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ACS84, a novel hydrogen sulfide-releasing compound, protects against amyloid β-induced cell cytotoxicity

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ABSTRACT

Hydrogen sulfide (H_2S) is a novel neurotransmitter. We studied here the effect of ACS 84, a new H_2S releasing compound, on the cytotoxicity induced by amyloid beta (Aβ) in microglia. Treatment with $A\beta_{1-40}$ (25 μmol/L) for 24 h significantly inhibited MTT reduction and increased lactate dehydrogenase release in BV-2 microglia cells. Pretreatment with ACS 84 (10 μM) for 30 min attenuated the above cytotoxicity caused by $A\beta_{1-40}$, suggesting that ACS 84 may protect microglia against $A\beta_{1-40}$-induced cell injury. ACS 84 also significantly attenuated nitric oxide release and TNF-α production in BV-2 cells treated with Aβ peptides ($A\beta_{1-40}$ or $A\beta_{1-42}$), but had no significant effect on the up-regulated protein expression of cyclooxygenase 2. These data suggest that ACS 84 may produce anti-inflammatory effect via inhibition of the release of inflammatory cytokines but not via suppression of the prostanoids production. Furthermore, pretreatment with ACS 84 also attenuated mitochondrial membrane potential loss ($\Delta\psi_m$) caused by $A\beta_{1-40}$ in both microglia and neurons. To examine the underlying signaling mechanism, we detected the phosphorylation of p38-, JNK- and ERK-MAPKs. It was found that $A\beta_{1-40}$ stimulated phosphorylation of all above three types of MAKPs. However, ACS 84 only attenuated the activation of p38 and JNK, but had no significant effect on that of ERK. Taken together, our data suggest that ACS 84 may protect Aβ-induced cell injury via anti-inflammation and preservation of mitochondrial function in a p38 and JNK dependent mechanism. Our work suggests that ACS 84 may have potential for the treatment of neurodegenerative diseases.

1. Introduction

It was a conventional view that hydrogen sulfide (H_2S) is a toxic gas. Recently, this gas was found to be produced endogenously by pyridoxal-5′-phosphate-dependent enzymes (cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE)) (Stipanuk and Beck, 1982), and pyridoxal-5′-phosphate-independent enzyme (3-mercaptopyruvate sulfurtransferase (3MST)) (Shibuya et al., 2009). Moreover, H_2S produces both physiological and pathological functions in various body systems, especially in brain (Boehning and Snyder, 2003; Hu et al., 2010a,b; Kimura, 2002; Kimura and Kimura, 2004; Qu et al., 2006; Tay et al., 2010). For these reasons, H_2S is recognized to be a new gasotransmitter alongside nitric oxide and carbon monoxide (Elrod et al., 2007; Qu et al., 2006; Szabo, 2007). We and other group recently reported that H_2S protected neurons against oxidative stress (Kimura and Kimura, 2004; Lu et al., 2008). We also demonstrated that H_2S produces anti-inflammatory effects in brain cells and Parkinson's Disease (PD) animal models (Hu et al., 2007, 2009, 2010a,b). However, whether H_2S could also produce neuroprotective effects against the pathological process of Alzheimer disease (AD) is still unknown.

Amyloid-β peptide (Aβ), normally secreted by neurons is a major constituent of senile plaques and a hallmark of AD, which can be found in low concentrations (about 0.1–1 nM) in cerebrospinal fluid (CSF) and plasma (Seubert et al., 1992). It is now well documented that fibrillar forms of Aβ serve as an inflammatory stimulus for microglial cells (Combs et al., 2000; Gasic-Milenkovic et al., 2003; Ito et al., 2006; Vukic et al., 2009; Yamada et al., 2009; Yates et al., 2000). Recent experimental evidence has indicated that several H_2S-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) (S-diclofenac and S-aspirin)

Abbreviations: H_2S, hydrogen sulfide; Aβ, amyloid beta; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; AD, Alzheimer disease; CSF, cerebrospinal fluid; NSAIDs, nonsteroidal anti-inflammatory drugs; DMEM, Dulbecco's modified Eagle's medium; COX-2, cyclooxygenase; LDH, lactate dehydrogenase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.

