Biochemical and Biophysical Research Communications 414 (2011) 49–52

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Protective potential of resveratrol against oxidative stress and apoptosis in Batten disease lymphoblast cells

Dong-Ho Yoon [a], Oh-Youn Kwon [b], Ji Young Mang [b], Myung Jin Jung [b], Do Yeon Kim [b], Yoon Kyung Park [b], Tae-Hwe Heo [c,*], Sung-Jo Kim [a,*]

[a] Department of Biotechnology, Hoseo University, 165, Baebang, Asan, Chungnam 336-795, Republic of Korea
[b] Bokja Girl's High School, Sunghwang, Chonan, Chungnam 330-130, Republic of Korea
[c] College of Pharmacy, Catholic University of Korea, Bucheon 420-743, Republic of Korea

ARTICLE INFO

Article history:
Received 2 September 2011
Available online 14 September 2011

Keywords:
Batten disease
Resveratrol
Antioxidant
Apoptosis

ABSTRACT

Batten disease (BD) is the most common form of a group of disorders called neuronal ceroid lipofuscinosis, which are caused by a *CLN3* gene mutation. A variety of pathogenic lysosomal storage disorder mechanisms have been suggested such as oxidative stress, endoplasmic reticulum (ER) stress, and altered protein trafficking. Resveratrol, a stilbenoid found in red grape skin, is a potent antioxidant chemical. Recent studies have suggested that resveratrol may have a curative effect in many neurodegenerative diseases. Therefore, we investigated the activities of resveratrol at the levels of oxidative and ER stress and apoptosis factors using normal and BD lymphoblast cells. We report that the BD lymphoblast cells contained low-levels of superoxide dismutase-1 (SOD-1) due to the long-term stress of reactive oxygen species. However, when we treated the cells with resveratrol, SOD-1 increased to levels observed in normal cells. Furthermore, we investigated the expression of glucose-regulated protein 78 as an ER stress marker. BD cells underwent ER stress, but resveratrol treatment resolved the ER stress in a dose-dependent manner. We further demonstrated that the levels of apoptosis markers such as apoptosis induce factor, cytochrome c, and cleavage of poly (ADP)-ribose polymerase decreased following resveratrol treatment. Thus, we propose that resveratrol may have beneficial effects in patients with BD.

1. Introduction

Neuronal ceroid lipofuscinoses (NCLs) are lysosomal storage disorders that occur with an incidence of approximately 1 in 12,500 live births [1–4]. Based on the onset age of clinical symptoms, NCLs are classified into four major subtypes: infantile (INCL), late-infantile (LNCL), juvenile (JNCL), and adult types [4]. Batten disease (BD), or JNCL, is the most common of the NCLs and is sometimes used as the term for all forms of NCL. BD is an inherited disorder characterized by neurodegeneration, usually with retinal degeneration at 5–7 years of age, due to a *CLN3* gene mutation [5].

Oxidative stress has been implicated in the progression of BD [5] as with Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis [6]. Oxidative stress alters the antioxidant defense system by regulating the expression of antioxidant enzymes such as superoxide dismutase-1 (SOD-1) [7]. Recently, it was reported that brain cells in an INCL model undergo endoplasmic reticulum (ER) stress [8] and show elevated levels of glucose-regulated protein 78 (GRP78/BiP), which can be used as an ER stress marker [9]. Oxidative stress mediated by reactive oxygen species (ROS) can directly induce ER stress as well as mitochondrial and nuclear damage, leading to apoptosis [10]. Further, INCL cells cleave poly ADP-ribose polymerase (PARP), a compelling sign of apoptosis.

Because oxidative damage is related to neurological complications, antioxidants have been used as therapeutics for neurodegenerative disease. Resveratrol, a stilbenoid found in grapes and red wine, is one of the most potent antioxidant chemicals and has been studied for its beneficial effects in neurodegenerative diseases [7,11,12]. Furthermore, resveratrol is protective against the apoptotic cascade induced by 1-methyl-4-phenylpyridinium oxidative stress by acting on apoptosis-inducible factor (AIF) and cytochrome c [13].

