Biochemical and Biophysical Research Communications 457 (2015) 95–100

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Docosahexaenoic acid prevents paraquat-induced reactive oxygen species production in dopaminergic neurons via enhancement of glutathione homeostasis

Hyoung Jun Lee [a,1], Jeongsu Han [a,1], Yunseon Jang [a], Soo Jeong Kim [a], Ji Hoon Park [a], Kang Sik Seo [a], Soyeon Jeong [a,b], Soyeon Shin [a,b], Kyu Lim [a,b], Jun Young Heo [c,*], Gi Ryang Kweon [a,b,*]

[a] Department of Biochemistry, College of Medicine, Chungnam National University, Daejeon, Republic of Korea
[b] Infection Signaling Network Research Center, Chungnam National University, Daejeon, Republic of Korea
[c] Brainscience Institute, Chungnam National University, Daejeon, Republic of Korea

ARTICLE INFO

Article history:
Received 12 December 2014
Available online 27 December 2014

Keywords:
Parkinson's disease
Docosahexaenoic acid
Paraquat
Glutathione homeostasis

ABSTRACT

Omega-3 polyunsaturated fatty acid levels are reduced in the substantia nigra area in Parkinson's disease patients and animal models, implicating docosahexaenoic acid (DHA) as a potential treatment for preventing Parkinson's disease and suggesting the need for investigations into how DHA might protect against neurotoxin-induced dopaminergic neuron loss. The herbicide paraquat (PQ) induces dopaminergic neuron loss through the excessive production of reactive oxygen species (ROS). We found that treatment of dopaminergic SN4741 cells with PQ reduced cell viability in a dose-dependent manner, but pretreatment with DHA ameliorated the toxic effect of PQ. To determine the toxic mechanism of PQ, we measured intracellular ROS content in different organelles with specific dyes. As expected, all types of ROS were increased by PQ treatment, but DHA pretreatment selectively decreased cytosolic hydrogen peroxide content. Furthermore, DHA treatment-induced increases in glutathione reductase and glutamate cysteine ligase modifier subunit (*GCLm*) mRNA expression were positively correlated with glutathione (GSH) content. Consistent with this increase in *GCLm* mRNA levels, Western blot analysis revealed that DHA pretreatment increased nuclear factor-erythroid 2 related factor 2 (Nrf2) protein levels. These findings indicate that DHA prevents PQ-induced neuronal cell loss by enhancing Nrf2-regulated GSH homeostasis.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN) area [1]. Sporadic PD is linked to environmental factors including pesticides, solvents, metals, carbon monoxide, and herbicides. In particular, the herbicide paraquat (PQ) has been linked to an increased incidence of PD through an excessive production of reactive oxygen species (ROS) [2,3], which leads to oxidative challenges in the brain such as increased DNA damage, lipid peroxidation, and mitochondrial dysfunction [4]. Thus, maintaining a balance of intracellular ROS content is essential for preventing the progression of PD.

Docosahexaenoic acid (DHA) is the major omega-3 polyunsaturated fatty acid (ω-3 PUFA) found in the phospholipid fraction of the brain [5,6]. PUFA appears to be essential for cognition through its regulation of neuronal activity, as declines in membrane PUFA concentrations lead to cognitive impairment [7]. Several studies indicate that levels of DHA and other PUFAs are reduced in brain tissue of PD patients and animal models [8]. Importantly, DHA cannot be synthesized *de novo* in mammals due to the lack of a specific enzyme that converts ω-6 into ω-3 PUFA [9]. Therefore, a shortage of ω-3 PUFA in the brain must be offset through dietary intake. According to previous studies, administration of DHA has neuroprotective actions in animal models of PD [10,11]. Specifically, Bousquet et al. report that a diet high in ω-3 PUFA increases the number of tyrosine hydroxylase-immunoreactive neurons in the SN and dopamine content in the striatum following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment [12].

* Corresponding authors at: Department of Biochemistry, College of Medicine, Chungnam National University, Munhwa-dong, Jungu, Daejeon 301-747, Republic of Korea. Fax: +82 42 580 8121.
E-mail addresses: junyoung3@gmail.com (J.Y. Heo), mitochondria@cnu.ac.kr (G.R. Kweon).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.12.085
0006-291X/© 2014 Elsevier Inc. All rights reserved.

However, the protective mechanism of DHA in models of PQ-induced PD needs to be clarified.

Nrf2 protects neurons from acute injury and counteracts ROS-mediated damage in neurodegenerative diseases. In response to oxidative stress, Nrf2 dissociates from its cytoplasmic inhibitor Keap1 and then translocates into the nucleus, where it binds to the antioxidant responsive elements in the promoters of target genes [13,14]. This leads to transcriptional induction of several cellular defense genes, such as glutathione biosynthetic enzymes (e.g., *GCLm* and *GCLc*) and GSH-dependent antioxidant enzymes (e.g., *glutathione peroxidase 2, glutathione S-transferases*).

