Mitochondrion 17 (2014) 106–115

Contents lists available at ScienceDirect

Mitochondrion

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

Inhibition of stress induced premature senescence in presenilin-1 mutated cells with water soluble Coenzyme Q_{10}

Dennis Ma [a], Kyle Stokes [a], Kevinjeet Mahngar [a], Danijela Domazet-Damjanov [a], Marianna Sikorska [b], Siyaram Pandey [a,*]

[a] Department of Chemistry and Biochemistry, University of Windsor, 401 Sunset Avenue, Windsor, ON N9B 3P4, Canada
[b] Institute For Biological Sciences, NRC, Ottawa, ON, Canada

ARTICLE INFO

Article history:
Received 20 May 2014
received in revised form 28 June 2014
accepted 9 July 2014
Available online 15 July 2014

Keywords:
Coenzyme Q_{10}
Alzheimer's disease
Senescence
Autophagy
Mitochondria
Reactive oxygen species

ABSTRACT

A water-soluble formulation of CoQ_{10} (WS-CoQ_{10}) was shown to stabilize mitochondria and prevent oxidative stress-induced neuronal death. Presenilin-1 (PS-1)-mutated Alzheimer's Disease (AD) fibroblasts (PSAF) were used for studying the effects of PS-1 mutation. PS-1 mutation correlated to increased reactive oxygen species (ROS) production and stress induced premature senescence (SIPS) in PSAF; WS-CoQ_{10} treatment decreased ROS generation, increased population doublings, and postponed SIPS. Treated PSAF had higher PCNA expression, and lower levels of MnSOD, p21, p16Ink4A, and Rb. WS-CoQ_{10} caused the resumption of autophagy in PSAF. Thus, WS-CoQ_{10} as inhibitor of SIPS and ameliorator of autophagy could be an effective prophylactic/therapeutic agent for AD.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is distinguished by the presence of neurofibrillary tangles and amyloid plaques in the brain, and associated with synaptic loss and death of neurons in the hippocampus and cerebral cortex (Alzheimer, 1907). It is proposed that these brain lesions may appear before the onset of symptoms associated with AD, implying that neuronal injury precedes disease diagnosis (Savva et al., 2009). In fact, the earliest event in AD, in vulnerable neurons, includes increased production of reactive oxygen species (ROS) (Bonda et al., 2010; Zhu et al., 2013).

Increased ROS production in AD has been attributed to perturbed redox interactions of iron and copper, dysfunction of proteolysis, and dysfunction of mitochondria (Brunk and Terman, 2002; Casadesus et al., 2004; Castellani et al., 1999; Mezzetti et al., 1998; Nixon et al., 2005; Smith et al., 1997; Zhu et al., 2007). This increased mitochondrial dysfunction yields inefficient flow of electrons through the electron transport chain leading to elevated ROS production. For cells with low antioxidant capabilities and long life spans, such as neurons, this oxidative stress may have adverse consequences (Moreira et al., 2010). This oxidative stress can exacerbate the dysfunction of mitochondria creating a vicious cycle of mitochondrial oxidative injury and ROS production yielding damage to mitochondrial DNA, proteins and lipids. This dysfunction has been reported in cells of peripheral tissues of AD patients, including skin fibroblasts, which make them a practical in vitro model of AD (Cecchi et al., 2002). Prolonged exposure of sublethal doses of oxidative stress, can result in stress-induced premature senescence (SIPS), yielding changes in cellular morphology, cell cycle arrest, and increase in senescence-associated beta-galactosidase (SA-β-gal) activity (Toussaint et al., 2000). As this phenomenon has been well characterized in fibroblasts models, and because fibroblasts from AD patients produce increased endogenous ROS produced from defective mitochondria, these fibroblasts are an ideal model for studying SIPS collectively with mitochondrial dysfunction (Naderi et al., 2006a; Toussaint et al., 2000).

Alzheimer's disease can be sporadic or familial. In the cases where this disease is genetically linked, it is often associated but not limited to mutations in the amyloid precursor protein (APP), presenilin-1 (PS-1),

Abbreviations: Aβ, amyloid-beta peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; CoQ_{10}, Coenzyme Q_{10}; DCF, 2′,7′-dichlorofluorescein; DIC, differential interference contrast; H_2DCFDA, 2′-7′-dichlorofluorescin diacetate; HBSS, Hank's Balanced Salt Solution; LC3, microtubule-associated protein 1 light chain 3; MDC, monodansylcadaverine; MMP, mitochondrial membrane potential; MnSOD, manganese superoxide dismutase; NHF, Apparently healthy non-fetal human skin fibroblasts; PBS, phosphate buffered saline; PCNA, proliferating cell nuclear antigen; PS-1, presenilin-1; PS-2, presenilin-2; PSAF, PS-1 mutated AD familial type 3 fibroblasts; PTS, polyoxyethanyl α-tocopheryl sebacate; Rb, retinoblastoma; RFU, relative fluorescence units; ROS, reactive oxygen species; SA-β-gal, senescence-associated beta-galactosidase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SIPS, stress-induced premature senescence; TBST, Tris-Buffered Saline Tween-20; TMRM, tetramethylrhodamine methyl ester; v-ATPase, vacuolar [H+] ATPase; WS-CoQ_{10}, water soluble Coenzyme Q_{10}.