In this report, we investigated the possibility that resveratrol acts on BD lymphoblast cells to alleviate the pathogenic conditions of BD. We treated BD cells with resveratrol and examined the expression levels of SOD-1, the ER stress marker GRP78/BiP, and the apoptosis markers AIF, cytochrome c, and PARP [9].

* Corresponding authors. Fax: +82 2 2164 4059 (T.-H. Heo), +82 41 548 6231 (S.-J. Kim).
E-mail addresses: thhur92@catholic.ac.kr (T.-H. Heo), sungjo@hoseo.edu (S.-J. Kim).

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doi:10.1016/j.bbrc.2011.09.019

2. Materials and methods

2.1. Cell lines and culture conditions

The cells used were lymphoblast cell lines from controls (American Type Culture Collection, Rockville, MD, USA) and from patients with BD (Coriell Institute for Medical Research). Cells were cultured in RPMI-1640 medium (Hyclone; ThermoScientific, Rockford, IL, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gemcell; Gemini BioProducts, West Sacramento, CA, USA), 100 unit/ml penicillin-streptomycin (Hyclone), 1% L-glutamine (Well Gene Inc., Seoul, South Korea) in 100 mm dishes (SPL Life Science, Seoul, South Korea) in a CO_2 incubator (Thermo Scientific) at $1–2 \times 10^6$ cells/ml.

2.2. Western blot analysis

Resveratrol (Sigma Aldrich, St. Louis, MO, USA) was prepared by dissolving it in DMSO (Sigma Aldrich). Normal and BD patient lymphoblast cells were treated with resveratrol (0.1, 1, or 10 μM) for 24 h. Proteins were extracted in a buffer (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin) containing protease-inhibitor cocktail (Sigma). Thirty micrograms of total protein from each sample were resolved by electrophoresis using 10% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA, USA) under denaturing and reducing conditions. Proteins were then transferred to polyvinylidene fluoride membranes (Bio-Rad). The membranes were blocked with 5% non-fat dry milk (Bio-Rad) and then subjected to immunoblot analysis. The primary antibodies used were: anti-SOD-1 (Abcam, Cambridge, MA, USA), anti-GRP78/BiP (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-AIF (Santa Cruz Biotechnology), anti-cytochrome c (Abcam), anti-PARP (Cell Signaling Technology, Danvers, MA, USA), and anti-β-actin (Sigma). The secondary antibodies used were goat anti-rabbit IgG-HRP and rabbit anti-mouse IgG-HRP (Santa Cruz Biotechnology). Chemiluminescent detection was performed using the SuperSignal West Pico Luminal/Enhancer Solution (Pierce, Rockford, IL, USA), according to the manufacturer's instructions.

2.3. Statistical analysis

Results are expressed as the mean of at least three determinations ± standard deviation (SD). Statistical analyses were performed using Student's *t*-test and Microsoft Excel 2010 ($p < 0.05$).

3. Results

3.1. BD cells downregulated SOD-1

Little is known about SOD-1 expression levels in patients with neurodegenerative diseases. SOD-1 mutations can cause familial amyotrophic lateral sclerosis [14–16] and oxidative stress can damage the SOD-1 protein [15]. Thus, we examined SOD-1 expression levels to investigate the impact of oxidation conditions in BD on the endogenous anti-oxidative system (Fig. 1). Decreased SOD-1 expression was observed in BD cells compared with that in normal control cells.

3.2. Recovery of SOD-1 expression by resveratrol treatment

The SOD-1 protein was monitored by Western blot to determine whether expression patterns changed following resveratrol treatment. SOD-1 expression increased substantially in BD cells that had been treated with resveratrol for 24 h with a peak at 1 μM, compared with that in DMSO-treated control cells (Fig. 1).

