NeuroToxicology 30 (2009) 622–627

Contents lists available at ScienceDirect

NeuroToxicology

S-Adenosylhomocysteine increases β-amyloid formation in BV-2 microglial cells by increased expressions of β-amyloid precursor protein and presenilin 1 and by hypomethylation of these gene promoters

Hung-Chi Lin [a], Huei-Min Hsieh [b], Yu-Hsuan Chen [c], Miao-Lin Hu [a,*]

[a] Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan
[b] Department of Nutritional Science, Fu-Jen Catholic University, Taipei, Taiwan
[c] Department of Medical Laboratory Science and Biotechnology, Central Taiwan University of Science and Technology, Taiwan

ARTICLE INFO

Article history:
Received 12 December 2008
Accepted 30 March 2009
Available online 8 April 2009

Keywords:
S-Adenosylhomocysteine
β-Amyloid
Presenilin 1
Hypomethylation

ABSTRACT

S-Adenosylhomocysteine (SAH) has been implicated as a risk factor for neurodegenerative diseases such as Alzheimer's disease. As SAH is a potent inhibitor of all cellular methyltransferases, we herein examined the hypothesis that SAH may increase the formation of amyloid β-peptide (Aβ) in BV-2 mouse microglial cells through hypomethylation of presenilin 1 protein (PS1) and β-site amyloid precursor protein cleaving enzyme 1 (BACE1), both of which cleave Aβ precursor protein (APP) to form Aβ. The results showed that SAH increased Aβ protein formation in a concentration-dependent manner (10–500 nM), and this effect of SAH was accompanied by significantly increased expression of APP and PS1 proteins, although SAH only significantly increased the expression of BACE1 at the highest concentration used (500 nM). SAH (500 nM) markedly induced hypomethylation of APP and PS1 gene promoters. Incubation of cells with 5′-azc (20 μM), also an inhibitor of DNA methyltransferases enhanced Aβ protein expression and APP and PS1 gene promoters hypomethylation. By contrast, pre-incubation of cells with betaine (1.0 mM), 30 min followed by incubation with SAH (500 nM) or 5′-azc (20 μM) for 24 h markedly prevented the expression of Aβ protein (by 50%, $P < 0.05$) and the gene promoter hypomethylation of APP and PS1. Taken together, this study demonstrates that SAH increases the production of Aβ in BV-2 cells possibly by increased expression of APP and induction of hypomethylation of APP and PS1 gene promoters.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Many lines of evidence sustain the amyloid theory as the cause of Alzheimer's disease (AD) (Selkoe, 2000; De Strooper and Konig, 1999). Amyloid β-peptide (Aβ) principally constitutes the extracellular deposit in senile or diffusive plaques and cerebral vasculatures in AD brain (Selkoe, 2000; De Strooper and Konig, 1999). Aβ is formed from β-amyloid precursor protein (APP) as catalyzed by aspartyl protease β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and presenilins 1 (PS1) and presenilins 2 (PS2) (Sinha et al., 1999; Liao et al., 2004; Saura et al., 2004; Evin et al., 2003). Recent studies demonstrate that BACE1 levels and activities are increased in postmortem AD brains, suggesting a role in AD (Harada et al., 2006; Li and Sudhof, 2004). PS1 is an essential part of the γ-secretase protein complex that induces proteolysis of β-amyloid precursor protein. Mutations of PS1 are responsible for the early onset of AD due to increased production of β-amyloid plaques (Feng et al., 2001; McGuire and Davis, 2001). Overexpression of PS1 leads to increased production of Aβ while increased methylation of the PS1 promoter silences the gene and decreases PS1 expression leading to decreased Aβ formation (Scarpa et al., 2003). The β-amyloid precursor protein (APP) genes are frequently methylated and their demethylation with age may have some significance in the development of Aβ deposition in the age brain (Tohgi et al., 1999; Izumi et al., 1992).