The upregulation of antioxidant enzyme capacity through the administration of DHA ameliorates organelle injury and cellular toxicity [15,16]. For instance, DHA increases the activity of heme oxygenase-1 in rats with renal injury or cerebral ischemia. Also, administration of DHA induces the upregulation of thioredoxin and glutathione (GSH) in hippocampal cells, leading to an attenuation of amyloid beta-induced neuronal toxicity [17].

Based on previous studies, we expected that DHA may help prevent PQ-induced PD by enhancement of total GSH expression via DHA related Nrf2 induction. However, the relationship between DHA and antioxidant enzymes within the context of PD remains unclear. The aim of the present study was to test whether DHA can overcome PQ-induced ROS production by changing the antioxidant content of dopaminergic neurons, which would suggest a role for DHA as an alternative preventative therapy for PD.

2. Materials and methods

2.1. Reagents

DHA was purchased from Cayman Chemical (Ann Arbor, MI, USA). PQ and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). MitoSOX™, dihydroethidium (DHE), dichlorofluorescein-diacetate (DCF-DA), anti-Nrf2 antibody, and mBCL were purchased from Invitrogen (Camarillo, CA, USA). Anti-β-actin rabbit antibody was purchased from Santa Cruz (Santa Cruz, CA, USA). Trizol was purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Cell lines and culture conditions

The dopaminergic neuronal cell line SN4741 has been previously described [18]. SN4741 cells were maintained in RF medium, which was comprised of Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Camarillo, CA, USA) supplemented with 10% fetal bovine serum, 1% glucose, L-glutamine (2 mM), and penicillin–streptomycin. Cells were typically incubated at 33 °C with 5% CO_2 as described previously [18].

2.3. Cell viability and cytotoxicity

Cell viability was assessed by MTT assay as previously described [19]. Cells were cultured at 4.5×10^3 cells per well in 96-well cell culture plates. Cultured cells were treated with PQ (100–600 μM) for 24 h with or without pretreatment of 25 μM DHA, eicosapentaenoic acid (EPA), or arachidonic acid (AA) for 3 h. The treated cells were incubated with 0.5 mg/ml MTT solution for 1 h and then dissolved in DMSO and measured at 540 nm using a MultiSkan Ascent microplate reader (Thermo Electron Corporation, Bremen, Germany).

2.4. Detection of intracellular ROS

ROS generation was analyzed using the fluorescent dyes MitoSOX, DCF-DA, and DHE. Briefly, after exposure to 400 μM PQ for 24 h with or without 25 μM DHA pretreatment for 3 h, cells were incubated with one of the fluorescent dyes (10 μM) in Krebs-HEPES buffer (pH 7.4) at 33 °C for 20 min. Next, cells were washed with Hank's Balanced Salt Solution (HBSS) and identified by fluorescence microscopy (Olympus, Tokyo, Japan). Other cells were detached using 0.05% trypsin–EDTA, centrifuged in tubes, and resuspended in HBSS buffer (pH 7.4). Cells were identified using a FACScan (BD Bio-science, San Jose, CA, USA), and data analysis was performed using a FACSDiva (BD Bio-science, San Jose, CA, USA).

2.5. Changes in intracellular GSH content

Monochlorobimane (mBCl) is a specific probe that is used to measure intracellular GSH content in dopaminergic neurons [20]. Cultured cells were exposed to 400 μM PQ for 24 h in the presence or absence of 25 μM DHA pretreatment for 3 h. Treated cells were then exposed to 100 μM mBCl for 1 h in a buffer including 2 mM $CaCl_2$, 5 mM HEPES, 140 mM NaCl, 10 mM glucose, 6 mM KCl, 1 mM $MgCl_2$, and 100 μM mBCl at room temperature. Intracellular GSH content was identified by fluorescence microscopy.

2.6. Western blot analysis

SN4741 cells were cultured at 5×10^5 cells per well in 6-well cell culture plates. SN4741 cells were exposed to 400 μM PQ for 24 h with or without 25 μM DHA pretreatment for 3 h. Proteins were extracted in RIPA lysis buffer (100 mM Tris–HCl (pH 8.5), 200 mM NaCl, 5 mM EDTA, and 0.2% SDS). Quantification of protein levels was performed using the Bradford method [21]. Isolated protein (20 μg) was resolved using 12% SDS–PAGE and transferred onto polyvinylidene fluoride membranes, which were blocked with 5% skim milk in TBST (10 mM Tris–HCl (pH 7.6), 150 mM NaCl, and 0.1% Tween 20). The membranes were incubated with primary antibodies against Nrf2 (1:1000) and β-actin (1:5000), and proteins were detected with horseradish peroxidase-coupled secondary antibody according to the manufacture's protocol. Detection of antibody-labeled proteins was performed using an ECL chemiluminescence system (INtRON BioTechnology, Korea).

