Toxicology Letters 238 (2015) 70–82

Contents lists available at ScienceDirect

Toxicology Letters

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

Pyrroloquinoline quinone-conferred neuroprotection in rotenone models of Parkinson's disease

Jiaojiao Qin[a,1], Meilong Wu[a,b,1], Shu Yu[a], Xiaorong Gao[a], Jingjing Zhang[a], Xingyue Dong[a,b], Jinyan Ji[a,b], Yuxi Zhang[a,b], Lin Zhou[a,b], Qi Zhang[a,*], Fei Ding[a,*]

[a] Jiangsu Key Laboratory of Neuroregeneration, Co-innovation Center of Neuroregeneration, Nantong University, 19 Qixiu Road, Nantong JS 226001, PR China
[b] Medical School, Nantong University, 19 Qixiu Road, Nantong JS 226001, PR China

HIGHLIGHTS

- PQQ conferred protection in rotenone-induced PD models both *in vitro* and *in vivo*.
- PQQ increased antioxidant ability and mitochondrial function in PD models.
- PQQ affected dopamine redistribution in rotenone-induced PD models.

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 22 July 2015
Accepted 9 August 2015
Available online 11 August 2015

Keywords:
Pyrroloquinoline quinone (PQQ)
Rotenone
Neuroprotection
Reactive oxygen species (ROS)
Parkinson's disease (PD)
Dopamine

ABSTRACT

Pyrroloquinoline quinone (PQQ), a redox cofactor in the mitochondrial respiratory chain, has proven to protect neurons against glutamate-induced damage both *in vitro* and *in vivo*. This study was aimed to investigate the possible neuroprotective effects of PQQ in rotenone-induced Parkinson's disease (PD) model. Pre-treatment with PQQ prevented cultured SH-SY5Y cells from rotenone-induced apoptosis, accompanied by modulation of apoptosis-related proteins (Bcl-2, Bax and Smac), restoration of the mitochondrial membrane potential, inhibition of intracellular reactive oxygen species (ROS) production, suppression of tyrosine residues nitration, and dopamine redistribution. PQQ also exerted protective effects in an *in vivo* PD model, which was created by rotenone injection into the medial forebrain bundle of rats. Co-injection with PQQ and rotenone improved the apomorphine-evoked rotation, decreased neuronal loss, increased the ROS-scavenging ability, regulated intracellular expressions of mitochondrial complex subunits (Ndufs1-4), tyrosine hydroxylase, and vesicular monoamine transporter 2. Taken together, our results collectively suggest that PQQ confers neuroprotection in rotenone-induced PD model probably through complex and multifaceted mechanisms, at least involving oxidative stress, mitochondrial integrity, and dopamine functions.

1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder-characterized by dopaminergic degeneration in the substantia nigra pars compacta (SNc) and subsequent reduction in dopamine levels in the striatum. Despite considerable advances in the treatment of PD, the pathogenesis-targeted therapies are still lacking (Jankovic and Poewe, 2012). The pathogenic mechanisms contributing to neurodegeneration in PD are complex and multifaceted, but mitochondrial dysfunction and oxidative stress are considered to be central to the pathogenesis of PD (Sanders and Timothy Greenamyre, 2013). Inhibition of mitochondrial complex I has long been one of the leading hypotheses to explain SNc dopamine neuron death in PD (Abou-Sleiman et al., 2006). Many environmental risk factors are implicated in PD, including some commercial pesticides that inhibit complex I of the electron transport chain (ETC) (Cannon and Greenamyre, 2013). Rotenone, one of these pesticides, is usually used to reproduce the pathological features of PD in animal models because rotenone acts specifically at the ETC complex I, and concurrently produces oxidative damage in the cell (Panov et al., 2005; Sherer et al., 2003).

* Corresponding authors. Fax: +86 513 85511585.
E-mail addresses: zhangqi@ntu.edu.cn (Q. Zhang), dingfei@ntu.edu.cn (F. Ding).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.toxlet.2015.08.011
0378-4274/

Pyrroloquinoline quinone (PQQ) was initially found in bacteria as a redox cofactor (Hauge, 1964; Salisbury et al., 1979), and was later identified as an essential nutrient for plants, animals and humans (Cline and Tsien, 1991; Felton and Anthony, 2005; Kasahara and Kato, 2003; Rucker et al., 2009, 2005; Stites et al., 2000). PQQ has a strong ability to scavenge reactive oxygen species (ROS) and attenuate oxidative stress in mitochondria (Misra et al., 2004, 2012), and thereby induces protective effects against oxidative stress-induced cell damage in heart, liver, and brain (Hobara et al., 1988; Ohwada et al., 2008; Tao et al., 2007; Zhu et al., 2004). In particular, PQQ has been shown to protect human neuroblastoma SH-SY5Y cells against 6-hydroxydopamine-induced cell death and DNA fragmentation (Hara et al., 2007). We previously demonstrate that PQQ prevents glutamate-induced neurotoxicity both *in vitro* and *in vivo* (Zhang et al., 2013, 2012, 2011). These findings highlight the neuroprotective potential of PQQ.

