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).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
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).

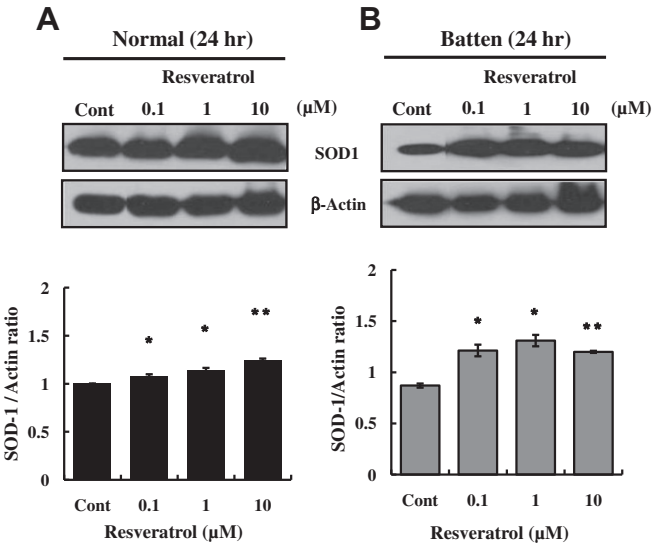

Fig. 1. Western blot analysis for superoxide dismutase-1 (SOD-1) in cultured normal and Batten disease (BD) lymphoblast cells. (A) SOD-1 protein in normal cells treated for 24 h with resveratrol. (B) Western blot analysis for the SOD-1 protein in BD cells treated with resveratrol for 24 h. β-actin was 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

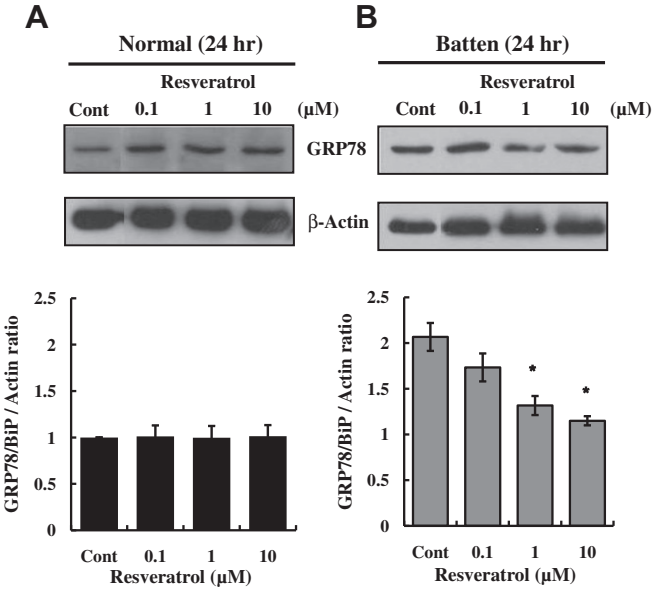

Fig. 2. Western blot analysis for the endoplasmic reticulum stress marker protein, glucose-regulated protein 78 (GRP78/BiP) from cultured normal and Batten disease (BD) lymphoblast cells. (A) GRP78/BiP protein in normal cells after a 24 h treatment with resveratrol. (B) Western blot analysis for the 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; $^*p < 0.05$.

GRP78/BiP expression was observed in untreated BD cells compared with that in normal control cells. GRP78/BiP expression decreased substantially in BD cells that had been treated with resveratrol for 24 h (Fig. 2) compared with that in normal control cells.

3.4. Impact of resveratrol on apoptosis marker expression in BD cells

Our results indicated that resveratrol treatment alleviated ER stress in BD cells. This led us to postulate that resveratrol may protect BD cells from the programmed cell death induced by ER stress. Thus, the expression patterns of apoptosis marker proteins were examined in resveratrol-treated BD cells. Overall, resveratrol treatment lowered the levels of apoptotic marker proteins (Fig. 3). In particular, AIF expression in BD cells treated with 10 μM of resveratrol decreased markedly compared with that in the low-concentration samples and untreated controls at 24 h. Cytochrome c and cleaved PARP expression in BD cells decreased significantly in a dose-dependent manner following resveratrol treatment.