2.7. Real-time polymerase chain reaction (PCR) analysis

Total RNA was isolated using Trizol according to the manufacturer's instructions. Real-time quantitative PCR was performed using cDNA, SYBR Green PCR Master Mix (iCycleriQ Real-Time PCR Detection System; Bio-Rad), and specific primers. Primers and their sequences were as follows: *SOD1* F (5′-GAGACCTGGGCAATGTGACT-3′), *SOD1* R (5′-GTTTACTGCGCAATCCCAAT-3′), *SOD2* F (5′-CCGAGGAGAAGTACCACGAG-3′), *SOD2* R (5′-GCTTGATAGCCTCCAGCAAC-3′), *Catalase* F (5′-ACATGGTCTGGGACTTCTGG-3′), *Catalase* R (5′-CAAGTTTTGATGCCCTGGT-3′), *GR* F (5′-CACGACCATGATTCCAGATG-3′), *GR* R (5′-CAGCATAGACGCCTTTGACA), *GPx1* F (5′-GTCCACCGTGTATGCCTTCT-3′), *GPx1* R (5′-TCTGCAGATCGTTCATCTCG-3′), *GCLm* F (5′-TGGAGCAGCTGTATCAGTGG-3′), and *GCLm* R (5′AGAGCAGTTCTTT CGGGTCA-3′). All primers were designed by the Primer3 program with an appropriated size (less than 150 bp) for the Rotor-Gene 6000 real time instrument. Relative expression of these genes was quantified and normalized to 18s ribosomal RNA using Rotor-Gene 6000 real-time rotary analyzer software (Qiagen, CA, USA).

2.8. Statistical analysis

All results were obtained from at least three independent experiments. Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). Data are

shown as mean ± standard error of the mean (SEM). Comparisons between groups were performed using one-way analysis of variance (ANOVA). A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. Prevention of PQ-induced cytotoxicity by DHA treatment in dopaminergic neurons

DHA, the major ω-3 PUFA, has neuroprotective effects in a 6-OHDA-induced model of PD [22], but no study has demonstrated a role of DHA in PQ-induced dopaminergic neuron loss. First, to evaluate the viability of dopaminergic neurons after PQ treatment, we performed MTT assay, which showed that PQ decreased the number of viable cells in a dose-dependent manner (Fig. 1A). Before verifying the protective effect of DHA in dopaminergic neurons, we optimized the duration (3, 6, or 24 h) and concentration (0–25 μM) of DHA treatment. Because 25 μM DHA treatment for 24 h had no effect on cell survival (Fig. S1A), and DHA in the absence of cells did not interfere with MTT activity (data not shown), we used these treatment parameters in subsequent experiments. Pretreatment with DHA enhanced cell survival by 30% for dopaminergic neurons exposed to 400 μM PQ (Fig. 1B). Furthermore, pretreatment with EPA, another ω-3 PUFA, improved the survival of dopaminergic neurons exposed to 600 uM PQ, but treatment with AA, a ω-6 PUFA, had no effect (Fig. S1B and C). These results indicate that administration of DHA improved the viability of PQ-exposed dopaminergic neurons.

3.2. Attenuation of PQ-induced ROS production by DHA treatment in dopaminergic neurons

One previous study reports that PQ induces ROS production via redox cycling [23]. Thus, we hypothesized that DHA may exert antioxidant effects by protecting against PQ-induced ROS production. Using the fluorescent dyes Mitosox, DHE, and DCF-DA to detect intracellular ROS, we measured fluorescence intensity in dopaminergic neurons after treatment of PQ with or without DHA pretreatment. We found that PQ treatment elevated mitochondrial and cytosolic superoxide and cytosolic hydrogen peroxide and that DHA pretreatment specifically attenuated PQ-induced cytosolic hydrogen peroxide production, evidenced by decreased DCF-DA intensity (Fig. 2A). To verify changes in ROS generation by administration of DHA, we performed fluorescence-activated cell sorting analysis. PQ treatment increased the intensities of all three fluorescent dyes (Figs. 2B, C and S2A, B). However, DHA administration only decreased DCF-DA fluorescence intensity, indicative of cytosolic hydrogen peroxide. These results suggest that DHA treatment attenuates PQ-mediated ROS generation.

3.3. Increase of GSH pool by DHA treatment in dopaminergic neurons

DHA enhances antioxidant enzyme activity and decreases nitric oxide production in cultured hippocampal neurons [24]. To determine whether the protective effect of DHA is related to increased expression levels of antioxidant enzymes, we measured mRNA levels of key antioxidant enzymes in dopaminergic neurons using real-time PCR. Unexpectedly, DHA treatment had no effect on *SOD1*, *SOD2*, *GPx*, or *catalase* mRNA levels. However, *GR* and *GCLm* mRNA levels were significantly increased by administration of DHA (Fig. 3A). *GCL* plays a role in attenuating oxidative damage and maintaining cellular redox homeostasis, and induction of this enzyme is regulated by the Nrf2 transcriptional factor [13,25]. Consistently, Nrf2 protein expression levels were significantly increased in dopaminergic neurons by DHA administration (Fig. 3B). As *GR* and *GCLm* are involved in intracellular GSH homeostasis [26], we next measured GSH content using cellular a GSH fluorescent dye, mBCl. As expected, GSH content was significantly increased by DHA treatment. However, cellular GSH content was not different between the DHA and DHA + PQ conditions (Fig. 3C). These findings suggest that DHA contributes to the upregulation of GSH-related enzymes *GR* and *GCL* through the Nrf2 pathway.

