Neuroscience Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Research

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

Rebamipide reduces amyloid-β 1–42 (Aβ42) production and ameliorates Aβ43-lowered cell viability in cultured SH-SY5Y human neuroblastoma cells

Kenta Fukui [a], Kazuma Yachi [a], Hidemi Yoshida [a,*], Kunikazu Tanji [b], Tomoh Matsumiya [a], Ryo Hayakari [a], Kazushi Tsuruga [c], Hiroshi Tanaka [c,d], Tadaatsu Imaizumi [a]

[a] Department of Vascular Biology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan
[b] Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan
[c] Department of Pediatrics, Hirosaki University School of Medicine and Hospital, Hirosaki 036-8563, Japan
[d] Department of School Health Science, Faculty of Education, Hirosaki University, Hirosaki 036-8560, Japan

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 24 March 2017
Accepted 24 May 2017
Available online xxx

Keywords:
Rebamipide
Aβ
TACE
NEP
MMP-14
SIRT1
SH-SY5Y cells
Alzheimer's disease

ABSTRACT

Amyloid-beta (Aβ) peptides, Aβ 1–42 (Aβ42) and Aβ43, in particular, have been implicated in the pathophysiology of neurodegenerative disease such as Alzheimer's disease (AD). Rebamipide (REB), a gastrointestinal protective drug, can cross the blood-brain barrier after oral administration; however, the effects of REB on neuronal cells have not yet been reported. In this study, we investigated the effects of REB on Aβ43-induced cytotoxicity (monomers, 10 μM) in cultured SH-SY5Y human neuroblastoma cells. Addition of REB (10–1000 nM) into the media partially ameliorated the reduced cell viability observed after Aβ43 treatment, which was determined by the MTT assay. REB reduced the levels of intracellular Aβ oligomers (100–150 kDa) that were formed from the exogenous addition of Aβ43 monomers. In addition, REB (30 nM) reduced endogenous Aβ42 secretion, which was analyzed by the enzyme-linked immunosorbent assay. Furthermore, REB enhanced the expression of tumor necrosis factor-α-converting enzyme/a disintegrin and metalloproteinase-17, neprilysin, matrix-metalloproteinase-14 (MMP-14)/membrane type-1 MMP, cyclooxygenase-2, and sirtuin 1, even in cells challenged with Aβ43. These results suggest that REB improves the cell viability by inducing genes that regulate Aβ levels and also genes that are cytoprotective. The secondary use of REB may have potential in the prevention of Aβ-mediated diseases, particularly AD.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia (Kumar et al., 2015), affecting about 1 in 9 people aged above 65 years or 1 in 3 people aged above 85 years (Alzheimer's Association, 2016). Recent evidence has demonstrated that amyloid-beta (Aβ) oligomers generate symptoms of AD pathology, even in the absence of senile plaques, as seen in transgenic mice that lack the ability to generate Aβ fibrils (Tomiyama et al., 2010). Furthermore, Ohnishi et al. (2015) reported that Aβ assemblies, termed amylospheroids (ASPD), cause presynaptic calcium overload by interacting with sodium pumps (specifically, the neuron-specific Na^+/K^+-ATPase α3 subunit), which can contribute to neurodegeneration. These findings have clearly identified the smaller Aβ aggregates as primary AD-causing toxins and strongly support the Aβ oligomer hypothesis as a molecular basis for AD (Bao et al., 2012; Ferreira and Klein, 2011; Klein, 2013).

Aβ 1–40 (Aβ40) and Aβ 1–42 (Aβ42) are two major forms of Aβ peptides produced from the amyloid precursor protein (APP)

