Biochemical and Biophysical Research Communications 464 (2015) 794–799

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

L-F001, a multifunctional ROCK inhibitor prevents paraquat-induced cell death through attenuating ER stress and mitochondrial dysfunction in PC12 cells

Wei Shen [a], Lan Wang [a], Rongbiao Pi [b, c], Zhifeng Li [d], Rikang Wang [d, *]

[a] Department of Neurology, Puai Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430033, China
[b] Department of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510080, China
[c] International Joint Laboratory (SYSU-PolyU HK) of Novel Anti-Dementia Drugs of Guangdong, Guangzhou 510006, China
[d] National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China

ARTICLE INFO

Article history:
Received 23 June 2015
Accepted 7 July 2015
Available online 14 July 2015

Keywords:
L-F001
ROCK inhibitor
Paraquat
Cell death
Endoplasmic reticulum stress
Mitochondria

ABSTRACT

Paraquat (PQ) was demonstrated to induce dopaminergic neuron death and is used as a Parkinson's disease (PD) mimetic. Amounting evidences demonstrated that Rho/ROCK may a novel target for the therapy of PD. Previously we synthesized L-F001 and proved it is a potent ROCK inhibitor with multi-functional effects, including anti-oxidative stress. In this study, we investigated the effects and also the molecular mechanisms of L-F001 in preventing PQ-induced cytotoxicity in PC12 cells. L-F001 effectively prevented PQ-induced apoptotic cell death, which involves the scavenger of ROS and also attenuated the declined of mitochondrial membrane potential and intracellular level of GSH induced by PQ. Moreover, PQ quickly induced alterations of GRP78 and CHOP, two hallmarks of endoplasmic reticulum (ER) stress and subsequently induced dysfunction of the mitochondria (such as the decrease in membrane potential and increase in ROS). These changes all were potently attenuated by L-F001. In summary, L-F001 attenuated PQ-induced apoptosis through modulating mitochondrial dysfunction and ER stress as well as the ROS production elicited by PQ. These data indicated that L-F001 could possibly be used to treat PD and other neurodegenerative disorders with similar pathologic mechanisms.

1. Introduction

Parkinson's disease (PD), the second age-related neurodegenerative disease, affects millions of people worldwide [1], and is characterized by selective loss of dopaminergic neurons in the substantia nigra of the brain [2]. Strong evidence supports the role for oxidative stress and mitochondrial dysfunctions in the dopaminergic neuronal death in PD [3]. Several studies have documented that mitochondrial dysfunction and oxidative stress in the brain of PD are associated to pesticides exposure [3,4].

Paraquat (PQ) is used as a herbicide worldwide. Epidemiological studies indicated PQ exposure as a potential risk factor for the onset of PD [5,6]. PQ was demonstrated to induce dopaminergic neuron death through inducing reactive oxigen species (ROS) and induced PD-like pathology in different animal models [7–9]. Furthermore, PQ was shown to induce endoplasmic reticulum (ER) stress [10], suggesting multiple targets or complex mechanisms of PQ.

Amounting evidences demonstrated that Rho/Rho-associated kinase (ROCK) pathway may be the novel target for the therapy of PD [11,12], and ROCK inhibitors have been suggested as candidate anti-PD drugs [12–14]. Previously we employed the pharmacophore of fasudil, a ROCK inhibitor [15], and alpha-lipoic acid (LA), a potent anti-oxidant, we developed a novel multitargeted and neuroprotective drug, L-F001 (see Fig. 1). we confirmed that L-F001 is a novel multitargeted and neuroprotective drug in non-catecholaminergic neurons, which was capable of scavenging free radicals, increasing the level of glutathione, and preventing HT 22 cell death caused by glutamate [16]. However, the effects of L-F001 on PQ toxicity remains unclear in dopaminergic neuronal cells. Therefore, here we investigated the effects of L-F001 on PQ-induced cytotoxicity in pheochromocytoma 12 (PC12) cells as well as its mechanism(s).