3.4. Abolishment of protective effect of DHA by GSH depletion in dopaminergic neurons

GR is a cellular antioxidant enzyme that reduces glutathione disulfide to the sulfhydryl form GSH [27], and *GCL* is a rate-limiting enzyme for the production of GSH from glutamate and cysteine. To verify the importance of GSH function as a preventative mechanism of DHA, we measured cell viability in the presence of inhibitors of two major enzymes involved in GSH homeostasis: buthionine sulfoximine (BSO; specific inhibitor of *GCL*) and 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU; specific inhibitor of *GR*). The protective effect of DHA against PQ was abolished by treatment of 100 μM BSO or 25 μM BCNU. Furthermore, the 60% viability of cells exposed to PQ declined to 15% and 25% with BSO and BCNU treatment, respectively (Fig. 4A and B). These results indicate that administration of DHA protects against PQ-induced dopaminergic neuronal loss through enhancing GSH homeostasis.

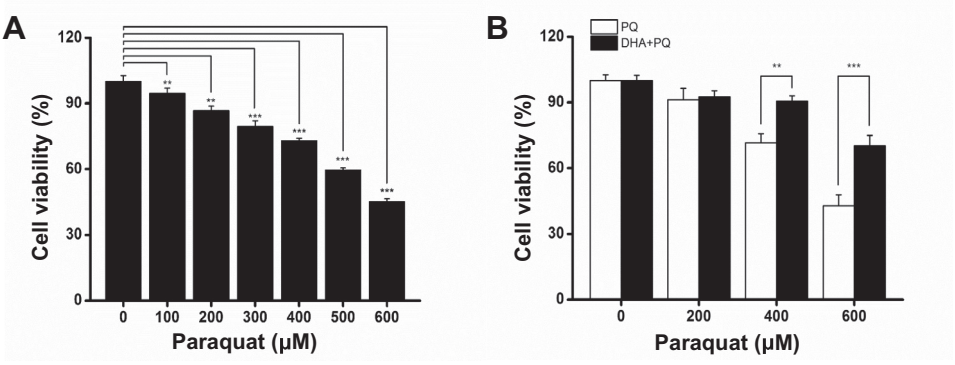

Fig. 1. DHA prevented PQ-induced cytotoxicity in dopaminergic neurons. The viability of dopaminergic neurons was measured using an MTT assay. (A) Dopaminergic neurons were exposed to increasing concentrations of PQ (0–600 μM) for 24 h ($n = 5$). (B) Dopaminergic neurons were exposed to various concentrations of PQ for 24 h with or without pretreatment with 25 μM DHA for 3 h ($n = 5$). $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA), compared with PQ only treated condition.

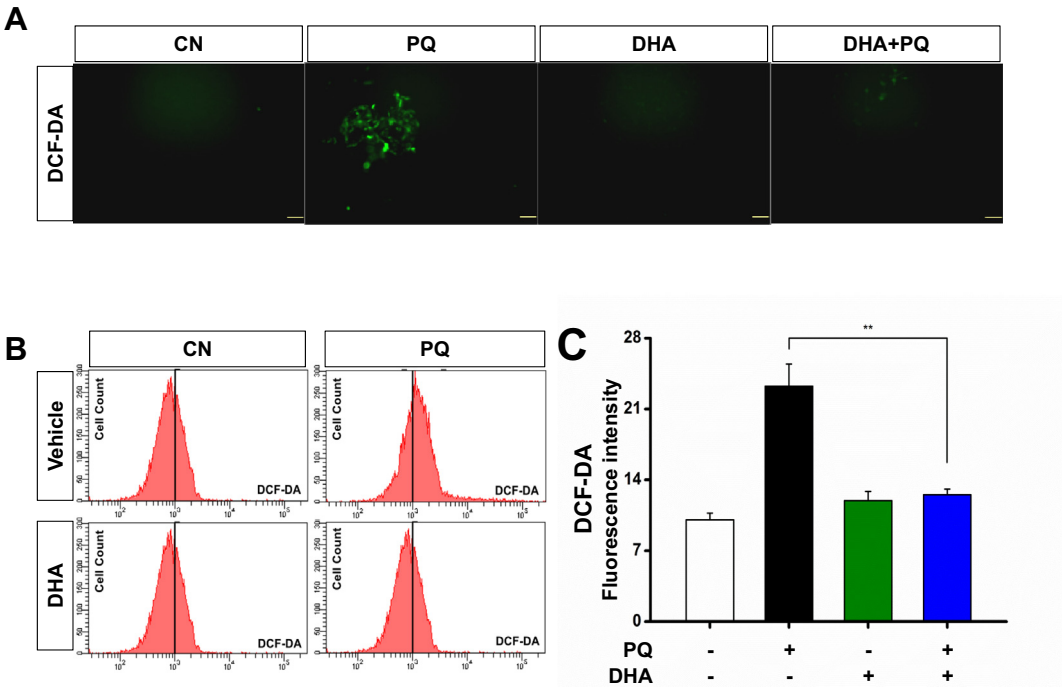

Fig. 2. DHA attenuated PQ-induced ROS production in dopaminergic neurons. Dopaminergic neurons were pretreated with 25 μM DHA for 3 h and treated with 400 μM PQ for 24 h. Cells were then stained with DCF-DA. (A) To identify ROS production, cellular ROS marker DCF-DA signals were assessed using a fluorescent microscope (100×). Scale bars: 100 μm. (B) And measured using flow cytometry. (C) Quantification of DCF-DA fluorescence intensity ($n = 5$). ns, not significant, $^{**}P < 0.01$ (one-way ANOVA), compared with PQ only treated condition.