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were developed by combining a H_2S releasing moiety, (5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADT-OH), with the NSAIDs. These compounds are metabolized by carboxylesterases and generate H_2S slowly in the body (Li et al., 2007; Rossoni et al., 2008; Sparatore et al., 2009). It has been proved that these compounds may also produce the anti-inflammatory effects (Lee et al., 2010a,b). A novel H_2S-releasing compound ACS 84, a derivative of L-DOPA methyl ester, has been recently synthesized and reported potential for treatment of Parkinson's disease (Lee et al., 2010a,b). In the present study, we examined the effect of ACS 84 on cell toxicity induced by Aβ exposure. This is particularly interesting since dopamine D2 receptors correlation with memory dysfunction was reported in AD (Kemppainen et al., 2003). Moreover, dopamine may modulate cholinergic cortical excitability in AD patients (Martorana et al., 2009). We found in the present study that ACS 84 protects against Aβ toxicity in BV-2 cells. Our results imply that ACS 84 may also be beneficial to the treatment of AD.

2. Materials and methods

The study protocol was approved by the Institutional Animal Care and Use Committees of National University of Singapore.

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin/streptomycin were purchased from Gibco BRL. Griess reagent system (G2930) was purchased from Promega Corporation (Promega, USA). TNF-α, 'Quantikine' ELISA kits were purchased from R&D Systems (Abingdon, Oxon, UK). Antibodies against phospho-JNK (Thr183 and Tyr185), phospho- and total ERK1/2, total and phospho-p38 (Thr180 and Tyr182), anti-GADD and total JNK1/3 were purchased from Santa Cruz (Santa Cruz Biotechnology Inc., Santa Cruz, CA). Anti-cyclooxygenase (COX-2) was purchased from Cayman (Cayman Chemical, USA). Anti-β-actin was purchased from Sigma–Aldrich (St. Louis, MO, USA). ACS 84 was prepared as previously described (Lee et al., 2010a,b). $A\beta_{1-40}$ peptide, $A\beta_{1-42}$ peptide, JC-1 and all other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). $A\beta_{1-40}$ peptides were resuspended in sterile ddH_2O. Fibrillar $A\beta_{1-40}$ (Aβ) was prepared by reconstitution of the lyophilized peptides in sterile distilled water, followed by incubation for one week at 37 °C (Combs et al., 2001). $A\beta_{1-40}$ was freshly prepared before each treatment.

2.2. Cell culture and treatment

BV-2 cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 0.05 U/ml penicillin, and 0.05 mg/ml streptomycin and maintained at 37 °C with 95% humidified air and 5% CO_2. Cells were seeded into 35-mm diameter dishes and were used for experiments after reaching 80% confluence. Cells were treated with 25 μM $A\beta_{1-40}$ for the indicated times. Alternatively, cells were preincubated for 30 min with various concentrations of ACS 84 before addition of Aβ.

Neurons were prepared from the hippocampi of Sprague Dawley rats on postnatal day 1–2 as described previously (Combs et al., 2001). In brief, brain tissue was isolated and mechanically dispersed and seeded in culture dishes precoated with 0.01% poly-L-lysine (Sigma–Aldrich). Cells were cultured in neurobasal™ medium (GBICO) supplemented with 2% B-27 supplement (GBICO), 1% glucose (Sigma–Aldrich). Cells were cultured for 5–10 days to obtain morphologically mature neurons.

2.3. Cell viability

Cell viability change was evaluated by the lactate dehydrogenase (LDH) assay, which measures the release of LDH from dead cells, and the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reduction assay, which measures the conversion of the tetrazolium salt to a colored formazan product by living cells. Cells seeded in 96 wells were pretreated with various concentrations of ACS 84 for 30 min followed by incubating with $A\beta_{1-40}$ (25 μmol/L) for 24 h. Following this incubation, cells were treated with 0.5 mg/mL MTT for 4 h followed by exposure to 100% dimethylsulfoxide for 10 min. The optical density of the dissolved formazan grains within the cells was measured spectrophotometrically at 570 nm. For lactate dehydrogenase (LDH) activity assay, cells were prepared as described above. The culture supernatants were collected and detected by using an *in vitro* toxicology assay kit (TOX 7, Sigma) according to the the manufacturer's instruction. The absorbances were read at 490 nm using a plate reader (TE-CAN infinite 200, Switzerland). The absorbances of control cells were set to 100%, and the percentage of viable cells collected from each treatment was calculated relative to the control group.

