Brain Research Bulletin 128 (2017) 22–28

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Neuroprotective effects of *N*-adamantyl-4-methylthiazol-2-amine against amyloid β-induced oxidative stress in mouse hippocampus

Jiae Kim[a], Chang Hun Cho[a], Hoh-Gyu Hahn[b], Soo-Young Choi[c], Sung-Woo Cho[a,*]

[a] Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea
[b] Chemical Kinomics Research Center, Korea Institute of Science and Technology, Seoul, 02456, Republic of Korea
[c] Department of Biomedical Science and Research Institute for Bioscience and Biotechnology, Hallym University, Chunchon, 24252, Republic of Korea

ARTICLE INFO

Article history:
Received 17 August 2016
Received in revised form 27 October 2016
Accepted 27 October 2016
Available online 2 November 2016

Keywords:
Amyloid β
Alzheimer's disease
Oxidative stress
N-Adamantyl-4-methylthiazol-2-amine

ABSTRACT

We previously reported that *N*-adamantyl-4-methylthiazol-2-amine (KHG26693) suppresses amyloid beta (Aβ)-induced neuronal oxidative damage in cortical neurons. Here we investigated the mechanism and antioxidative function of KHG26693 in the hippocampus of Aβ-treated mice. KHG26693 significantly attenuated Aβ-induced TNF-α and IL-1β enhancements. KHG26693 decreased Aβ-mediated malondialdehyde formation, protein oxidation, and reactive oxygen species by decreasing the iNOS level. KHG26693 suppressed Aβ-induced oxidative stress through a mechanism involving glutathione peroxidase, catalase, and GSH attenuation. Aβ-induced MMP-2, cPLA2, and pcPLA2 expressions were almost completely attenuated by KHG26693 treatment, suggesting that Aβ-induced oxidative stress reduction by KHG26693 is, at least partly, caused by the downregulation of MMP-2 and cPLA2 activation. Compared with Aβ treatment, KHG26693 treatment upregulated Nrf2 and HO-1 expressions, suggesting that KHG26693 protects the brain from Aβ-induced oxidative damage, likely by maintaining redox balance through Nrf2/HO-1 pathway regulation. KHG26693 significantly attenuated Aβ-induced oxidative stress in the hippocampus of Aβ-treated mice.

1. Introduction

Amyloid beta (Aβ) peptide accumulation and neurofibrillary tangle formation are major events in the pathology of Alzheimer's disease (AD) (Cerpa et al., 2008). Aβ fragment administration induces memory deficits and oxidative stress in neuronal and synaptosomal systems (Götz et al., 2001; Zampagni et al., 2012). Aβ-induced oxidative-stress damage in the brain is a key event in Aβ-induced toxicity, although its precise mechanism is unknown (Moreira et al., 2010; Turunc Bayrakdar et al., 2014; Yu et al., 2015). Aβ-induced toxicity is regulated through reactive oxygen species (ROS) generation that facilitates subsequent Aβ accumulation and accelerates AD progression (Kern and Behl 2009; Wang et al., 2012). Therefore, reducing Aβ-induced oxidative stress may be an attractive strategy for developing drugs for AD. Although our understanding of the pathogenesis of AD has remarkably increased, there are no therapies or consensus molecular targets for AD.

To identify more effective neuroprotective drugs against Aβ-induced cell death, we synthesized and characterized thiazole derivatives because of their therapeutic potential as anti-inflammatory and antioxidative agents (Gribkoff and Bozik, 2008; Anzini et al., 2010; Gallardo-Godoy et al., 2011; Geng et al., 2012; Geronikaki et al., 2013; Li et al., 2013; Burke et al., 2015; El-Achkar et al., 2015). For example, thiazol-2-amine derivatives possess desirable properties as improved lead compounds for treating brain disease, which include sufficient stability in liver microsomes, oral bioavailability, and accumulation at high concentrations in the mouse brain (Gallardo-Godoy et al., 2011; Li et al., 2013). Furthermore, several thiazole derivatives exert neuroprotective effects with ROS scavenging activity, suggesting their potential for treating brain diseases (Gribkoff and Bozik, 2008; Anzini et al., 2010). Previous studies show that 2-(4′-methylaminophenyl)benzothiazole efficiently enters the AD brain, where it binds primarily to