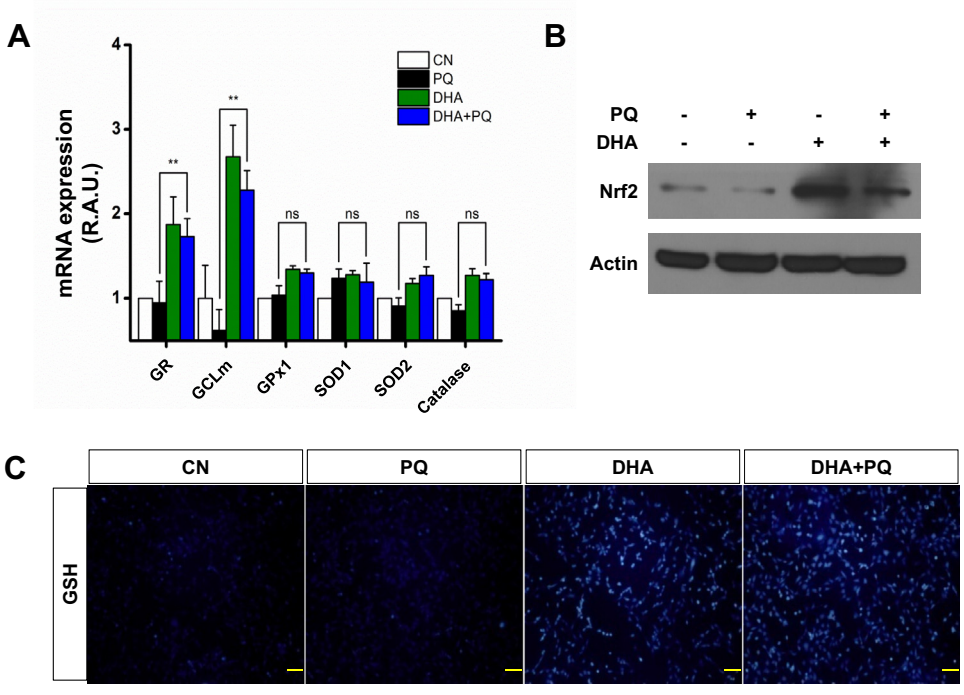

Fig. 3. DHA increased antioxidant enzyme GR and GSH content by elevating Nrf2 expression levels. Dopaminergic neurons were exposed to 400 μM PQ for 24 h with or without 25 μM DHA pretreatment for 3 h. (A) mRNA levels of *GR*, *GCLm*, *GPx1*, *SOD1*, *SOD2*, and *catalase* were measured using real-time PCR ($n = 3$). (B) Protein expression levels of Nrf2 were analyzed by Western blot analysis. Protein expression of β-actin was used as a loading control. (C) To identify cellular GSH expression, SN4741 cells were stained with the GSH-specific dye mBCL, and fluorescence was measured using fluorescence microscopy (×100). Scale bars: 100 μm. $^{**}P < 0.01$ (one-way ANOVA), compared with PQ only treated condition.