* Corresponding author.
E-mail address: wrk168ok@163.com (Rikang Wang).

http://dx.doi.org/10.1016/j.bbrc.2015.07.035
0006-291X/

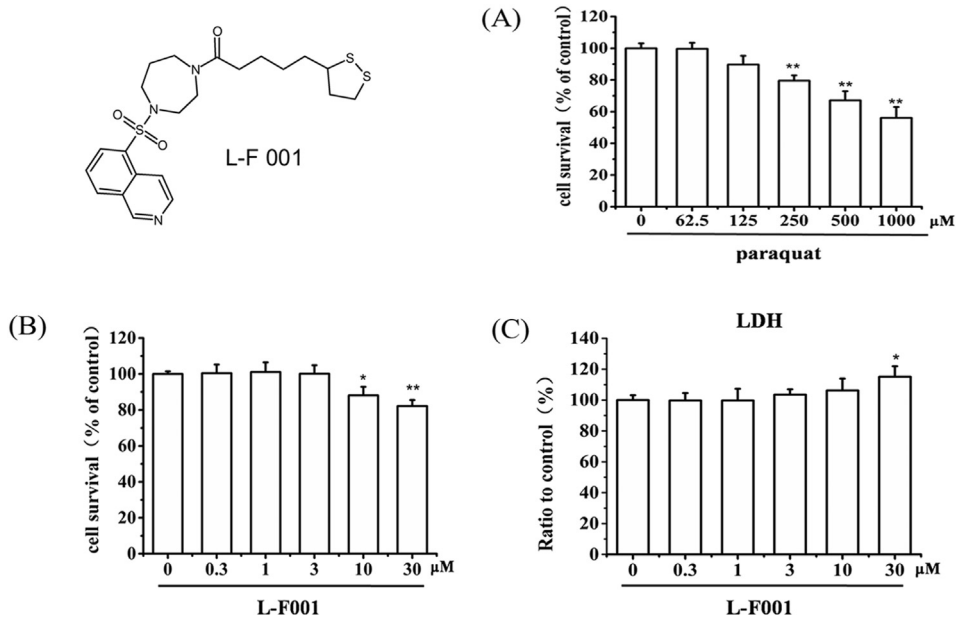

Fig. 1. Effects of PQ and L-F001 on the cell viability of PC12 cells. Cells were exposed to different concentrations of PQ **(A)** or L-F001 **(B)** as indicated for 24 h. Cell viability and LDH release **(C)** in PC12 cells was determined by MTT assay and LDH assay respectively, The data was presented as mean ± SD for six replicates. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group.

2. Materials and methods

L-F001 was synthesized as described before; 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Poly-D-lysine, 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) and DMSO were from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and horse serum were purchased from Gibco-BRL (NY, USA). Hoechst 33258 and BCA protein assay kit were from Beyotime Institute of Biotechnology (Haimen, China); JC-1 dye (Molecular Probes) was from BestBio (Shanghai, China). LDH release assay and Glutathione (GSH) assay kit were from Jiancheng Biochemical Company (Nanjing, China). L-F001 stock solution (100 mM/L) was dissolved in DMSO and stored at −20 °C. anti-GRP78 Antibody, anti- CHOP Antibody from Cell Signaling Technology (Woburn, USA).

2.1. Cell culture and treatment

PC12 cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS) and 10% horse serum, 100 μg of streptomycin/ml, and 100 U of penicillin/ml and incubated at 37 °C with 5% CO_2 humidified atmosphere. Cultured media was replaced twice a week with fresh medium as described above. Stock culture was routinely subcultured at 1:5 ratio at a weekly interval. For the experiments, cells were pre-incubated with various concentrations of L-F001 for 2 h without other description, and followed by treatment of PQ for 24 h. Control group was treated with 0.1% (v/v) DMSO as vehicle control.

2.2. MTT assay and LDH release assay

Cell viability was estimated using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay and LDH release assay as previously described. The release of LDH in the culture medium was determined using a commercially kit. Briefly, after 24 h treatments, 20 μl supernatant per well was transferred into a 96-well microplate to determine LDH levels according to the manufacturer's instructions before adding MTT, the rest of culture medium was removed and replaced with 90 μL of fresh DMEM. 10 μl of 5 mg/ml MTT in phosphate-buffered saline (PBS) was added to each well and the plates were incubated at 37 °C for another 3 h, then supernatants were discarded. 100 μL of DMSO solutions were added to each wells and the solutions were mixed thoroughly. Then the plates were incubated at 37 °C for another 10 min. Each sample was mixed again and the resultant formazan was measured by its absorbance at 570 nm using a Multiskan Ascent Microplate Reader (Thermo, USA). The experiments were repeated at least 3 times and compared with the control experiment.

