Behavioural Brain Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Phytochemical allylguaiacol exerts a neuroprotective effect on hippocampal cells and ameliorates scopolamine-induced memory impairment in mice

Hye-Sun Lim[a], Bu-Yeo Kim[a], Yu Jin Kim[a,b], Soo-Jin Jeong[a,c,*]

[a] Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea
[b] College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea
[c] Korean Medicine of Life Science, University of Science & Technology, Daejeon 34113, Republic of Korea

ARTICLE INFO

Keywords:
Allylguaiacol
Neuroprotective effect
Scopolamine
Memory impairment
Alzheimer's diseases

ABSTRACT

Allylguaiacol is a phytochemical occurring in various plants such as cloves, cinnamon, basil, and nutmeg. Pharmacological effects of allylguaiacol include antimicrobial, anti-inflammatory, anticancer, antioxidant, and neuroprotective activity. Although allylguaiacol is considered to have neuroprotective effects, there is no report on its regulatory mechanisms at the molecular level. In the present study, we investigated the mechanisms of allylguaiacol as an antioxidant and neuroprotective agent using hydrogen peroxide (H_2O_2)-treated HT22 hippocampal cells. Allylguaiacol increased the scavenging activities of free radicals 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and enhanced the expression of antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase. In addition, allylguaiacol inhibited H_2O_2-induced damage of HT22 with increasing production of brain-derived neurotrophic factor (BDNF), phosphorylation of phosphoinositide 3-kinase (PI3K), and cyclic AMP response element-binding protein (CREB). Furthermore, antibody microarray data revealed that phospho-regulation of nuclear factor kappa B (NF-κB) p65 and death domain-associated protein (DAXX) is involved in protection against neuronal cell damage. In a mouse model of short-term memory impairment, allylguaiacol (2.5 or 5 mg/kg) significantly ameliorated scopolamine-mediated cognitive impairment in a passive avoidance task. In addition, allylguaiacol significantly increased the expression of TrkA and B in the hippocampus from scopolamine-treated mice. Taken together, our findings suggest that allylguaiacol exerts a neuroprotective effect through the antioxidant activation and protein regulation of NF-κB p65 and DAXX-related signaling. The ameliorating effect of allylguaiacol may be useful for treatment of memory impairment in Alzheimer's and its related diseases.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that is prominently age dependent [1]. According to a World Health Organization (WHO) report in 2015, the incidence of AD is gradually increasing and affects approximately 48 million people worldwide [2]. Currently, four acetylcholinesterase inhibitors (tacrine, rivastigmine, galantamine, and donepezil) and one *N*-methyl-D-aspartate (NMDA) receptor agonist (memantine) have been approved by the U.S. Food and Drug Administration (FDA) and are prescribed for patients with AD. However, they have no curative activity except for alleviating small symptomatic problems. Thus, the development of complementary and alternative treatments for AD is necessary. AD is an irreversible disorder that is characterized by progressive memory loss and cognitive impairment because of oxidative stress caused by the generation of reactive oxygen species (ROS) [3,4]. Thus, protecting neuronal death by antioxidant therapy is considered an attractive noncholinergic therapeutic approach for patients with AD. Phytochemicals are often powerful antioxidants and have potential for the treatment of various diseases such as cancers [5,6], inflammatory diseases [7–9], and metabolic diseases [10,11]. They have also been found to show possible anti-AD therapeutic activities in preclinical or clinical investigations [12,13].

Allylguaiacol, also referred to as 4-allylguaiacol or eugenol (Fig. 1), is a component of botanical essential oils from cloves and other plants, such as cinnamon, basil, and nutmeg [14]. Allylguaiacol has antioxidant [15], anti-inflammatory [16], anticancer [17,18], and antibacterial and antiviral activities [19]. Interestingly, some studies have suggested the possibility of allylguaiacol as a potent drug for the treatment of AD or its related diseases. Allylguaiacol showed a neuroprotective effect in PC12 cells incubated with amyloid β [20] in a gerbil model of global ischemia [21], and anticholinesterase activity [22].

* Corresponding author at: Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 34054, Republic of Korea.
E-mail address: sjijeong@kiom.re.kr (S.-J. Jeong).

https://doi.org/10.1016/j.bbr.2017.11.003
Received 29 September 2017; Received in revised form 31 October 2017; Accepted 2 November 2017
0166-4328/ © 2017 Elsevier B.V. All rights reserved.

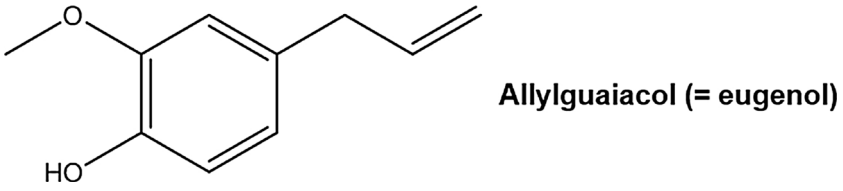

Fig. 1. Chemical structure of allylguaiacol (molar mass: 164.2 g/mol).

However, the molecular mechanisms responsible for anti-AD activity of allylguaiacol remain unclear. In the present study, we investigated the mechanisms of allylguaiacol action for neuroprotection and anti-oxidative stress using HT22 murine hippocampal cells. We further examined the effect of allylguaiacol in a scopolamine-mediated mouse model of short-term memory impairment.