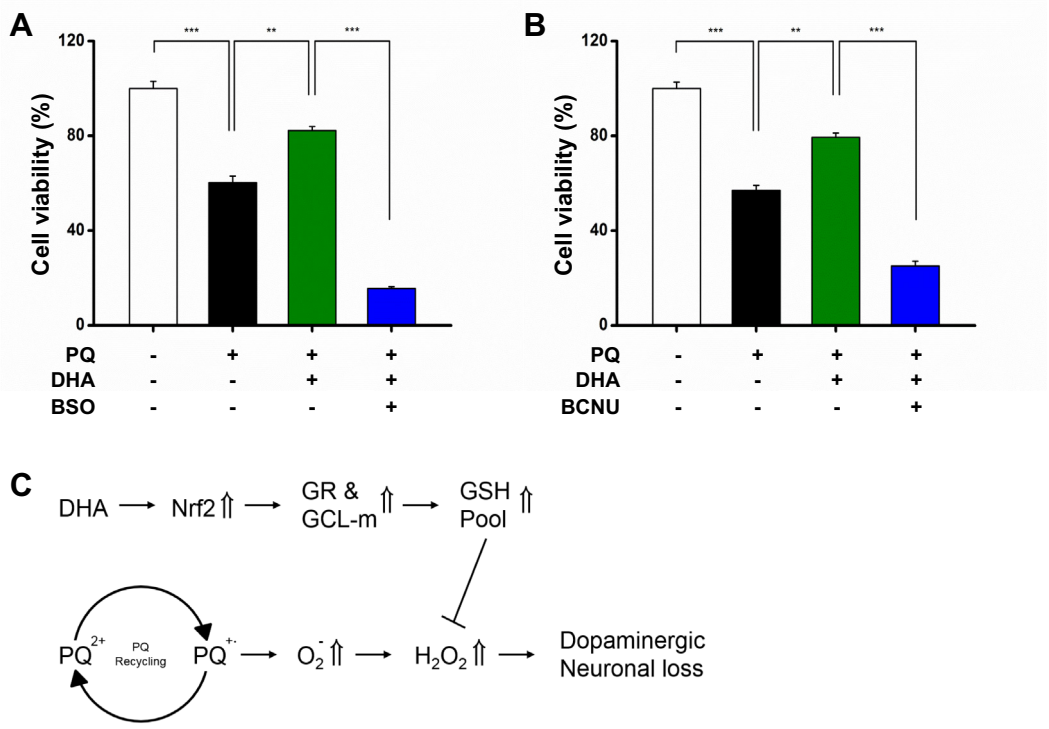

Fig. 4. DHA protected against PQ-induced toxicity through maintenance of GSH content. (A) Dopaminergic neurons were pretreated with 100 μM BSO for 6 h to inhibit *GCL* and were then treated with 25 μM DHA for 3 h followed by incubation with 400 μM PQ for 24 h ($n = 5$). (B) Dopaminergic neurons were pretreated with 25 μM BCNU for 6 h to inhibit *GR* and were then treated with DHA for 3 h followed by incubation with 400 μM PQ for 24 h. Cell viability was assessed using an MTT assay ($n = 5$). (C) A schematic overview of the protective role of DHA against PQ-induced neuronal cell loss on the basis of our results. $^{**}P < 0.01$ (one-way ANOVA), compared with each condition.

4. Discussion

We show that DHA has a protective role in controlling dopaminergic neuronal ROS removal. DHA increased GR and *GCLm* mRNA expression via Nrf2 against PQ-induced oxidative stress, and enhanced the accumulation of the intracellular GSH pool, which has a defensive role in cellular redox homeostasis through hydrogen peroxide elimination, thereby avoiding intracellular accumulation of excessive ROS and subsequent PQ-induced toxicity (Fig. 4C).

DHA, a major ω-3 PUFA in phospholipid fractions of the brain, is essential for maintaining normal neuronal functions and is thought to be effective in preventing many chronic diseases [28]. Previous studies report that DHA protects against neurotoxin-induced dopaminergic neuronal cell death [12,29]. Moreover, DHA levels are reduced in the SN region of PD patients compared to normal control tissue [8]. In the present study, we demonstrated that DHA protects against PQ-induced toxicity in dopaminergic neurons through modulating GSH homeostasis.

Severe oxidative stress leads to cellular GSH depletion in many human diseases [13], and low concentrations of GSH are detected in the SN area in a PD model [30]. In hippocampal neurons, the administration of DHA upregulates thioredoxin reductase and GSH-related antioxidant enzymes (e.g., *GPx*, *GR*) [17]. Furthermore, oral administration of DHA in aged rats potentiates *SOD* and *GPx* activity [31]. However, we found that DHA treatment only increased mRNA levels of *GR* and *GCLm*, which are regarded as key regulatory enzymes that modulate GSH content in dopaminergic neurons.

To clarify the relationship between GSH depletion and expression of the two major enzymes involved in GSH homeostasis, *GCL* and *GR*, we employed the specific inhibitors BSO and BCNU. Inhibition of *GCL* and *GR* accelerated PQ-induced cell loss and abolished the protective effect of DHA. On the other hand, GSH content was increased by administration of DHA. Therefore, DHA may protect against PQ-induced dopaminergic neuronal loss via upregulation of the GSH pool.

In a previous study, PQ-induced cytotoxicity was found to reduce protein levels of Nrf2 and downstream factors [32]. Although Nrf2 in known to be activated by oxidative stress, we found that DHA directly enhanced expression of Nrf2 independent of oxidative stress. In our study, despite the induction of oxidative stress, Nrf2-induced *GCLm* and *GR* mRNA expression, and intracellular GSH pools, did not show any difference between the DHA and DHA + PQ conditions in dopaminergic neuronal cells. We surmise that these phenomena are a result of low dose (400 μM) treatment of PQ in experiments. Although treatment with 400 μM PQ causes neuronal cell loss, the level of ROS production might not be sufficiently high enough to show any difference in the GSH pool. If PQ has been processed at high concentrations (>500 μM), these phenomena might represent a big difference between the DHA and DHA + PQ conditions via excessive ROS-induced GSH depletion by high dose PQ. DHA treatment increased Nrf2 protein levels, which was consistent with increased *GR* and *GCL* expression levels. Specifically, only the *GCLm* subunit was upregulated by DHA treatment. As low expression of the *GCLm* subunit in Nrf2 knock-out mice contributes to GSH depletion [26], this suggests that *GCLm* is regulated by Nrf2 expression after DHA treatment in dopaminergic neurons.