2.4. Measurement of nitric oxide and TNF-α productions

Nitrite was detected in the culture supernatant using a commercial kit (Promega, Madison, WI, USA). In accordance with the manufacturer's instructions, 50 μL aliquots of cell culture medium from each dish were collected and mixed with 100 μL of Griess reagent (50 μL of 1% sulfanilamide + 50 μL of 0.1% naphthylethy-

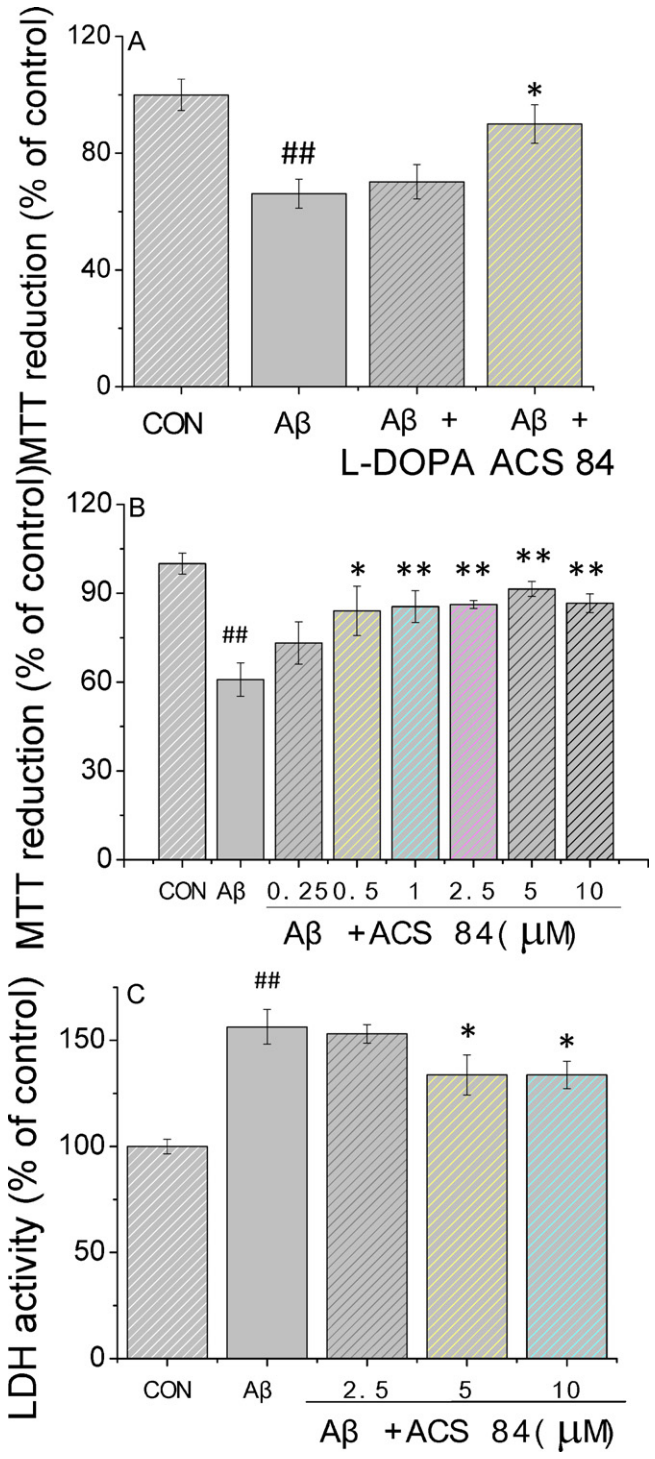

Fig. 1. Effects of ACS 84 and L-DOPA on Aβ-induced cytotoxicity in BV-2 cells. Cells were pretreated with ACS 84 or L-DOPA for 30 min and then incubated with aggregated $A\beta_{1-40}$ (25 μM) for 24 h. ACS 84, but not L-DOPA, attenuated $A\beta_{1-40}$-induced MTT reduction (A). Concentration dependent effect of ACS 84 (0.25–10 μM) on $A\beta_{1-40}$-induced MTT reduction (B) and LDH release (C). Values are expressed as the percentage of the values in the untreated control. Data represent as mean ± S.D. $n = 6$, $^{\#\#}p < 0.01$ versus control; $^{*}p < 0.05$; $^{**}p < 0.01$ versus Aβ group, respectively.