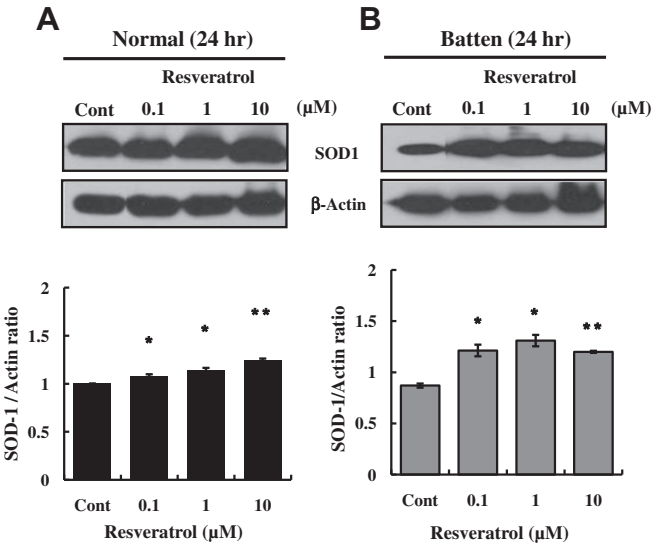

Fig. 1. Western blot analysis for superoxide dismutase-1 (SOD-1) in cultured normal and Batten disease (BD) lymphoblast cells. (A) SOD-1 protein in normal cells treated for 24 h with resveratrol. (B) Western blot analysis for the SOD-1 protein in BD cells treated with resveratrol for 24 h. β-actin was used as the loading control. Cont (control: DMSO). Densitometric analysis of the SOD-1 protein (bottom) was performed using the SOD-1 protein to β-actin ratio. Each result represents the mean ± SD of three experiments performed in triplicate; $^*p < 0.05$, $^{**}p < 0.001$.

3.3. Reduction in the expression of the ER stress marker protein GRP78/BiP by resveratrol

GRP78/BiP, an ER stress marker, was monitored by Western blot to determine whether expression patterns changed. Increased

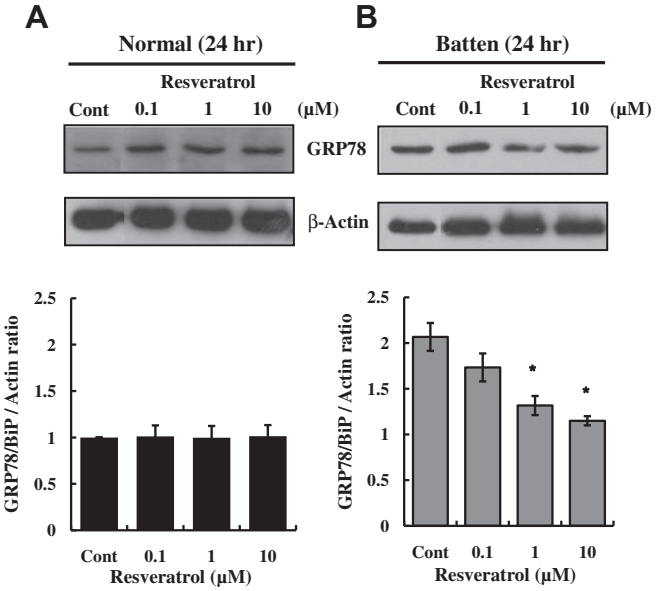

Fig. 2. Western blot analysis for the endoplasmic reticulum stress marker protein, glucose-regulated protein 78 (GRP78/BiP) from cultured normal and Batten disease (BD) lymphoblast cells. (A) GRP78/BiP protein in normal cells after a 24 h treatment with resveratrol. (B) Western blot analysis for the GRP78/BiP protein in BD cells treated for 24 h with resveratrol. β-Actin was used as the loading control. Cont (control: DMSO). Densitometric analysis of the GRP78/BiP protein (bottom) was performed using the GRP78/BiP protein to β-actin ratio. Each results represent the mean ± SD of three experiments performed in triplicate; $^*p < 0.05$.