2.3. Morphologic changes

PC12 cells grown on 48-well plates were treated with L-F001 and/or PQ as described above. After that, cells were fixed with 4% paraformaldehydeand stained with Hoechst 33258 (5 μg/mL) for 10 min at 37 °C in the dark. Then Hoechst 33258 was removed by washing with PBS, and morphologic changes were observed by phase-contrast microscopy and cells images were taken using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

2.4. Measurement of ROS

Intracellular ROS formation was measured by fluorescence using DCFH-DA. Briefly, after treatment, cells were washed and then stained with 10 μM DCFH-DA in serum-free medium for 30 min at 37 °C in the dark. the fluorescence from the DCF was analyzed using a fluorescence plate reader (Flex Station3, Molecular Devices, USA) at excitation and emission wavelengths of 488 and 525 nm, respectively, or taken images using a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

2.5. Estimation of intracellular glutathione (GSH)

Intracellular GSH concentration was measured a GSH assay kit (48T, Nanjing Jiancheng, China). By reacting with

dithiobisnitrobenzoicacid, reduced GSH could form a yellow compound, which is quantifiable at 405 nm using a Multiskan Ascent Microplate Reader (Thermo, USA) and reflect the content of the reduced GSH indirectly. In brief, after treatment, whole-cell lysate was prepared according to manufacturer's instructions. All GSH values were normalized to per μg protein of each sample and the levels of GSH measured in control conditions were set as the 100% values.

2.6. Mitochondrial membrane potential determination

Mitochondrial membrane potential was analyzed by using a fluorescent dye JC-1 (BestBio Shanghai China). JC-1 penetrates cells and healthy mitochondria. At low membrane potentials (apoptotic cells), JC-1 exists as a monomer which emits green fluorescence. JC-1 aggregates and emits red fluorescence at higher membrane potentials (non-apoptotic cells). Assays were initiated by incubating PC12 cells with JC-1 (5 mg/L) for 20 min at 37 °C in the dark and the fluorescence of separated cells were captured by inverted fluorescence microscopy (IX71, Olympus, Tokyo, Japan, at wavelengths of 490 nm excitation and 530 nm emission for green, and at 540 nm excitation and 590 nm emission for red). The ratios of red/green fluorescence were calculated.

2.7. Western blotting analysis

Western blotting analysis was performed as previously described [17]. Briefly, Cells from different experimental conditions were lysed with ice-cold RIPA lysis buffer and protein concentration was determined with a BCA protein assay kit according to the manufacturer's instructions. Equal amounts of lysate protein (20 μg/lane) were subjected to SDS-PAGE with 10% polyacrylamide gels and electropho-retically transferred to nitrocellulose membranes. Nitrocellulose blots were first blocked with 3% bovine serum albumin (BSA) in PBST buffer (PBS with 0.01% Tween 20, pH 7.4), and incubated overnight at 4 °C with primary antibodies in PBST containing 1% BSA. Immunoreactivity was detected by sequential incubation with horseradish peroxidase-conjugated secondary antibodies, and detected by the enhanced chemiluminescence technique.

3. Results

3.1. Effects of L-F001 on PQ-induced cytotoxicity in PC12 cells

Cell viability was analyzed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. PQ induced significant cell death in a concentration-dependent manner (Fig. 1A). L-F001 didn't affect cells viability under the concentration of 10 μM, but slightly induced cell death and LDH release at 30 μM (Fig. 1B, C). The cell death induced by PQ was significantly attenuated by L-F001 (3–10 μM), and LA (10 μM) (Fig. 2A) but not fasudil (10 μM). In addition, the anti-apoptotic effect of L-F001 on PC12 cells exposed to PQ was simply evaluated by Hoechst 33258 nuclei staining. The nuclei staining showed that PQ treatment induced nuclear condensation as well as the apoptotic bodies, which was attenuated by L-F001 (Fig. 2B).

3.2. Effects of L-F001 on PQ-induced ROS accumulation, GSH release and mitochondrial dysfunction in PC12 cells

Intracellular ROS level was evaluated using DCFH-DA. L-F001 (3 μM) significantly reduced the intracellular production of ROS induced by PQ (Fig. 3A). Further study We elucidated the effects of L-F001 on mitochondrial membrane potential and endogenous antioxidants using a fluorescent dye JC-1 and a GSH assay kit. The results showed that PQ greatly decreased the intracellular level of GSH, which was significantly reversed L-F001 and LA, but not fasudil (Fig. 3B). and L-F001 significantly prevented the declined of mitochondrial membrane potential induced by PQ (Fig. 3C).