Abbreviations: Aβ, amyloidβ; AD, Alzheimer's disease; cPLA2, cytosolic phospholipase A2; DCF, DA 2′,7′-dichlorofluorescin diacetate; CAT, catalase; DMSO, dimethyl-sulfoxide; EDTA, ethylenediaminetetraacetic acid; GPx, glutathione peroxidase; GSH, reduced glutathione; HO-1, heme oxygenase 1; IL-1β, Interleukin-1 beta; iNOS, including nitric oxide synthase; MDA, malondialdehyde; MMP-2, matrix metalloproteinase-2; Nrf2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha.

* Corresponding author at: Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, 88 Olympic-ro 43-Gil, Songpa-gu, Seoul, 05505, Republic of Korea.
E-mail address: swcho@amc.seoul.kr (S.-W. Cho).

http://dx.doi.org/10.1016/j.brainresbull.2016.10.010
0361-9230/

Aβ amyloid deposits (Klunk et al., 2003; Leuma Yona et al., 2008). However, the detailed mechanisms of action underlying thiazoles are not completely understood, and thiazole derivatives may act through different molecular mechanisms that suppress Aβ neurotoxicity. Therefore, evaluating compounds with distinct structures is required to improve strategies for identifying lead compounds to treat brain diseases.

We recently synthesized *N*-adamantyl-4-methylthiazol-2-amine (KHG26693), a new thiazole derivative (Yang et al., 2014), and reported that KHG26693 effectively suppresses Aβ-induced neuronal oxidative damage in primary cortical neuron cultures (Cho et al., 2016). However, the neuroprotective effects of KHG26693 on Aβ-induced toxicity *in vivo* are unknown. Here we investigated the antioxidative and anti-inflammatory activities of KHG26693 in the brain to improve the efficacy of KHG26693 for treating Aβ-mediated neurotoxicity.

2. Materials and method

2.1. Materials

$A\beta_{25-35}$, phosphate-buffered saline (PBS), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO). Antibodies against iNOS, MMP-2, p-cPLA2α, cPLA2α, HO-1, Nrf2, and β-actin were purchased from Cell Signaling Technology (Beverly, MA). *N*-Adamantyl-4-methylthiazol-2-amine (KHG26693) was synthesized and purified as previously described (Yang et al., 2014). All other commercial reagents were of the highest available purity.

2.2. Animals and drug treatment

Three-month-old female C57BL/6 mice (Harlan Sprague Dawley, Indianapolis, IN) were individually housed in plastic cages in a pathogen-free facility and maintained under a standard 12/12-h light/dark cycle with food and water ad libitum. This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Asian Institute for Life Sciences, Asian Medical Center, which abides by the Institute of Laboratory Animal Resources guidelines. The treatment groups (n = 6 each) were as follows: 1) control group, 2) control group treated with KHG26693, 3) $A\beta_{25-35}$ group, and 4) $A\beta_{25-35}$ group treated with KHG26693. $A\beta_{25-35}$ was dissolved in distilled water and aggregated for 5 days at 37 °C. The aggregated $A\beta_{25-35}$ peptide (10 nmol/5 μL) was intracerebroventricularly injected into the hippocampus once daily and KHG26693 (3 mg/kg) dissolved in dimethyl sulfoxide (DMSO) was injected intraperitoneally 6 h after injecting $A\beta_{25-35}$ for 7 days. Equivalent amounts of DMSO were administered to controls and Aβ-treated groups. Mice were sacrificed, the hippocampus was dissected, and hippocampal crude extracts were prepared using Dounce pestle fragmentation and sonication in HEPES buffer containing a mixture of protease inhibitors (1 μg/mL leupeptin, 1 μM dithiothreitol, 2 mM sodium orthovanadate, and 1 μM phenylmethylsulfonylfluoride), followed by centrifugation at 21,600 × *g* for 10 min at 4 °C.