The brain is highly susceptible to oxidative damage because of the high levels of polyunsaturated fatty acids and relatively low antioxidant activity (Mariani et al., 2005). Oxidative stress plays an important role in the pathogenesis of PD although the underlying mechanisms are not fully understood. Unfortunately, most of the commonly used antioxidants have had a limited success in the treatment of PD, possibly because ROS generation from the ETC complex I, II and III is modulated in an insult-specific manner and the ETC sites are differentially accessible to those common antioxidants (Liu and Schubert, 2009). Our recent study has shown that ROS produced by complex I and III inhibitors could be attenuated by PQQ, suggesting these two ROS-generating sites might be accessible to PQQ (Zhang et al., 2014).

In this study, we aimed to test whether and how PQQ conferred neuroprotection in rotenone models of PD both *in vitro* and *in vivo*. Our results showed that pretreatment with PQQ protected cultured SH-SY5Y cells against rotenone injury by scavenging ROS, maintaining mitochondrial function and regulating dopamine redistribution. We also found that injection with PQQ prevented rats from rotenone-induced neuronal degeneration.

2. Materials and methods

2.1. Chemicals and reagents

PQQ, rotenone, trypsin, Hoechst 33,342, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), monoclonal mouse anti-Bcl-2 antibody, monoclonal mouse anti-Bax antibody, monoclonal mouse anti-Smac antibody, monoclonal mouse anti-tyrosine hydroxylase (TH) antibody, and monoclonal mouse anti-β-actin antibody were purchased from Sigma (St. Louis, MO). Monoclonal mouse anti-dopamine antibody was purchased from Abcam (Cambridge, MA). Monoclonal mouse anti-nitrotyrosine antibody and polyclonal rabbit anti-vesicular monoamine transporter 2 (VMAT2) antibody were purchased from Millipore (Bedford. MA). Neurobasal medium, Dulbecco's modified eagle's medium (DMEM), B27 supplement and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY). IRDye 800-conjugated goat anti-mouse IgG and IRDye 800-conjugated donkey anti-rabbit IgG were purchased from Rockland (Gilbertsville, PA). Tissue lysis buffer, protease inhibitor cocktail and BCA-based protein quantification kit were obtained from Biocolor (Shanghai, China). Cell Counting Kit-8 (CCK-8) and Fluo-3/AM were obtained from Dojindo (Kumamoto, Japan). Annexin V was from PharMingen (San Diego, CA). Trizol reagent was from Invitrogen (Carlsbad, CA). MitoTracker Green FM was purchased from Life technologies (Carlsbad, CA). Tetramethylrhodamine methyl ester (TMRM) and Fast EvaGreen qPCR Master Mix were purchased from Biotium (Hayward, CA, USA). Omniscript Reverse Transcription (RT) kit was from Qiagen (Valencia, CA). The assay kits for superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) were obtained from Jiancheng Institute of Biotechnology (Nanjing, Jiangsu, China).

2.2. Cell culture and treatment

Human SH-SY5Y neuroblastoma cells and C6 glioma cells were cultured in DMEM supplemented with 10% FBS at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. PQQ at different concentrations (0.1–100 μM) was added to the culture medium for 24 h incubation, and then rotenone (100 μM) was added to the medium for additional 24 h incubation. The cells not exposed to rotenone were used as control. To detect a long-term protective effects of PQQ, lower concentration of rotenone (10 μM) was added to the medium for an additional 72 h incubation and then cell viability was measured.

2.3. Cell viability measurement

Following cell treatment, cell viability was assessed by CCK-8 method. Briefly, tetrazolium salt-8 (WST-8) solution was added to cultured SH-SY5Y cells or C6 cells in 96-well plate (10 μl/each well), followed by incubation at 37 °C for 2 h. The absorbance (optical density, OD) was measured by spectrophotometry at 450 nm with an ELx-800 microplate reader (Bio-Tek Inc., Winooski, VT).

2.4. Hoechst 33342 staining

The SH-SY5Y cells were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.2) at room temperature for 20 min, and then stained with 5 μg/ml Hoechst 33,342 dye for 10 min, followed by observation under a DMR fluorescence microscope (Leica Microsystems, Wetzlar, Germany) with fluorescence excitation at 340 nm and emission at 510 nm. The cells with fragmented or condensed DNA were counted as apoptotic cells, and the ratio of apoptotic cells to total cells was calculated.

2.5. Annexin V/PI staining and flow cytometry (FCM) analysis

The SH-SY5Y cells were harvested and resuspended in 1 × binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$) at a concentration of 1×10^6 cell/ml. In a 100 μl aliquot of the cell suspension, 5 μl of FITC-conjugated annexin V and 5 μl of 50 μg/ml propidium iodide (PI) were added. After 15 min incubation in the dark at room temperature, cells were analyzed for annexin V binding within 1 h by using Fluorescence Activated Cell Sorting (FACS) Caliber Systems (BD Bioscience, San Jose, CA).

2.6. ROS detection

The SH-SY5Y cells were harvested and incubated with DCFH-DA at a final concentration of 20 μM at 37 °C for 30 min in the dark, and then gently rinsed with PBS for 3 times. ROS levels were measured as the fluorescence of oxidation product of DCFH-DA, dichlorofluorescin (DCF) by FCM with excitation and emission wavelengths of 485 and 528 nm, respectively. A minimum of 10,000 individual events was recorded. The fluorescence intensity was expressed as the relative value to the control.

2.7. Mitochondrial membrane potential (MMP)

The fluorescent dye TMRM is a mitochondrial specific probe used to determine the change of MMP in intact cells. For indirect measurement of MMP, TMRM was added to the cultured SH-SY5Y