3.3. Effects of L-F001 on ER stress in PC12 cells

Finally, to identify the direct effect of L-F001 on GRP78 and CHOP, two hallmarkers of ER stress, were examined by Western blotting. The levels of GRP78 and CHOP were significantly increased with the presence of PQ for 12 h and 24 h, which was significantly inhibited by L-F001 (Fig. 4A/B).

4. Discussion

In this study, L-F001, a novel multifunctional ROCK inhibitor was demonstrated to be effective in preventing PQ-induced cell death

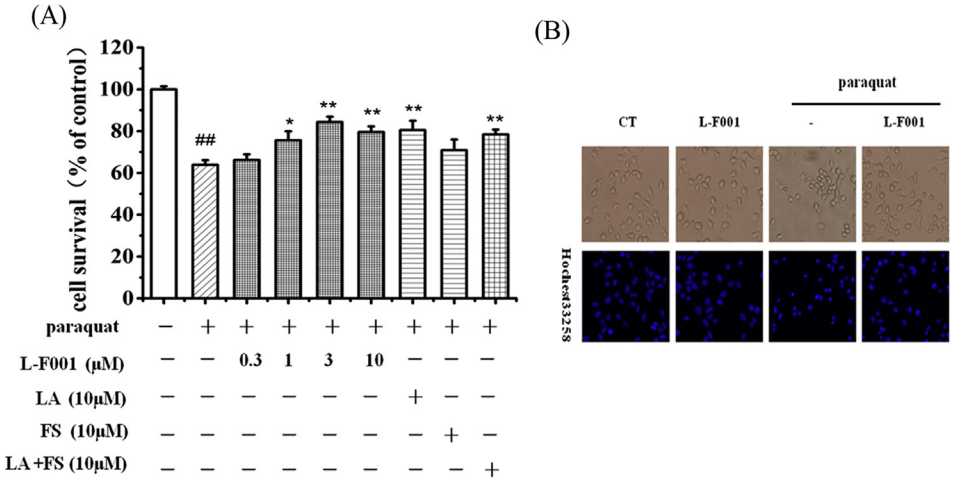

Fig. 2. L-F001 protected PC12 cells from PQ-induced cytotoxicity and attenuated apoptosis induced by PQ. (A) PC12 cells were pre-treated with L-F001 at different concentrations or LA (10 μM), FS (10 μM) for 2 h before 500 μM PQ was added. Cell viability in PC12 cells was determined by MTT assay. **(B)** Cells were either untreated or pretreated with L-F001 (3 μM) for 2 h before addition of 500 μM PQ. Images of nuclear chromatin were obtained using Fluorescence microscopy. The data was presented as mean ± SD for six replicates. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus PQ group. $^{\#\#}P < 0.01$ versus control group.