2.3. Measurement of TNF-α, IL-1β, MDA, ROS, and protein oxidation

TNF-α and IL-1β levels were measured using ELISA kits (R&D Systems; Minneapolis, MN) according to manufacturers' instructions and as previously described (Doverhag et al., 2010; Ju et al., 2015; Kim et al., 2016). The MDA level was measured as an index of lipid peroxidation as previously described (Kim et al., 2009; Uddin et al., 2015). Briefly, tissues were treated as described above and suspended in a reaction mixture containing 8.1% sodium dodecyl sulfate (SDS, 100 μL), 20% acetic acid (pH 3.5, 750 μL), 0.8% thiobarbituric acid (750 μL), and distilled water (300 μL). Samples were then boiled for 1 h at 95 °C and centrifuged at 4000 × *g* for 10 min. Absorbance of the supernatant was measured at 532 nm.

ROS levels were determined as previously described (Eom et al., 2015; Seo et al., 2015) with a slight modification using 2′,7′-dichlorofluorescin diacetate (DCF-DA), which is converted by ROS to fluorescent DCF. ROS concentrations were assessed using the oxidation-sensitive probe DCF-DA according to the manufacturer's protocol. The fluorescence intensity of the DCF product was measured using a SpectraMax GEMINI XS fluorescence spectrophotometer (Molecular Devices) at excitation and emission wavelengths of 485 nm and 538 nm, respectively. Challenge with H_2DCF-DA and measurement of fluorescence intensity was performed in the dark.

Measurement of protein carbonyls for protein oxidation was performed spectrophotometrically by the methods of Reznick and Packer (1994) using the extinction coefficient of $22{,}000 \times 10^6$ nmol/ml for aliphatic hydrazones. Results are expressed as the percentage of the control.

2.4. Measurement of superoxide dismutase (SOD), catalase, GPx, and GSH

SOD activity was measured by monitoring the inhibition of the ferricytochrome-c reduction reaction by xanthine/xanthine oxidase (Kim et al., 2009). The reaction mixture contained 10 μM cytochrome c, 50 μM xanthine, and sufficient xanthine oxidase to produce a reduction rate of cytochrome c = 0.025 absorbance units/min at 550 nm. The assay was performed at 25 °C in a cuvette containing 3 mL 50 mM potassium phosphate buffer (pH 7.8) and 0.1 mM EDTA. The catalase activity assay was performed by determining the rate of H_2O_2 decomposition at 240 nm according to a published method (Aebi, 1984). Glutathione peroxidase (GPx) activity was measured using a coupled reaction with glutathione reductase as described elsewhere (Rattanajarasroj and Unchern, 2010). Briefly, samples were added to GPx-detection working solution (10 mM NADPH, 84 mM GSH, glutathione reductase, 15 mM t-Bu-OOH) in a 96-well plate, and absorbance at 340 nm was measured for 3 min at 25 °C.

The reduced glutathione (GSH) level was measured as described elsewhere (Lombardi et al., 2002) with minor modifications. To assay GSH, 100 μL 6 mM 5′,5′-dithio-bis(2-nitrobenzoic acid), 25 μL protein-free extract, 875 μL NADPH (0.3 mM), and 10 μL GSH reductase (10 U/ml) were mixed, and the change in absorbance was measured at 412 nm.

2.5. Western blotting

Crude extracts were incubated in SDS-PAGE loading buffer and boiled for 5 min. The supernatant was collected, and the protein concentration was determined using bovine serum albumin as the standard. Proteins were separated using 12% SDS-PAGE and transferred to a nitrocellulose membrane. Immunoreactive bands of iNOS, MMP-2, p-cPLA2α, cPLA2α, HO-1, Nrf2, and β-actin in the crude extracts were detected and analyzed using an enhanced chemiluminescence kit according to the manufacturer's instructions (Amersham Bioscience, Piscataway, NJ, USA) and as previously described (Dilshara et al., 2015; Eom et al., 2015). Densitometry was performed using ImageJ software (NIH, Bethesda, MD).

2.6. Statistical analysis

Data are expressed as mean ± SD for the groups. Individual differences between the groups were analyzed using one-way analysis of variance. Student's *t* test was used to analyze differences between