Abbreviations: Aβ, amyloid-beta; AD, Alzheimer's disease; ADAM, a disintegrin and metalloproteinase; APP, amyloid precursor protein; ASPD, amylospheroids; BCA, bicinchoninic acid; BACE1, β-site APP-cleaving enzyme-1; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CA, carnosic acid; COX, cyclooxygenase; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; ECE1, endothelin-converting enzyme 1; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; HO-1, hemoxygenase-1; HRP, horseradish peroxidase; HSP, heat-shock protein; IDE, insulin-degrading enzyme; iNOS, inducible nitric oxide synthase; MMP, matrix-metalloproteinase; MT1-MMP, membrane type-1 matrix-metalloproteinase; NEP, neprilysin; NGF, nerve growth factor; NO, nitric oxide; NT-3, neurotrophic factor-3; PARP, poly-(ADP-ribose) polymerase; PBS, phosphate-buffered saline; PGE2, prostaglandin E2; PGEP4, prostaglandin EP4; PS1, presenilin-1; PVDF, polyvinylidene fluoride; REB, rebamipide; RES, resveratrol; ROS, reactive oxygen species; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SIRT1, sirtuin 1; SDS, sodium dodecyl sulphate; TACE, tumor necrosis factor-α-converting enzyme; TBST, tris-bufferd saline with Tween 20; VEGF, vascular endothelial growth factor.

* Corresponding author.
E-mail addresses: hidemi_yoshida@gakushikai.jp, hidemiyo@hirosaki-u.ac.jp (H. Yoshida).

http://dx.doi.org/10.1016/j.neures.2017.05.005
0168-0102/

(Selkoe, 2001). Compared to Aβ40, Aβ42 exhibits higher neurotoxicity as a result of its higher hydrophobicity, thus, leading to faster oligomerization and aggregation (Blennow et al., 2006). Interestingly, Aβ43 is also frequently detected in AD brains (Sandebring et al., 2013; Welander et al., 2009), and it is more neurotoxic than Aβ42 and can promote Aβ42 polymerization (Saito et al., 2011; Meng et al., 2015). These findings highlight both Aβ42 and Aβ43 as important therapeutic targets.

Functions of rebamipide (REB), a gastrointestinal protective drug (Mucosta™), include stimulation of prostaglandin and mucus glycoprotein synthesis, inhibition of reactive oxygen species (ROS) generation, reduction of inflammatory cytokines and chemokine secretion, and attenuation of neutrophil activation mainly in the gastric mucosa (Haruma and Ito, 2003; Arakawa et al., 2005). In the gastric mucosa, REB stimulates the cyclooxygenase-2 (COX-2)-prostaglandin E2 (PGE2) pathway (Kleine et al., 1993; Sun et al., 2000) and induces heat-shock protein 70 (HSP70) expression (Hahm et al., 1997), both of which have been demonstrated to protect against gastric damages caused by ethanol/hydrochloric acid and reactive oxygen metabolites, respectively.

The radioactivity of ^{14}C-labeled REB in the brain after a single oral dose in rats is 41% and 71% of plasma and blood levels, respectively (Shioya et al., 1989). In humans, the maximum concentration of REB in plasma after fasting oral administration of 100 mg is 216 μg/L (582 nM) (Maiguma et al., 2013). Therefore, 240 nM (41% of 582 nM) is estimated to be the maximum concentration of REB in the human brain; however, the effects of REB on neuronal cells have not yet been elucidated. In this study, we therefore examined the effects of REB on Aβ production and Aβ-induced neurotoxicity using cultured human neuroblastoma cells as a model of human neurons.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle Medium (DMEM)/Nurient F-12 Ham (F12) (1:1) containing GlutaMAX™-I, Advanced DMEM/F12, fetal bovine serum (FBS), Zymax™ horseradish peroxidase (HRP)-conjugated anti-goat IgG, and Lipofectamine™ RNAiMAX were purchased from Invitrogen™/Thermo Fisher Scientific Inc. (Frederick, MD, USA). Bovine serum albumin (BSA) and 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonate (CHAPS) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). REB and carnosic acid (CA) were willingly donated by Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) and Nagase Co., Ltd. (Osaka, Japan), respectively. Resveratrol (RES) was purchased from Calbiochem/Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) and the enzyme-linked immunosorbent assay (ELISA) kit for Aβ42 (Human/Rat βAmyloid (1–42) ELISA Kit Wako, High-Sensitive) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Human Aβ42 and Aβ43 monomers were from Peptide Institute, Inc. (Ibaraki, Japan). The CellQuanti-MTT™ assay kit was from BioAssay Systems (Hayward, CA, USA). The NucleoSpin® RNA II total RNA isolation kit was from Macherey-Nagel GmbH & Co. KG (Düren, Germany). An iScript™ Advanced cDNA synthesis kit and SsoAdvanced™ Universal SYBR® Green Supermix solution for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Oligonucleotide primers for qPCR were custom synthesized by Fasmac/Greiner Japan (Atsugi, Japan). The bicinchoninic acid (BCA™) protein assay kit was from Pierce®/Thermo Scientific Inc. (Waltham, MA, USA). Polyvinylidene fluoride (PVDF) membranes and the Luminata™ Crescendo Western HRP substrate were from Millipore Corporation (Billerica, MA, USA). Antibodies against tumor necrosis factor-α-converting enzyme (TACE)/a disintegrin and metalloprotease-17 (ADAM17) (raised against Val200, rabbit, #3976) and matrix-metalloproteinase-14 (MMP-14)/membrane type-1 MMP (MT1-MMP) (D1E4, rabbit monoclonal, #13130) were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Anti-neprilysin (NEP) antibody (N2C1, internal, rabbit, #GTX111680) was from GeneTex Inc. (Irvine, CA, USA). Antibodies to sirtuin 1 (SIRT1) (E104, C-terminal, rabbit, #ab32441) and β-actin (ACTN (C4), mouse monoclonal, #ab3280) were from Abcam plc (Cambridge, UK). An anti-Aβ 1–16 monoclonal antibody (6E10, mouse ascites, #SIG-39300) was from BioLegend, Inc. (San Diego, CA, USA), formerly known as Covance. An anti-COX-2 antibody (H-3, mouse monoclonal, #sc-37686) and anti-rabbit and anti-mouse IgG-HRP were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Silencer® Select Validated siRNA for target gene TACE (alias ADAM17, #s13718), NEP (alias MME, #s8845), MMP-14 (#s8877), COX-2 (alias PTGS2, #s11473), or SIRT1 (#s23769) was from Ambion/Life Technologies/Applied Biosystems (Carlsbad, CA, USA). Nonsilencing negative control siRNA (1027281) was from Qiagen (Hilden, Germany).