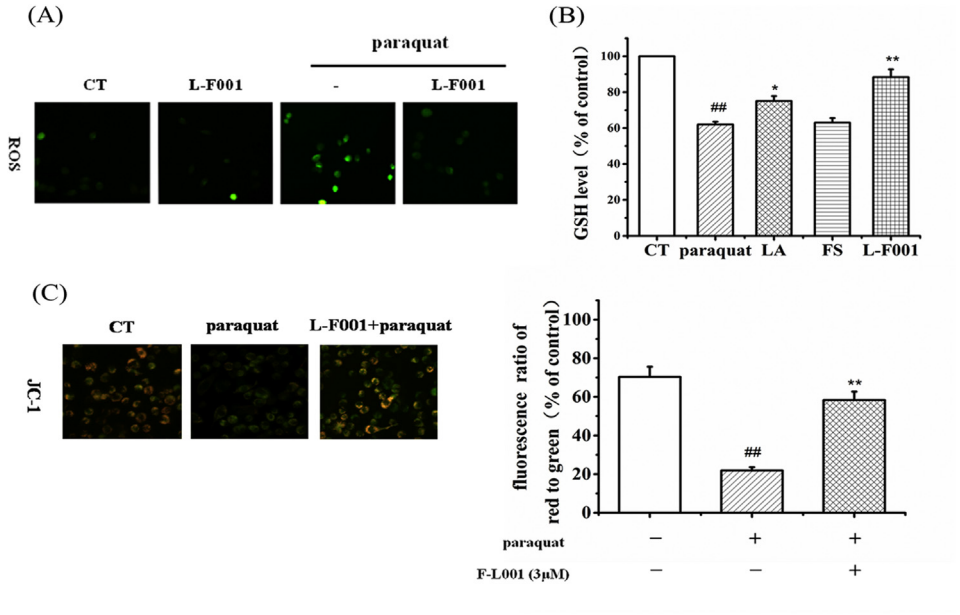

Fig. 3. L-F001 inhibits PQ-induced ROS formation, GSH depletion and mitochondrial dysfunction in PC12 cells. **(A)** PC12 cells were pretreated with or without of L-F001 (3 μM) for 2 h and then treated with or without PQ (500 μM) for 12 h, the fluorescence intensity of DCFH-DA showed that L-F001 blocked the ROS accumulation induced by PQ. Images of ROS were obtained using Fluorescence microscopy. **(B)** PC12 cells were treated with LA, fasudil at 10 μM and L-F001 at 3 μM for 2 h ahead before exposed to PQ (500 μM) for 12 h. The level of GSH was measured using commercial assay kits. The basal contents of GSH in untreated control cells were taken as 100%. **(C)** PC12 cells were incubated with JC-1 (5 mg/L) for 20 min at 37° in the dark and the mitochondrial membrane potential assay was performed. The shift of fluorescence from red to green indicated by JC−1 reflected the decline of the membrane potential and early apoptosis. The data was presented as mean ± SD for six replicates. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus PQ group. $^{\#\#}P < 0.01$ versus control group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

through cleavaging ROS and recovering mitochondrial membrane potential and intracellular level of GSH as well as to attenuate PQ-induced ER stress.

PD is characterized by selective loss of dopaminergic neurons in the substantia nigra of the brain [2], Several recent studies have shown that activation of the RhoA/ROCK pathway is involved in the MPTP-induced dopaminergic cell degeneration and possibly in Parkinson's disease [18], moreover, treatment with the ROCK inhibitor did not induce a significant reduction in the dopaminergic cell loss and was suggested as candidate neuroprotective drugs for

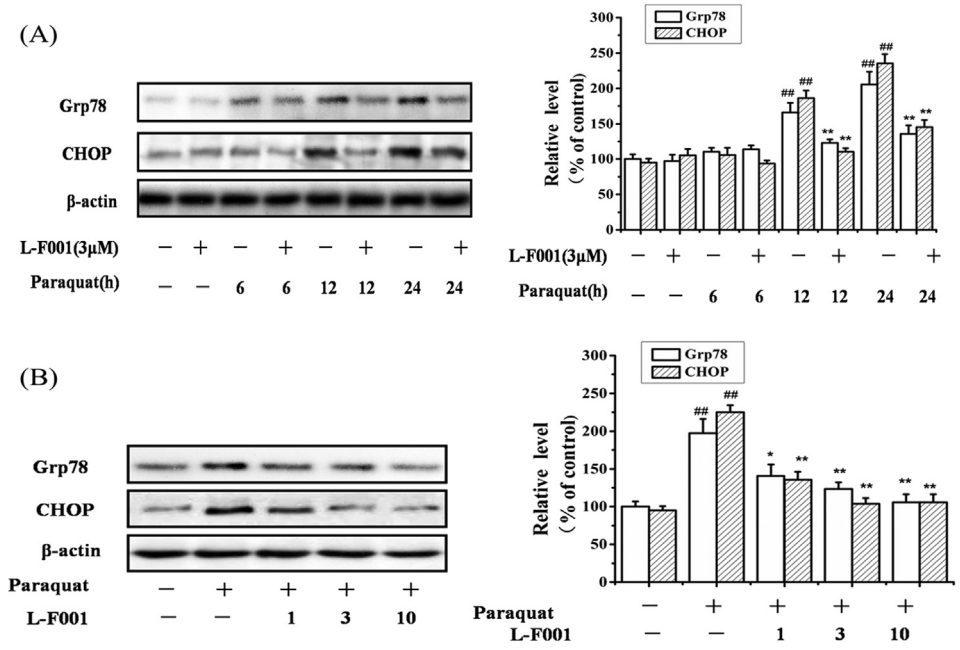

Fig. 4. L-F001 protected PC12 from PQ insult via attenuating endoplasmic reticulum stress (ERS). **(A)** Cells were pretreated with or without L-F001 for 2 h then co-treated PQ (500 μM) for 0, 6, 12 and 24 h. GRP78 and CHOP were detected by Western blot. Quantitative data of GRP78 and CHOP were determined by densitometry of the blots, and values represent the means ± SEM of three independent experiments. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus the vehicle group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus the PQ alone treated group at relevant time-point. **(B)** Cells were pretreated with L-F001 at different concentrations for 2 h before exposed to PQ (500 μM) for 12 h. GRP78 and CHOP were detected by Western blot. The density of bands of GRP78; and CHOP were expressed as a percentage of control, and values represent the means ± SEM of three independent experiments. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the untreated control group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus the PQ group.