* Corresponding author. Tel.: +1 519 253 3000x3701; fax: +1 519 973 7098.
E-mail address: spandey@uwindsor.ca (S. Pandey).

http://dx.doi.org/10.1016/j.mito.2014.07.004

and presenilin-2 (PS-2). The transmembrane protein PS-1 in particular, responsible for the development of AD familial type 3 when its gene is mutated, is a component of the γ-secretase complex responsible for APP processing to the amyloid-beta peptide (Aβ) (Citron et al., 1997; De Strooper et al., 1998). Various mutations in PS-1 have yielded aberrant mitochondrial activity and increased mitochondrial ROS production (Schuessel et al., 2006; Strazielle et al., 2009). More recently, PS-1 was shown to be vital for lysosomal proteolysis and autophagy (Lee et al., 2010), a cellular stress response and degradative mechanism mediated by lysosomes (de Duve, 1963; de Duve and Wattiaux, 1966; Lee et al., 2012). Currently, there are no effective therapies for AD. However, as this autophagic deficiency, a direct consequence of PS-1 malfunction, represents a putative scaffold for AD pathogenesis (Lee et al., 2010), it presents novel therapeutic opportunities.

Coenzyme Q_{10} (CoQ_{10}) is a potent antioxidant in mitochondrial membranes (Forsmark-Andrée et al., 1997; Noack et al., 1994) and an electron transport chain intermediate (Beyer, 1992; Do et al., 1996; Ernster and Dallner, 1995). More specifically, CoQ_{10} facilitates efficient transport of electrons and defends mitochondria against oxidative injury. However, the levels of CoQ_{10} have been reported to decline with age, making mitochondria increasingly vulnerable and hazardous (Ernster and Dallner, 1995); this malfunction may contribute to the development of neurodegenerative diseases. As such, considerable interest has been placed on the therapeutic use of CoQ_{10} in neurodegenerative diseases (Matthews et al., 1998). Nevertheless, the preclinical and clinical evaluation of CoQ_{10} has been hindered by its hydrophobicity, severely limiting its use in vitro and in vivo. In efforts to overcome this bottleneck, water soluble formulations of CoQ_{10} have been devised (Bergamini et al., 2012). One formulation, water soluble Coenzyme Q_{10} (WS-CoQ_{10}), made as described in the US Patent No. 6,045,826 (Borowy-Borowski et al., 2000), provided robust antioxidant and mitochondrial stabilizing effects in mammalian cells as well as neuroprotection in various neurodegenerative models (McCarthy et al., 2004; Naderi et al., 2006b; Sandhu et al., 2003; Somayajulu et al., 2005; Somayajulu-Nițu et al., 2009). In light of these findings, we evaluated the effects of WS-CoQ_{10} in a cellular model of AD.

In search of an in vitro model of AD, we cultured PS-1 mutated AD familial type 3 fibroblasts (PSAF) and apparently healthy human skin fibroblasts (NHF) from age matched healthy donors in parallel and monitored for any signs of cellular morphological and changes in the diseased fibroblasts. Indeed, after culturing these cells in parallel, PSAF had higher levels of endogenous ROS, a residual growth rate, and expressed various proteins involved in cell cycle arrest, demonstrating characteristic features of SIPS. Thus, we used PSAF as a cellular model to study the deficiency due to the PS-1 mutation in AD fibroblasts and to study the potential therapeutic modulator WS-CoQ_{10} in ameliorating the effects of the PS-1 mutation. After culturing PSAF with 50 μg/mL WS-CoQ_{10} in parallel to PSAF without supplementation, these supplemented fibroblasts demonstrated minimal features of SIPS, had lower levels of ROS at both the whole cell and mitochondrial level and had a modest increase autophagic activity. Provided that WS-CoQ_{10} is effective in amending or partially amending primary putative abnormalities involved in AD etiology, including mitochondrial dysfunction, increased ROS generation, and defective autophagic execution, it presents a potential novel prophylactic and therapeutic agent for AD.