2.2. Cell culture

SH-SY5Y human neuroblastoma cells (ECACC No. 94030304) were obtained from the European Collection of Authenticated Cell Cultures (Porton Down, UK), and cultured in DMEM/F12 containing GlutaMAX™-I supplemented with 10% FBS (Meng et al., 2015). Once the cells were 80% confluent, they were used for experimental purpose; immediately, the medium was replaced with fresh Advanced DMEM/F12 containing GlutaMAX™-I supplemented with 3% FBS. In experiments with REB, cells were pretreated with REB for 1 h prior to the addition of Aβ peptides. Aβ peptides, CA, and RES were dissolved in DMSO; the maximum final concentration of DMSO in culture medium was 1%, and control cells were treated with vehicle alone.

2.3. Cell viability

Cell viability was measured by using a CellQuanti-MTT™ assay kit according to the manufacturer's protocol with slight modifications. The assay is based on the conversion of MTT (a pale yellow substrate) to a formazan (a purple compound) by metabolically active cells. The cellular reduction reaction requires the pyridine nucleotide cofactors, NADH/NADPH, and is only catalyzed by living cells. SH-SY5Y cells were cultured in 96-well culture plates at 4×10^4 cells per well in 100 μL of medium. The cells were pretreated with REB for 1 h and then treated with Aβ peptides for 24 h. The MTT reagent (15 μL) was then added per well, and the cells were incubated for an additional 4 h. The formazan crystals were solubilized by adding 100 μL/well of solubilizer (10% sodium dodecyl sulphate (SDS), 3.2 mM HCl) and the plates were placed overnight at 37 °C. The absorbance (A570) of the resulting colored solution was measured with a microplate reader (iMark, Bio-Rad). Each experiment was performed in triplicate.

2.4. ELISA

Cell culture supernatants were mixed with 0.2% BSA and 0.075% CHAPS to minimize the loss of Aβ42 by adhesion to storage tubes. Aliquots of the samples were stored at −80 °C until further use. The level of Aβ42 in the medium was determined using an ELISA kit, according to the manufacturer's instructions.

Please cite this article in press as: Fukui, K., et al., Rebamipide reduces amyloid-β 1–42 (Aβ42) production and ameliorates Aβ43-lowered cell viability in cultured SH-SY5Y human neuroblastoma cells. Neurosci. Res. (2017), http://dx.doi.org/10.1016/j.neures.2017.05.005