Parkinson's disease [19–21]. It's also well known that PQ triggers oxidative stress and dopaminergic cell death [10]. In this study, L-F001 was effective in preventing PQ-induced apoptotic cell death, which involves the scavenger of ROS and prevented the declined of mitochondrial membrane potential induced by PQ, supporting that ROCK plays an essential role in fragmentation and phagocytosis of apoptotic cells [21,22] and RhoA/ROCK signaling pathway plays an important role in mitochondria-mediated neuronal apoptosis [23]. Meanwhile, the majority of Rho proteins appear to be concentrated in the mitochondrial membranes while a small fraction is seen in the cytoplasm and in the cytosolic lysosomal compartments, suggesting that rho protein(s) might be involved in the mitochondrial function or assembly [24].

On the other hand, PQ induced the generation of ROS and led to oxidative stress damage-related cell death and inflammation [25], while GSH plays a protective role in the ROS generation in PQ-induced oxidative stress [26,27]. Our results also demonstrated that L-F001 inhibits the declined of intracellular level of GSH induced by PQ, suggesting that the protection of L-F001 might also be GSH-dependent. Recently, many studies demonstrated that both mitochondrial dysfunction and ER stress–mediated cell death pathways are involved in toxic chemical (arsenic)-induced apoptosis in mammalian cells by increasing the Bax/Bcl-2 ratio [28–30], which are involved in the pathogenesis of PD [31]. PD features oxidative stress and accumulation of ER stress-related proteins such as CHOP and GRP78 associated loss of dopaminergic neurons [32–34]. As well known that CHOP is a transcription factor downstream of PRKR-like ER kinase (PERK)/ATF4 which decreased Bcl-2 transcription and promote ROS production. It has been reported that PERK pathway activation and inhibition of the expression of ROCK-1 and ROCK-2 in ER stress early reduced the loss of retinal ganglion cells (RGCs) in glaucoma rat model [35]. However, whether ROCK inhibitor regulating ER stress are unknown. Our results indicated that PQ also quickly induced alterations of GRP78 and CHOP and subsequently dysfunction of the mitochondria (such as the decrease in membrane potential and increase in ROS). And the mechanism of ROCK inhibitor L-F001 in preventing PQ-induced apoptosis might be mediated by ER stress and mitochondrial dysfunction, which respectively inhibited upregulation of GRP78 and CHOP elicited by PQ. However, we currently have no direct evidence to prove that L-F001 primarily acts on ER stress and/or through other oxidative stress (such as mitochondrial ROS formation), which results in ER stress and mitochondrial dysfunction blockade to prevent PQ cytotoxicity. Further investigations are required to answer these questions.

Taken together, our results indicate that L-F001 exerts strikingly protective effects against PQ-induced cytotoxicity in PC12 cells through attenuating ER stress and mitochondrial dysfunction. Thus, L-F001 could be a promising candidate for the PQ-induced toxicity as well as other related neurodegenerative diseases [12,36].

Conflict of interest

The authors declare no conflict of interests.

Acknowledgments

This study was supported by Programme of young medical backbone personnel in Wuhan (2014) to W. Shen, Guangdong Provincial International Cooperation Project of Science & Technology (No. 2013B051000038) to R. Pi and Natural Science Foundation of Jiangxi Province (No. 20151BAB215030)/Key projects of Natural Science Foundation of Jiangxi Province (No. 20151BDH80081) to R. Wang.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.035.