2. Materials and methods

2.1. Water-soluble CoQ_{10} (WS-CoQ_{10})

A water-soluble formulation of CoQ_{10} was prepared by directly combining CoQ_{10} (Kyowa Hakko, New York, NY, USA) and polyoxyethanyl α-tocopheryl sebacate (PTS) in a molar ratio of 1:2 mol/mol (1:3 w/w) and heating to a temperature higher than their respective melting points to form a clear melt. This water-soluble melt can be diluted with aqueous solutions to a desired concentration. PTS was synthesized by conjugating polyethylene glycol 600 to α-tocopherol via bi-functional sebacic acid (Sigma-Aldrich, St. Louis, MO, USA) as previously described (Borowy-Borowski et al., 2004).

2.2. Cell culture

Apparently healthy non-fetal human skin fibroblasts (NHF) and PS-1 mutated AD familial type 3 fibroblasts (PSAF) from a healthy female and a female AD patient respectively (Coriell Institute for Medical Research, Cat. No. AG09309 & AG04159, Camden, NJ, USA) were used extensively in this study. Experiments were repeated with additional PS-1 mutated fibroblasts from 2 male AD patients (Coriell Institute for Medical Research, Cat. No. AG09035 & AG07671, Camden, NJ, USA). All fibroblasts in this study were cultured in Eagle's Minimum Essential Medium with Earle's Salts and Non-Essential Amino Acids supplemented with 15% (v/v) fetal bovine serum (Thermo Scientific, Waltham, MA, USA) and 10 mg/mL gentamicin (Gibco BRL, VWR, Mississauga, ON, Canada). PSAF were grown in medium with or without supplementation with 50 μg/mL WS-CoQ_{10} or with the PTS carrier molecule both provided by Zymes LLC, Hasbrouck Heights, NJ, USA. Both of these untransformed fibroblasts were grown at 37 °C and 5% CO_2.

2.3. Measurement of total cell reactive oxygen species (ROS)

The production of ROS was measured by membrane permeable 2′-7′-dichlorofluorescin diacetate (H_2DCFDA) (Life Technologies Inc., Cat No. D-399, Burlington, ON, Canada) which is oxidized to fluorescent 2′,7′-dichlorofluorescein (DCF) by ROS following cleavage of its acetate groups by intracellular esterases. Cells were incubated with 10 μM H_2DCFDA dissolved in DMSO for 30 min at 37 °C and DCF fluorescence was detected using fluorescence microscopy with a Leica DMIRB inverted fluorescence microscope (Leica Microsystems, Wetzlar, Germany), using a Spectra Max Gemini XS multi-well plate fluorescence reader (Molecular Devices, Sunnyvale, CA, USA) at Ex. 495 and Em. 529 with a black opaque 96-well microplate, and image-based cytometry with a Tali® Image-Based Cytometer (Life Technologies Inc., Cat No. T10796, Burlington, ON, Canada). Fluorescence readings were expressed as relative fluorescence units (RFU).

2.4. Cell growth rate determination with cell counting

To determine the growth rate of PSAF and NHF, approximately 1.0×10^5 cells were seeded and grown for 48 and 96 h. Cells were harvested and the number of live cells was quantified with a Trypan Blue solution (Sigma-Aldrich Canada, Cat. No. T8154, Mississauga, ON, Canada) and a hemocytometer.

2.5. Western blot analyses

Protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane which was blocked with 5% w/v milk TBST (Tris-Buffered Saline Tween-20) solution for 1 h. Membranes were incubated with primary antibodies overnight at 4 °C: manganese superoxide dismutase (MnSOD) (Cat. No. sc-30080, rabbit polyclonal IgG, 1:1000), p21 (Cat. No. sc-817, mouse monoclonal IgG, 1:500), proliferating cell nuclear antigen (PCNA) (Cat. No. sc-7907, rabbit polyclonal IgG, 1:500), p53 (Cat. No. sc-98, mouse monoclonal IgG, 1:1000), β-Actin (Cat. No. sc-81178, mouse monoclonal IgG, 1:1000), Beclin 1 (Cat. No. sc-11427, rabbit polyclonal IgG, 1:500) (Santa Cruz Biotechnology, Inc., Paso Robles, CA, USA), Rb (Cat. No. R6775, rabbit polyclonal IgG, 1:1000), p16Ink4A (Cat. No. P0968, mouse monoclonal IgG, 1:1000) (Sigma-Aldrich Canada, Mississauga, ON, Canada), and microtubule-associated protein 1 light chain 3 (LC3) (Cat. No. NB100-2220, rabbit polyclonal IgG, 1:500) (Novus Biologicals, Littleton, CO, USA). Membranes were washed with TBST, incubated with a rabbit anti-mouse (1:2000) or