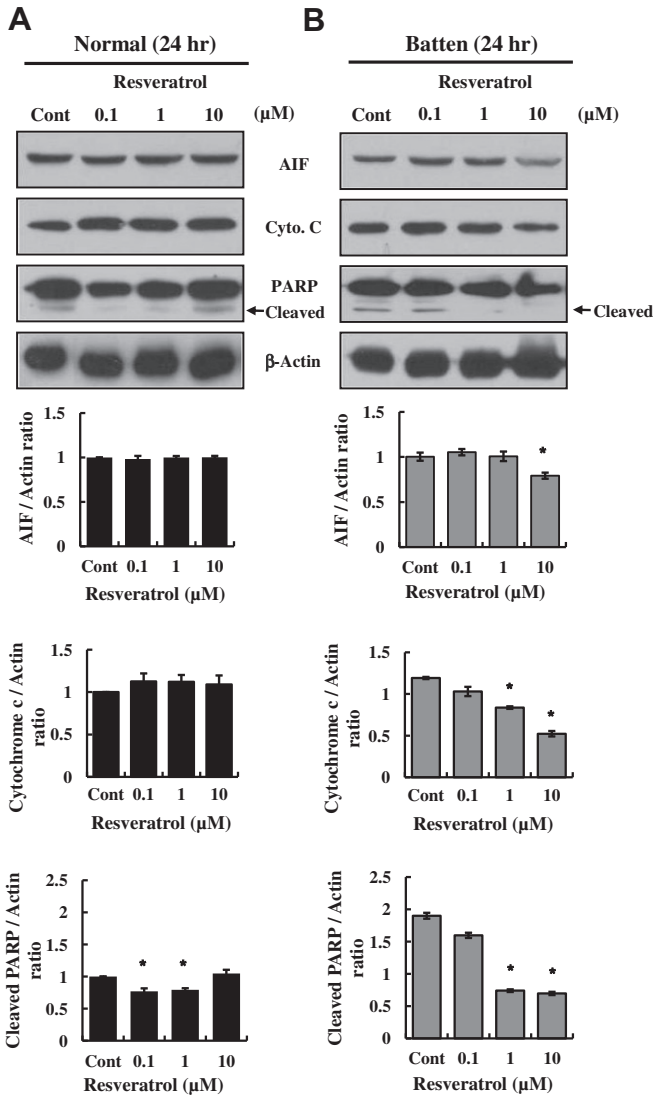

Fig. 3. Western blot analysis for apoptosis inducible factor (AIF), cytochrome c, and poly ADP-ribose polymerase (PARP) proteins in cultured normal and Batten disease (BD) lymphoblast cells. (A) AIF protein in normal cells treated for 24 h with resveratrol (upper panel). Cytochrome c protein in normal cells treated for 24 h with resveratrol (middle panel). PARP protein in normal cells treated for 24 h with resveratrol (low panel). (B) AIF protein in BD cells treated for 24 h with resveratrol (upper panel). Cytochrome c protein in BD cells treated for 24 h with resveratrol (middle panel). PARP protein in BD cells treated for 24 h with resveratrol (low panel). β-Actin was used as the loading control. Cont (control: DMSO). Densitometric analysis of each protein (bottom) was performed using the AIF or cytochrome c protein to β-actin ratio. Each result represents the mean ± SD of three experiments performed in triplicate; $^{*}p < 0.05$.

4. Discussion

Oxidative stress is linked with neurodegenerative conditions. Cells have several defense and repair mechanisms against ROS, which is toxic. However, the antioxidant enzymes SOD, catalase, glutathione peroxidase, and glutathione reductase show reduced activities in the brains of patients with AD and may contribute to the pathogenic condition [17]. SOD-1 is a 32-kDa homodimer with one copper- and one zinc-binding site and catalyzes the disproportionation of superoxide to produce dioxygen and hydrogen peroxide [15]. Little data are available regarding the SOD expression pattern in BD cells, but we found that SOD-1 enzyme expression was below the normal level in BD cells.

We postulated that antioxidants are beneficial to BD cells, because antioxidants may be able to scavenge ROS and upregulate endogenous antioxidants. Indeed, there is a report that resveratrol upregulates SOD activity in the brains of healthy rats [18]. Here, we used resveratrol and showed that the level of SOD-1 enzyme in BD cells was elevated in a dose-dependent manner and that this trend was more significant in BD cells than that in normal cells (Fig. 1).