Homocysteine (Hcy) has been shown to induce neuronal apoptosis and to increase DNA damage, PARP activation and p53 induction (Kruman et al., 2000). Like Hcy, S-adenosylhomocysteine (SAH) which is a potent inhibitor of methyltransferases (Chiang et al., 1996; Zappia et al., 1969; Akamatsu and Law, 1970) has been implicated as a risk factor for neurodegenerative diseases. For instance, it has been shown that SAH is elevated in the plasma and brain tissues of patients with AD (Ennedy et al., 2004) and that the elevated brain SAH is sufficient to inhibit methyltransferase

* Corresponding author. Tel.: +886 4 22812363; fax: +886 4 22812363.
E-mail address: mlhuhu@dragon.nchu.edu.tw (M.-L. Hu).

0161-813X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2009.03.011

activity and is inversely correlated with cognition of the patients (Vollset et al., 2001) suggesting that elevated SAH may contribute to the disease.

In this study, we hypothesized that SAH may enhance cellular Aβ formation through the up-regulation of APP-PS1-BACE system. To examine this hypothesis, we employed BV-2 mouse microglial cells which have been shown to be able to produce Aβ (Lewandowska et al., 1999). It is interesting to note that some communication exists between microglial cells and neurons since the former are able to produce β-endorphin whose release is regulated by a classical neurotransmitter, such as noradrenalin (Sacerdote et al., 1993).

2. Materials and methods

2.1. Reagents

All chemicals used are of reagent grade. β-Amyloid (Aβ) and *S*-adenosylhomocysteine (SAH) were from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum, trypsin, penicillin, streptomycin, sodium pyruvate, and non-essential amino acids were from GIBCO/BRL (Rockville, MD USA). Antibodies for PS1, APP, Aβ and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture

BV-2 cells (immortalized mouse microglia) were grown in DMEM medium containing 10% (v/v) FBS, 0.37% (w/v) $NaHCO_3$, penicillin (100 unit/ml), streptomycin (100 (g/ml), 0.1 mM NEAA, and 1 mM sodium pyruvate in a 5% CO_2 incubator at 37 °C. The cells were harvested at ca. 90% confluence (10^6 cells/dish), and the survival rates were always higher than 95% by Trypan-blue assay. Cells were then incubated with SAH at 37 °C for 24 and 48 h. Aβ was dissolved in PBS and SAH was dissolved in dimethylsulphoxide (DMSO); the final concentration of DMSO was 0.2%, which did not affect cell viability.

2.3. Immunoblotting

AβAPP, BACE1 and PS1 proteins were assayed by Western blotting as described previously (Fuso et al., 2005). A portion (40 μg) of each protein extract was run on 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, then blotted onto nitrocellulose (BIO-RED). Nitrocellulose membrane were incubated with AβAPP, BACE1 and PS1 monoclonal antibodies followed by incubation with horseradish peroxidase-conjugated antimouse IgG (Santa Cruz, CA) and then visualized using the ECL chemiluminescent detection kit (Amersham Co, Bucks, UK). Total protein contents were determined using a BCA protein kit (Bio-Rad, Hercules, CA).

2.4. RT-PCR (RNA isolation and sequencing)

Total cellular RNA was isolated from cell culture (RNAzol-kit) and reverse-transcribed into cDNA (MMLV-Reverse Transcriptase, Gibco/BRL, Bethesda, MD, USA) by using oligo $(dT)_{15}$ as primers and then co-amplified with four primers bases on PS1 and β-actin (internal control) sequences. The primers for amplifying PS1 cDNA (L42110) were 5′-ACGACCCCAGGGTAACTCCCG-3′, located in the 5′-untranslated region, and 5′-CTCTCTGGCCCACAGTCTCGG-3′, located in the 3′-untranslated region. The primers for amplifying β-actin cDNA (M10277) were 5′-AAGAGAGGCATCCTCACCCT-3′ and 5′-TACATGGCTGGGGTGTTGAA-3′. PCR amplification was performed with a thermal cycler, as follows: denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 90 s (32 cycle), followed by a final incubation at 72 °C for 7 min. The sizes of the amplification products of PS1 and β-actin were 227 base pairs and 218 base pairs, respectively. The PCR products were subjected to 1% agarose gel electrophoresis and stained with ethidium bromide. The relative PS1 levels were quantitated by Matrox Inspector 2.1 software.