The herbicide PQ induces dopaminergic neuronal cell death through excessive ROS production [2,3,23]. We previously reported that PQ induces excessive mitochondrial superoxide production in dopaminergic neurons via mitochondrial complex I, which leads to an increase of cytosolic ROS [33]. In the present study, we confirmed PQ-induced ROS production with three different fluorescent

dyes and found an increase in not only mitochondrial superoxide, but also cytosolic superoxide and hydrogen peroxide. Interestingly, we conclude that DHA treatment selectively reduced cytosolic hydrogen peroxide. Because the GSH is known to reduce hydrogen peroxide to water through the glutathione-ascorbate cycle, our results show that DHA specifically diminished the DCF-DA intensity, which is an indication of total cellular ROS, but not the Mitosox and DHE, which detect mitochondrial and cytosolic superoxide, respectively. These results suggest that DHA partially rescued the PQ-induced cell toxicity. Due to differences in working sites between PQ and DHA, DHA might be more effective in preventing the effects of other PD-related neurotoxins such as lipopolysaccharides.

On the basis of the present results, and as shown in Fig. 4C, we propose that DHA serves a protective function in dopaminergic neurons against PQ-induced oxidative stress, and suggest a role for DHA as an alternative preventative therapy for PD.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Science, ICT & Future Planning (MSIP) (2012R1A2A2A01046957) and research fund of Chungnam National University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.085.