ER stress is implicated in NCLs and may lead to BD pathogenesis [8,9], but the mechanisms are poorly understood. Molecular chaperones, including GRP78/BiP, are a group of proteins that assist in the proper folding of synthesized proteins in the ER [19]. Under normal conditions, chaperones are expressed and maintained at steady-state levels, but ER stress can increase protein marker expression. Although resveratrol is a popular antioxidant, little is known about its ability to ameliorate ER stress or BD. A recent report on the effects of antioxidants [20,21] on ER stress led us to study the possibility that resveratrol may have beneficial activities against BD. We were able to show that ER stress clearly occurred in BD cells (Fig. 2B) and that this was normalized by resveratrol in a dose-dependent manner. These results suggest that resveratrol stimulates the scavenging of ROS, which plays a role ameliorating ER stress and downregulating GRP78/BiP.

Oxidative stress and enhanced ER stress can trigger intracellular signaling pathways leading to apoptosis [22]. Because resveratrol increased antioxidant enzymes and reduced ER stress in BD cells, we investigated the expression levels of apoptosis related molecules. The molecular mechanisms of the apoptosis cascades include pro-apoptotic proteins such as AIF and cytochrome c [23]. We determined that AIF, cytochrome c, and cleaved PARP were detectable in BD cells as a sign of apoptosis but were diminished by resveratrol treatment.

Some antioxidants are unstable in culture media and readily undergo oxidation to generate products such as H_2O_2 [24–27]. Because H_2O_2 has cellular effects, researchers should be aware of the side effects of H_2O_2. Resveratrol did not generate H_2O_2 but was partially unstable in DMEM media [27]. We adopted the same incubation time (24 h) in RPMI media throughout the study to minimize artifacts. It will be necessary to address artifacts in antioxidant studies of BD cell cultures in a future study.

We identified resveratrol as a BD cell protective agent. Resveratrol-mediated induction of antioxidant enzymes and the reduction in ER stress may be associated with decreased expression of pro-apoptotic markers in BD cells. More detailed signal transduction and *in vivo* studies to unveil the underlying mechanism may lead to the possibility of resveratrol therapy for patients with BD.

Acknowledgment

This research was supported by the Academic Research fund of Hoseo University in 2010 (2010-0102).

