from AD patients (Selkoe, 2001; Ghiso and Frangione, 2002). Previous studies suggest that Aβ accumulation in cellular compartments interferes with normal function and promotes degeneration (LaFerla et al., 2007). Also, Aβ peptides accumulated in synaptic terminals, especially in mitochondria, impairs the synaptic function (Selkoe, 2002). Such a synaptic damage might lead to impaired neurotransmission, and ultimately contribute to cognitive decline in aging persons and AD patients (Caspersen et al., 2005; Reddy and Beal, 2008).

Meanwhile, the cognitive functions are highly dependent on central cholinergic neurotransmission (Sarter and Parikh, 2005). Although other neural systems were known to be involved in learning and memory process, the cholinergic system plays a critical role in modulating cognitive performances (Hasselmo and Sarter, 2011). Indeed, acetylcholine (ACh) secreted from cholinergic nervous system governs the acquisition of information (Schliebs and Arendt, 2006). Furthermore, choline acetyltransferase (ChAT), the presynaptic enzyme responsible for the ACh biosynthesis, is presently the most specific indicator for monitoring the functional state of cholinergic neurons in the central and

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http://dx.doi.org/10.1016/j.yrtph.2016.04.006
0273-2300/

peripheral nervous systems. Notably, ChAT activity has been found to be significantly reduced in AD patients (Ikonomovic et al., 2005; Ozturk et al., 2006). It is reported that the degree of reduction of cerebral ChAT activity is significantly correlated with the severity of dementia (Sharp et al., 2009). Therefore, it is generally accepted that the cellular loss and dysfunction of cholinergic neurons results in the development of dementia in AD and other types of brain disease. In fact, direct treatment or injection of Aβ peptides not only exerted cytotoxicity and ACh depletion in neuronal cultures, but also caused neurodegeneration in animals, leading to cognitive deficits (Pedersen et al., 1996; Kayed and Lasagna-Reeves, 2013; Zussy et al., 2013). In addition, transgenic AD model mice exhibited accumulation of Aβ peptides in their brain, lading to memory impairment as seen in AD patients (Zhang et al., 2011). Accordingly, animal models challenged with Aβ peptides has been used for assessment of the anti-amnesic activity of AD therapeutics and functional materials.

Ginseng, the root of *Panax* sp., has been widely used in traditional oriental medicine for the prevention and treatment of a variety of diseases, and as general health elixirs and performance enhancer. The major bioactive ingredients in ginseng are triterpenoid saponins, known as ginsenosides (Jia and Zhao, 2009). Ginsenosides have been suggested to have a promising therapeutic potential as cognition-enhancing drugs or functional (Lee et al., 2008; Smith et al., 2014). In fact, it was reported that ginsenosides were effective in the attenuation of learning deficits due to brain damage and aging in animals and in humans (Lee et al., 2010).

The beneficial effects of ginseng root of *Panax ginseng* C.A. Meyer (Korean ginseng) on learning and memory function are often attributed to ginsenosides Rb1 and Rg1. Several studies provide evidences of their neurotrophic, neuroprotective, and anti-aging properties (Chang and Wang, 2008; Xu et al., 2009; Scholey et al., 2010). Although *Panax quinquefolius* (American ginseng) contains many saponins in common with *Panax ginseng* and other *Panax* sp., it has its own characteristic saponin profile including a high concentration of ginsenoside Rb1 (Scholey et al., 2010).

AD therapeutics available to date, such as acetylcholinesterase (AChE) inhibitors, are only a palliative treatment of memory dysfunction without potential protection against the progressive neurodegeneration (Ogura et al., 2000). The essential requirements for a promising candidate for AD would be protective or recovering potentials against Aβ-mediated synaptic damage and progressive neuronal degeneration. Although Cereboost™, an American ginseng root extract, was suggested to have a potential as a cognition-enhancing functional food in humans (Scholey et al., 2010), underlying mechanism(s) were not well defined. Therefore, we investigated whether Cereboost™ improves the cognitive dysfunction of $A\beta_{1\text{-}42}$-challenged mice by restoring neuronal function, in addition to the ACh-increasing activity.