References

[1] G.W. Ross, H. Petrovitch, R.D. Abbott, J. Nelson, W. Markesbery, D. Davis, J. Hardman, L. Launer, K. Masaki, C.M. Tanner, L.R. White, Parkinsonian signs and substantia nigra neuron density in decendents elders without PD, Ann. Neurol. 56 (2004) 532–539.
[2] P.R. Castello, D.A. Drechsel, M. Patel, Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain, J. Biol. Chem. 282 (2007) 14186–14193.
[3] Z.E. Suntres, Role of antioxidants in paraquat toxicity, Toxicology 180 (2002) 65–77.
[4] A. Czerniczyniec, A.G. Karadayian, J. Bustamante, R.A. Cutrera, S. Lores-Arnaiz, Paraquat induces behavioral changes and cortical and striatal mitochondrial dysfunction, Free Radic. Biol. Med. 51 (2011) 1428–1436.
[5] K.A. Youdim, A. Martin, J.A. Joseph, Essential fatty acids and the brain: possible health implications, Int. J. Dev. Neurosci. 18 (2000) 383–399.
[6] M. Akbar, F. Calderon, Z. Wen, H.Y. Kim, Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival, Proc. Natl. Acad. Sci. USA 102 (2005) 10858–10863.
[7] A.L. Petursdottir, S.A. Farr, J.E. Morley, W.A. Banks, G.V. Skuladottir, Effect of dietary n-3 polyunsaturated fatty acids on brain lipid fatty acid composition, learning ability, and memory of senescence-accelerated mouse, J. Gerontol. A Biol. Sci. Med. Sci. 63 (2008) 1153–1160.
[8] D.T. Dexter, C.J. Carter, F.R. Wells, F. Javoy-Agid, Y. Agid, A. Lees, P. Jenner, C.D. Marsden, Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease, J. Neurochem. 52 (1989) 381–389.
[9] M. Bousquet, K. Gue, V. Emond, P. Julien, J.X. Kang, F. Cicchetti, F. Calon, Transgenic conversion of omega-6 into omega-3 fatty acids in a mouse model of Parkinson's disease, J. Lipid Res. 52 (2011) 263–271.
[10] G. Hacioglu, Y. Seval-Celik, G. Tanriover, O. Ozsoy, E. Saka-Topcuoglu, S. Balkan, A. Agar, Docosahexaenoic acid provides protective mechanism in bilaterally MPTP-lesioned rat model of Parkinson's disease, Folia Histochem. Cytobiol. 50 (2012) 228–238.
[11] G.P. Eckert, U. Lipka, W.E. Muller, Omega-3 fatty acids in neurodegenerative diseases: focus on mitochondria, Prostaglandins Leukot. Essent. Fatty Acids 88 (2013) 105–114.
[12] M. Bousquet, M. Saint-Pierre, C. Julien, N. Salem Jr., F. Cicchetti, F. Calon, Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's disease, FASEB J. 22 (2008) 1213–1225.
[13] S.C. Lu, Glutathione synthesis, Biochim. Biophys. Acta 2013 (1830) 3143–3153.
[14] T. Nguyen, P.J. Sherratt, P. Nioi, C.S. Yang, C.B. Pickett, Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1, J. Biol. Chem. 280 (2005) 32485–32492.
[15] C. Kusunoki, L. Yang, T. Yoshizaki, F. Nakagawa, A. Ishikado, M. Kondo, K. Morino, O. Sekine, S. Ugi, Y. Nishio, A. Kashiwagi, H. Maegawa, Omega-3 polyunsaturated fatty acid has an anti-oxidant effect via the Nrf-2/HO-1 pathway in 3T3-L1 adipocytes, Biochem. Biophys. Res. Commun. 430 (2013) 225–230.
[16] S.H. Liu, C.D. Chang, P.H. Chen, J.R. Su, C.C. Chen, H.C. Chaung, Docosahexaenoic acid and phosphatidylserine supplementations improve antioxidant activities and cognitive functions of the developing brain on pentylenetetrazol-induced seizure model, Brain Res. 1451 (2012) 19–26.
[17] V. Casanas-Sanchez, J.A. Perez, N. Fabelo, A.V. Herrera-Herrera, C. Fernandez, R. Marin, M.C. Gonzalez-Montelongo, M. Diaz, Addition of docosahexaenoic acid, but not arachidonic acid, activates glutathione and thioredoxin antioxidant systems in murine hippocampal HT22 cells: potential implications in neuroprotection, J. Neurochem. (2014).
[18] J.H. Son, H.S. Chun, T.H. Joh, S. Cho, B. Conti, J.W. Lee, Neuroprotection and neuronal differentiation studies using substantia nigra dopaminergic cells derived from transgenic mouse embryos, J. Neurosci. 19 (1999) 10–20.
[19] J. van Meerloo, G.J. Kaspers, J. Cloos, Cell sensitivity assays: the MTT assay, Methods Mol. Biol. 731 (2011) 237–245.
[20] J.C. Fernandez-Checa, N. Kaplowitz, The use of monochlorobimane to determine hepatic GSH levels and synthesis, Anal. Biochem. 190 (1990) 212–219.
[21] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[22] H. Kabuto, M. Amakawa, M. Mankura, T.T. Yamanushi, A. Mori, Docosahexaenoic acid ethyl ester enhances 6-hydroxydopamine-induced neuronal damage by induction of lipid peroxidation in mouse striatum, Neurochem. Res. 34 (2009) 1299–1303.
[23] D. Bonneh-Barkay, S.H. Reaney, W.J. Langston, D.A. Di Monte, Redox cycling of the herbicide paraquat in microglial cultures, Brain Res. Mol. Brain Res. 134 (2005) 52–56.
[24] X. Wang, X. Zhao, Z.Y. Mao, X.M. Wang, Z.L. Liu, Neuroprotective effect of docosahexaenoic acid on glutamate-induced cytotoxicity in rat hippocampal cultures, NeuroReport 14 (2003) 2457–2461.
[25] M. Mani, S. Khaghani, T. Gol Mohammadi, Z. Zamani, K. Azadmanesh, R. Meshkani, P. Pasalar, E. Mostafavi, Activation of Nrf2-antioxidant response element mediated glutamate cysteine ligase expression in hepatoma cell line by homocysteine, Hepat. Mon. 13 (2013) e8394.
[26] C.J. Harvey, R.K. Thimmulappa, A. Singh, D.J. Blake, G. Ling, N. Wakabayashi, J. Fujii, A. Myers, S. Biswal, Nrf2-regulated glutathione recycling independent of biosynthesis is critical for cell survival during oxidative stress, Free Radic. Biol. Med. 46 (2009) 443–453.
[27] B. Mannervik, The enzymes of glutathione metabolism: an overview, Biochem. Soc. Trans. 15 (1987) 717–718.
[28] A.P. Simopoulos, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases, Exp. Biol. Med. (Maywood) 233 (2008) 674–688.
[29] M. Cansev, I.H. Ulus, L. Wang, T.J. Maher, R.J. Wurtman, Restorative effects of uridine plus docosahexaenoic acid in a rat model of Parkinson's disease, Neurosci. Res. 62 (2008) 206–209.
[30] G. Harish, A. Mahadevan, M.M. Srinivas Bharath, S.K. Shankar, Alteration in glutathione content and associated enzyme activities in the synaptic terminals but not in the non-synaptic mitochondria from the frontal cortex of Parkinson's disease brains, Neurochem. Res. 38 (2013) 186–200.
[31] L.H. Jiang, S. Yan, J. Wang, Q.Y. Liang, Oral administration of docosahexaenoic acid activates the GDNF-MAPK-CERB pathway in hippocampus of natural aged rat, Pharm. Biol. 51 (2013) 1188–1195.
[32] G.L. Hong, J.M. Liu, G.J. Zhao, L. Wang, G. Liang, B. Wu, M.F. Li, Q.M. Qiu, Z.Q. Lu, The reversal of paraquat-induced mitochondria-mediated apoptosis by cycloartenyl ferulate, the important role of Nrf2 pathway, Exp. Cell Res. 319 (2013) 2845–2855.
[33] H.J. Kwon, J.Y. Heo, J.H. Shim, J.H. Park, K.S. Seo, M.J. Ryu, J.S. Han, M. Shong, J.H. Son, G.R. Kweon, DJ-1 mediates paraquat-induced dopaminergic neuronal cell death, Toxicol. Lett. 202 (2011) 85–92.