2. Materials and methods

2.1. Cell culture and drug treatment

HT22 cells were maintained in Dulbecco's modified Eagle's medium (Hyclone/Thermo, Rockford, IL) supplemented with 10% fetal bovine serum (Hyclone/Thermo) and penicillin/streptomycin under an atmosphere of 5% CO_2 at 37 °C. HT22 cells were cotreated with allylguaiacol and H_2O_2 (500 μM) for 6 h. Allylguaiacol and H_2O_2 were obtained from Sigma-Aldrich, St. Louis, MO.

2.2. Cell viability test

A cell counting kit (CCK) assay was performed to assess the viability of allylguaiacol in HT22 cells. Cells were plated in 96-well microplates at a density of 5×10^3/well and treated with various concentrations (0, 12.5, 25, or 50 μg/mL) of allylguaiacol for 24 h. CCK-8 solution (Dojindo, Kumamoto, Japan) was added, and the cells were incubated for 4 h. The absorbance of the well contents at 450 nm was determined using an Epoch Microplate Spectrophotometer (BioTek Instruments, Winooski, VT). The cell viability was calculated using the following equation.

$$\text{Cell viability}(\%) = \frac{\text{Mean OD in allylguaiacol-treated cells}}{\text{Mean OD in untreated cells}} \times 100$$

2.3. Cytotoxicity test

To determine the cytotoxic effect of allylguaiacol, lactose dehydrogenase (LDH) release from H_2O_2-damaged HT22 cells was determined using a CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega, Madison, WI). Cells were lysed to induce maximal LDH release and supernatants were collected to measure the release. Cell lysates or supernatants were reacted with substrate mixture at room temperature for 30 min in the dark. After adding stop solution, absorbance at 490 nm was determined using an Epoch Microplate Spectrophotometer (BioTek Instruments). The cytotoxicity of allylguaiacol was calculated using the following formula.

$$\text{Cytotoxicity}(\%) = \frac{\text{Experimental LDH release}}{\text{Maximum LDH release}} \times 100$$

2.4. Enzyme-linked immunosorbent assay for total BDNF production

HT22 cells were cotreated with H_2O_2 and various concentrations of allylguaiacol for 6 h. Total BDNF production in the culture supernatants was estimated using a total BDNF ELISA kit from R&D Systems (Minneapolis, MN).

2.5. Western blotting analyses

Cells were lysed in a CelLytic M lysis buffer (Sigma-Aldrich) containing a protease inhibitor cocktail (GenDEPOT, Barker, TX) to prepare whole cell extracts. The protein concentration was determined using a Bradford reagent (Sigma-Aldrich). Equal amounts of cell extract (20–30 μg) were resolved by 4%–20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was incubated with 5% skim milk in Tris-buffered saline containing Tween 20 (TBST), followed by an overnight incubation at 4 °C with the appropriate primary antibodies; anti- manganese superoxide dismutase (MnSOD) (Enzo Life Sciences, Inc., Farmingdale, NY, USA), anti-catalase (Abcam, Cambridge, MA, USA), phospho anti-cyclic AMP response element-binding protein (CREB) (Cell signaling technology, Inc., Danvers, MA, USA), anti-CREB (Cell signaling), phospho anti-phosphoinositide 3-kinase (PI3K) (Cell signaling), anti-PI3K (Cell signaling), phorpho anti-nuclear factor kappa B (NF-κB) p65 (S468) (Cell signaling), anti-NF-κB p65 (Cell signaling), phorpho anti-death domain-associated protein (DAXX) (Cell signaling), anti-DAXX (Cell signaling), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, Dallas, TX, USA). The membranes were washed three times with TBST, and then incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h at room temperature. The membranes were again washed three times with TBST, and then immunoreactivity was visualized using an enhanced chemiluminescence (ECL) kit (Thermo Scientific, Rockford, IL, USA). Image capture was performed using ImageQuant LAS 4000 mini Luminescent Image Analyzer (GE Healthcare Bio-Sciences, Piscataway, NJ, USA).

2.6. 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

ABTS radical cations were produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 h. Absorbance of the reactant was later adjusted to 0.7 at 734 nm. Aliquots of allylguaiacol solution (100 μl) at various concentrations were mixed with 100 μl $ABTS^{\cdot +}$ solution. The reaction mixture was incubated for 5 min in the dark at room temperature. The absorbance of the resulting solution was measured at 734 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments). The radical scavenging capacity of the allylguaiacol-treated samples was calculated using the following equation.

$$\text{Scavenging activity}(\%) = \frac{1-\text{Absorbance of allylguaiacol-treated sample}}{\text{Absorbance of untreated sample}} \times 100$$

2.7. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

To determine DPPH radical scavenging activity of allylguaiacol, a 100 μl aliquot of allylguaiacol at various concentrations was mixed with 100 μl DPPH solution (0.15 mM in methanol). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm using an Epoch Microplate Spectrophotometer (BioTek Instruments). The radical

Download English Version:

https://daneshyari.com/en/article/8837982

Download Persian Version:

https://daneshyari.com/article/8837982

Daneshyari.com