References

[1] R.L. Nussbaum, C.E. Ellis, Alzheimer's disease and Parkinson's disease, N. Engl. J. Med. 348 (2003) 1356–1364.
[2] C.W. Olanow, W.G. Tatton, Etiology and pathogenesis of Parkinson's disease, Annu. Rev. Neurosci. 22 (1999) 123–144.
[3] C. Henchcliffe, M.F. Beal, Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis, Nat. Clin. Pract. Neurol. 4 (2008) 600–609.
[4] P. Jenner, Parkinson's disease, pesticides and mitochondrial dysfunction, Trends Neurosci. 24 (2001) 245–247.
[5] C.M. Tanner, F. Kamel, G.W. Ross, J.A. Hoppin, S.M. Goldman, M. Korell, C. Marras, G.S. Bhudhikanok, M. Kasten, A.R. Chade, K. Comyns, M.B. Richards, C. Meng, B. Priestley, H.H. Fernandez, F. Cambi, D.M. Umbach, A. Blair, D.P. Sandler, J.W. Langston, Rotenone, paraquat, and Parkinson's disease, Environ. Health Perspect. 119 (2011) 866–872.
[6] C. Berry, C. La Vecchia, P. Nicotera, Paraquat and Parkinson's disease, Cell Death Differ. 17 (2010) 1115–1125.
[7] H. Klintworth, K. Newhouse, T. Li, W.S. Choi, R. Faigle, Z. Xia, Activation of c-Jun N-terminal protein kinase is a common mechanism underlying paraquat- and rotenone-induced dopaminergic cell apoptosis, Toxicol. Sci. 97 (2007) 149–162.
[8] J.R. Cannon, J.T. Greenamyre, Neurotoxic in vivo models of Parkinson's disease recent advances, Prog. Brain Res. 184 (2010) 17–33.
[9] A.L. McCormack, D.A. Di Monte, Effects of L-dopa and other amino acids against paraquat-induced nigrostriatal degeneration, J. Neurochem. 85 (2003) 82–86.
[10] S.J. Chinta, A. Rane, K.S. Poksay, D.E. Bredesen, J.K. Andersen, R.V. Rao, Coupling endoplasmic reticulum stress to the cell death program in dopaminergic cells: effect of paraquat, Neuromolecular Med. 10 (2008) 333–342.
[11] A. Borrajo, A.I. Rodriguez-Perez, B. Villar-Cheda, M.J. Guerra, J.L. Labandeira-Garcia, Inhibition of the microglial response is essential for the neuroprotective effects of Rho-kinase inhibitors on MPTP-induced dopaminergic cell death, Neuropharmacology 85 (2014) 1–8.
[12] J.L. Labandeira-Garcia, A.I. Rodríguez-Perez, B. Villar-Cheda, A. Borrajo, A. Dominguez-Meijide, M.J. Guerra, Rho kinase and dopaminergic degeneration: a promising therapeutic target for Parkinson's disease, Neuroscientist. (2014 Oct 16) pii: 1073858414554954, [Epub ahead of print] Review, PubMed PMID: 25323761.
[13] M. Chen, A. Liu, Y. Ouyang, Y. Huang, X. Chao, R. Pi, Fasudil and its analogs: a new powerful weapon in the long war against central nervous system disorders? Expert Opin. Invest. drugs 22 (2013) 537–550.
[14] R. Guan, X. Xu, M. Chen, H. Hu, H. Ge, S. Wen, S. Zhou, R. Pi, Advances in the studies of roles of Rho/Rho-kinase in diseases and the development of its inhibitors, Eur. J. Med. Chem. 70 (2013) 613–622.
[15] K. Yamashita, Y. Kotani, Y. Nakajima, M. Shimazawa, S. Yoshimura, S. Nakashima, T. Iwama, H. Hara, Fasudil, a Rho kinase (ROCK) inhibitor, protects against ischemic neuronal damage in vitro and in vivo by acting directly on neurons, Brain Res. 1154 (2007) 215–224.
[16] M. Chen, A. Liu, M. Tan, Z. Xie, A. Uri, Z. Chen, G. Huang, Y. Sun, H. Ge, P. Liu, M. Li, X. Li, S. Wen, R. Pi, Simply combining fasudil and lipoic acid in a novel multitargeted chemical entity potentially useful in central nervous system disorders, RSC Adv. 4 (2014) 37266–37269.
[17] R. Wang, J. Yang, L. Peng, J. Zhao, N. Mu, J. Huang, P. Lazarovici, H. Chen, W. Zheng, Gardenamide A attenuated cell apoptosis induced by serum deprivation insult via the ERK1/2 and PI3K/AKT signaling pathways, Neuroscience 286 (2015) 242–250.
[18] L. Tonges, T. Frank, L. Tatenhorst, K.A. Saal, J.C. Koch, E.M. Szego, M. Bahr, J.H. Weishaupt, P. Lingor, Inhibition of rho kinase enhances survival of dopaminergic neurons and attenuates axonal loss in a mouse model of Parkinson's disease, Brain J. Neurol. 135 (2012) 3355–3370.
[19] C.I. Dubreuil, M.J. Winton, L. McKerracher, Rho activation patterns after spinal cord injury and the role of activated Rho in apoptosis in the central nervous system, J. Cell Biol. 162 (2003) 233–243.
[20] B. Villar-Cheda, A. Dominguez-Meijide, B. Joglar, A.I. Rodriguez-Perez, M.J. Guerra, J.L. Labandeira-Garcia, Involvement of microglial RhoA/Rho-kinase pathway activation in the dopaminergic neuron death. Role of angiotensin via angiotensin type 1 receptors, Neurobiol. Dis. 47 (2012) 268–279.
[21] K.A. Orlando, R.N. Pittman, Rho kinase regulates phagocytosis, surface expression of GlcNAc, and Golgi fragmentation of apoptotic PC12 cells, Exp. Cell Res. 312 (2006) 3298–3311.
[22] K.A. Orlando, N.L. Stone, R.N. Pittman, Rho kinase regulates fragmentation and phagocytosis of apoptotic cells, Exp. Cell Res. 312 (2006) 5–15.
[23] Y. Zhang, X. Gu, X. Yuan, Phenylalanine activates the mitochondria-mediated apoptosis through the RhoA/Rho-associated kinase pathway in cortical neurons, Eur. J. Neurosci. 25 (2007) 1341–1348.
[24] C. Hauser, S. Avraham, H. Avraham, Association of rho proteins with the mitochondria organelle, Int. J. Oncol. 3 (1993) 1103–1110.