2.5. Bisulfite genomic modification and sequencing analysis of PS1, APP and methylation-specific PCR (MSP)

For bisulfite modification, 3 μg genomic DNA was extracted using the QIAamp Mini Kit (Qiagen, Hilden, Germany) and denatured in 1N NaOH followed by conversion in 0.5 mM hydroquinone and 3 M sodium bisulfite under an O_2 exclusive layer of mineral oil for 16 h at 50 °C. DNA was then purified with the Qiaex Kit (Qiagen), ethanol-precipitated and resuspended in ddH_2O. For amplification of genomic fragments after bisulfite modification independent of methylation status, primers were designed which anneal specifically to converted (C-to-T) sequences at CpG-free binding sites. PCR for PS1 (accession BD080745) was performed using the following primers and conditions—PS1 gene promoter, sense: 5′-TATTACTTACCTTG-GAGTCATT-3′ (position 11–32) and antisense: 5′-GGAAGACTGA-CACCAGGGAA-3′ (position 511–540); 94 °C for 10 min, 94 °C for 30 s, annealing at 64 °C for 60 s, elongation at 72 °C for 60 s and a final cycle at 72 °C for 7 min.

We used bisulfite-modified genomic DNA as template and designed the methylated primers as: PS1 methylated reaction, 5′-AGGGGACGCAGCGAAACCGGGGCCC-3′ (sense) and PS1 methylated reaction, 5′-ACCACAACCCCAAAACACCAACACCCA-3′ (antisense) were used for amplification of CpG-methylated sequences of PS1. PCR conditions were 94 °C for 5 min, 94 °C for 45 s, annealing for 45 s at 48 °C, elongation at 72 °C for 45 s and a final cycle at 72 °C for 7 min. For the unmethylated PCR, primers PS1 unmethylated reaction, 5′-AGGGGATGTAGTGAAATTGGGGTTT-3′ (sense) and PS1 unmethylated reaction, 5′-ATCACAACCCCAAA-CATCAACATCCA-3′ (antisense) were used under the following conditions: 94 °C for 5 min, 94 °C for 45 s, annealing for 45 s at 55 °C, elongation at 72 °C for 1 min and a final cycle at 72 °C for 7 min. APP gene was amplified using PCR and analyzed as described previously (Tohgi et al., 1999). PCR products for both amplifications were separated in an 8% nondenaturing polyacrylamide gel and visualized by silver staining and quantified using Matrox Inspector 2.1 software.

2.6. Statistical analyses

Data are expressed as means ± SD and analyzed statistically using one-way ANOVA followed by Duncan's Multiple Range test for comparison of group means. A *P*-value of <0.05 is considered statistically significant.

3. Results

3.1. Aβ protein expression and the effect of betaine

The expression of Aβ protein in BV-2 cells was significantly increased by SAH in a concentration-dependent manner (0–500 nM) and in a time-dependent manner (12, 24 and 48 h) (Fig. 1). SAH at the highest concentration (500 nM) significantly increased Aβ formation by 2.3-, 3.2- and 3.7-fold at 12, 24 and 48 h, respectively, as compared with the control of 12 h incubation. The formation of Aβ protein in BV-2 cells was also significantly increased (2.6-fold, $P < 0.05$) by incubation (20 μM, 24 h) with 5′-azc, an inhibitor of methyltransferases (Fig. 2). However, the increase in Aβ protein formation induced by SAH (500 nM) or 5′-

Download English Version:

https://daneshyari.com/en/article/2590091

Download Persian Version:

https://daneshyari.com/article/2590091

Daneshyari.com