References

[1] S.N. Phillips, J.W. Benedict, J.M. Weimer, D.A. Pearce, CLN3, the protein associated with batten disease: structure, function and localization, J. Neurosci. Res. 79 (2005) 573–583.
[2] H. Wei, S.J. Kim, Z. Zhang, P.C. Tsai, K.E. Wisniewski, A.B. Mukherjee, ER and oxidative stresses are common mediators of apoptosis in both neurodegenerative and non-neurodegenerative lysosomal storage disorders and are alleviated by chemical chaperones, Hum. Mol. Genet. 17 (2008) 469–477.
[3] S.J. Kim, Z. Zhang, C. Sarkar, P.C. Tsai, Y.C. Lee, L. Dye, A.B. Mukherjee, Palmitoyl protein thioesterase-1 deficiency impairs synaptic vesicle recycling at nerve terminals, contributing to neuropathology in humans and mice, J. Clin. Invest. 118 (2008) 3075–3086.
[4] S.S. Seehafer, D.A. Pearce, Spectral properties and mechanisms that underlie autofluorescent accumulations in Batten disease, Biochem. Biophys. Res. Commun. 382 (2009) 247–251.
[5] R.I. Tuxworth, H. Chen, V. Vivancos, N. Carvajal, X. Huang, G. Tear, The Batten disease gene CLN3 is required for the response to oxidative stress, Hum. Mol. Genet. 20 (2011) 2037–2047.
[6] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, Nat. Rev. Drug Discov. 3 (2004) 205–214.
[7] M.M. Khan, A. Ahmad, T. Ishrat, M.B. Khan, M.N. Hoda, G. Khuwaja, S.S. Raza, A. Khan, H. Javed, K. Vaibhav, F. Islam, Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease, Brain Res. 1328 (2010) 139–151.
[8] E. Bible, P. Gupta, S.L. Hofmann, J.D. Cooper, Regional and cellular neuropathology in the palmitoyl protein thioesterase-1 null mutant mouse model of infantile neuronal ceroid lipofuscinosis, Neurobiol. Dis. 16 (2004) 346–359.
[9] S.J. Kim, Z. Zhang, E. Hitomi, Y.C. Lee, A.B. Mukherjee, Endoplasmic reticulum stress-induced caspase-4 activation mediates apoptosis and neurodegeneration in INCL, Hum. Mol. Genet. 15 (2006) 1826–1834.
[10] E.K. Kim, E.J. Choi, Pathological roles of MAPK signaling pathways in human diseases, Biochim. Biophys. Acta 1802 (2010) 396–405.
[11] P. Marambaud, H. Zhao, P. Davies, Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides, J. Biol. Chem. 280 (2005) 37377–37382.
[12] H. Wei, Z. Zhang, A. Saha, S. Peng, G. Chandra, Z. Quezado, A.B. Mukherjee, Disruption of adaptive energy metabolism and elevated ribosomal p-S6K1 levels contribute to INCL pathogenesis: partial rescue by resveratrol, Hum. Mol. Genet. 20 (2011) 1111–1121.
[13] J. Bournival, P. Quessy, M.G. Martinoli, Protective effects of resveratrol and quercetin against MPP+ -induced oxidative stress act by modulating markers of apoptotic death in dopaminergic neurons, Cell Mol. Neurobiol. 29 (2009) 1169–1180.
[14] R.A. Conwit, Preventing familial ALS: a clinical trial may be feasible but is an efficacy trial warranted?, J Neurol. Sci. 251 (2006) 1–2.
[15] J.S. Valentine, P.A. Doucette, S. Zittin Potter, Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis, Annu. Rev. Biochem. 74 (2005) 563–593.
[16] H.X. Deng, A. Hentati, J.A. Tainer, Z. Iqbal, A. Cayabyab, W.Y. Hung, E.D. Getzoff, P. Hu, B. Herzfeldt, R.P. Roos, et al., Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase, Science 261 (1993) 1047–1051.
[17] J.K. Andersen, Oxidative stress in neurodegeneration: cause or consequence?, Nat Med. 10 (Suppl.) (2004) S18–25.
[18] M. Mokni, S. Elkahoui, F. Limam, M. Amri, E. Aouani, Effect of resveratrol on antioxidant enzyme activities in the brain of healthy rat, Neurochem. Res. 32 (2007) 981–987.
[19] R.O. Brady, J.N. Kanfer, R.M. Bradley, D. Shapiro, Demonstration of a deficiency of glucocerebroside-cleaving enzyme in Gaucher's disease, J. Clin. Invest. 45 (1966) 1112–1115.
[20] J. Lu, D.M. Wu, Y.L. Zheng, B. Hu, W. Cheng, Z.F. Zhang, Q. Shan, Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IkappaB kinase beta/nuclear factor-kappaB-mediated inflammatory pathways in mice, Brain Behav. Immun. (2011), doi:10.1016/j.bbi.2011.06.009.
[21] J.D. Malhotra, H. Miao, K. Zhang, A. Wolfson, S. Pennathur, S.W. Pipe, R.J. Kaufman, Antioxidants reduce endoplasmic reticulum stress and improve protein secretion, Proc. Natl. Acad. Sci. U S A 105 (2008) 18525–18530.
[22] Y. Tabata, K. Takano, T. Ito, M. Iinuma, T. Yoshimoto, H. Miura, Y. Kitao, S. Ogawa, O. Hori, Vaticanol B, a resveratrol tetramer, regulates endoplasmic reticulum stress and inflammation, Am. J. Physiol. Cell Physiol. 293 (2007) C411–418.
[23] K.A. Jellinger, Recent advances in our understanding of neurodegeneration, J. Neural. Transm. 116 (2009) 1111–1162.
[24] B. Halliwell, Free radicals and antioxidants – quo vadis?, Trends Pharmacol Sci. 32 (2011) 125–130.
[25] L.H. Long, B. Halliwell, Artefacts in cell culture: pyruvate as a scavenger of hydrogen peroxide generated by ascorbate or epigallocatechin gallate in cell culture media, Biochem. Biophys. Res. Commun. 388 (2009) 700–704.
[26] L.H. Long, B. Halliwell, Artefacts in cell culture: alpha-Ketoglutarate can scavenge hydrogen peroxide generated by ascorbate and epigallocatechin gallate in cell culture media, Biochem. Biophys. Res. Commun. 406 (2011) 20–24.
[27] L.H. Long, A. Hoi, B. Halliwell, Instability of and generation of hydrogen peroxide by, phenolic compounds in cell culture media, Arch. Biochem. Biophys. 501 (2010) 162–169.