[25] S. Liu, K. Liu, Q. Sun, W. Liu, W. Xu, P. Denoble, H. Tao, X. Sun, Consumption of hydrogen water reduces paraquat-induced acute lung injury in rats, J. Biomed. Biotechnol. 2011 (2011) 305086.
[26] S.G. Konstantinova, E.M. Russanov, Studies on paraquat-induced oxidative stress in rat liver, Acta Physiologica Pharmacol. Bulg. 24 (1999) 107–111.
[27] J.D. Adams Jr., B.H. Lauterburg, J.R. Mitchell, Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress, J. Pharmacol. Exp. Ther. 227 (1983) 749–754.
[28] Y.P. Yen, K.S. Tsai, Y.W. Chen, C.F. Huang, R.S. Yang, S.H. Liu, Arsenic induces apoptosis in myoblasts through a reactive oxygen species-induced endoplasmic reticulum stress and mitochondrial dysfunction pathway, Arch. Toxicol. 86 (2012) 923–933.
[29] C.L. Huang, Y.C. Lee, Y.C. Yang, T.Y. Kuo, N.K. Huang, Minocycline prevents paraquat-induced cell death through attenuating endoplasmic reticulum stress and mitochondrial dysfunction, Toxicol. Lett. 209 (2012) 203–210.
[30] H. Mortiboys, K.K. Johansen, J.O. Aasly, O. Bandmann, Mitochondrial impairment in patients with Parkinson disease with the G2019S mutation in LRRK2, Neurology 75 (2010) 2017–2020.
[31] B. Li, L. Xiao, Z.Y. Wang, P.S. Zheng, Knockdown of STIM1 inhibits 6-hydroxydopamine-induced oxidative stress through attenuating calcium-dependent ER stress and mitochondrial dysfunction in undifferentiated PC12 cells, Free Radic. Res. 48 (2014) 758–768.
[32] S.J. Chinta, J.K. Andersen, Dopaminergic neurons, Int. J. Biochem. Cell Biol. 37 (2005) 942–946.
[33] W.A. Holtz, K.L. O'Malley, Parkinsonian mimetics induce aspects of unfolded protein response in death of dopaminergic neurons, J. Biol. Chem. 278 (2003) 19367–19377.
[34] E.J. Ryu, H.P. Harding, J.M. Angelastro, O.V. Vitolo, D. Ron, L.A. Greene, Endoplasmic reticulum stress and the unfolded protein response in cellular models of Parkinson's disease, J. Neurosci. 22 (2002) 10690–10698.
[35] X.B. Sun, H.E. Lu, Y. Chen, X.H. Fan, B. Tong, Effect of lithium chloride on endoplasmic reticulum stress-related PERK/ROCK signaling in a rat model of glaucoma, Die Pharm. 69 (2014) 889–893.
[36] M. Raad, T. El Tal, R. Gul, S. Mondello, Z. Zhang, R.M. Boustany, J. Guingab, K.K. Wang, F. Kobeissy, Neuroproteomics approach and neurosystems biology analysis: ROCK inhibitors as promising therapeutic targets in neurodegeneration and neurotrauma, Electrophoresis 33 (2012) 3659–3668.