2. Materials and methods

2.1. Materials

Panax quinquefolius was procured from an Oriental drug market (Seoul, Korea), and extracted 3 times with 30% ethanol at 40 °C for 5 h. The extract (named Cereboost™) were filtrated, vacuum-evaporated, and spray-dried.

Synthetic peptide $A\beta_{1\text{-}42}$ (American Peptide Co., Sunnyvale, CA, USA), corresponding to the neurotoxic amino acid residues of the human $A\beta_{1\text{-}42}$, was dissolved in PBS at a concentration of 1 mg/ml by incubating at 37 °C for 7 days. $A\beta_{1\text{-}42}$ aliquots were then stored at −20 °C until use.

2.2. Measurement of enzyme activity and cytotoxicity

2.2.1. AChE inhibition

Seven-week-old male ICR mouse brain was quickly removed after transcardial perfusion with cold saline under anesthesia, and homogenized in 19 vol of cold PBS (pH 7.4) to prepare a 5% homogenate. Assay for AChE activity was performed at 25 °C by a slight modification of the method of Ellman et al. (1961). In brief, assay mixture (3.0 ml PBS) containing 0.5 mM acetylthiocholine iodide and 0.5 mM 5.5′-dithiobis-(2-nitrobenzoic acid) were prepared. Cereboost™ (50 μl, final concentration 1–1000 μg/ml) and brain homogenate (50 μl) were added to the assay mixture, and the change in absorbance at 412 nm during 5 min was monitored (Park et al., 2011). The relative enzyme activity (inhibited by Cereboost™) was calculated based on the absorbance change (full activity 100%) of assay mixture containing 50 μl PBS without Cereboost™.

2.2.2. Cytotoxicity

The protective effect of Cereboost™ against $A\beta_{1\text{-}42}$ cytotoxicity was determined via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using F3.ChAT human neural stem cells (Park et al., 2011). Briefly, the cells (1×10^4 cells/ml) were seeded in each well containing 100 μl Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% D-glutamine, 100 μg/ml gentamicin, and 2.5 U/ml amphotericin B in a 96-well plate. After 24-h incubation at 37 °C, the medium was replaced with fresh medium containing $A\beta_{1\text{-}42}$ peptide (5 μM) and/or Cereboost™ (10–1000 μg/ml). After 24-h incubation and washing twice with fresh medium, 50 μl of MTT (5 mg/ml DMEM, filtered) was added and incubated for additional 4 h (Datki et al., 2003; Resende et al., 2008; Park et al., 2015). The medium was discarded, and the formazan blue formed in the living cells was dissolved by adding 150 μl dimethyl sulfoxide. The optical density was measured at 570 nm in 30 min using an ELISA reader (Molecular Devices, Sunnyvale, CA, USA). The experiments were performed 3 times, and mean values were presented.

2.2.3. ChAT gene expression

F3.ChAT cells (1×10^6 cells/ml/well) were seeded in a 6-well plate. After 24-h incubation at 37 °C, the cells were treated with $A\beta_{1\text{-}42}$ (5 μM) and/or Cereboost™ (10–1000 μg/ml). After 24-h incubation, for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of ChAT mRNA expression, total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from total RNA using a WizScript™ RT Master kit (Wizbiosolutions, Seongnam, Gyeonggi, Korea) according to the supplier's instructions. RT-PCR products were separated electrophoretically on 1.2% agarose gel containing ethidium bromide and visualized under UV light. The primers used for the RT-PCR of ChAT are followings; sense: 5′-CTCTGACCTGTCAGAAGAAT-3′, antisense: 5′-GACGCTGACACTTACAGAAT-3′

2.3. Animals and treatment

Six-week-old male ICR mice (n = 10/group) were purchased from Daehan-Biolink (Eumseong, Chungbuk, Korea) and used after acclimatization for about 1 week. Mice were housed in a room with a constant temperature (23 ± 3 °C), relative humidity (50 ± 10%), and a 12-h light/dark cycle. Mice were fed with standard rodent diet and purified water *ad libitum*. All experimental procedures were carried out in accordance with the Standard Operating Procedures of Laboratory Animal Research Center, Chungbuk National University, Korea. The protocol was approved by the Institutional Animal Care and Use Committee of Chungbuk National University.

The mice were orally administered with Cereboost™ (30, 100 or