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Aldose reductase deficiency leads to oxidative stress-induced dopaminergic neuronal loss and autophagic abnormality in an animal model of Parkinson's disease

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ABSTRACT

Fungicide exposure causes degeneration of dopaminergic neurons and contributes to Parkinson's disease (PD). Benomyl inhibits enzymes responsible for detoxifying the reactive dopamine metabolite 3,4-dihydroxyphenylacetaldehyde. Aldose reductase (AR) is known as tetrahydrobiopterin (BH_4) reductase that generates BH_4, a cofactor for tyrosine hydroxylase (TH) involved in dopamine synthesis. AR also acts as an aldehyde reductase involved in detoxifying 3,4-dihydroxyphenylacetaldehyde. In PD patients, the level of AR is significantly lower in the cerebellum. To determine if AR deficiency contributes to PD, AR wild-type ($AR^{+/+}$) and knockout ($AR^{-/-}$) mice were administrated with 1-methyl-4-phenyl -1,2,3,6-tetrahydropyridine (MPTP). The MPTP-treated $AR^{-/-}$ mice showed more severe behavioral deficits and brain damage than that of $AR^{+/+}$ mice. Contrary to expectation, under normal or MPTP-treated condition, $AR^{-/-}$ mice showed a significant elevation of BH_4 and dopamine in the midbrain, suggesting that either AR does not contribute to BH_4 production, or other BH_4 synthetic pathways are induced. The $AR^{-/-}$ brain showed upregulation of peroxynitrite, inducible nitric oxide synthase and downregulation of antioxidant enzymes, Cu/Zn superoxide dismutase (SOD) and peroxiredoxin 2 (Prx2), which indicate an increase in oxidative stress. In line with the animal data, pretreating the SH-SY5Y cells with AR inhibitors (Fidarestat or Epalrestat) before MPP^+ treatment, increased severe cell death and mitochondrial fragmentation with downregulation of SOD were observed when compared to the MPP^+ treatment alone. Cycloxygenase 2 (COX2), which can lead to the oxidation of dopamine, was upregulated in $AR^{-/-}$ brains. Autophagic proteins, beclin-1 and LC3B were also downregulated. The loss of dopaminergic neurons was associated with activation of p-ERK1/2. These findings suggest that AR plays an important role in protecting dopaminergic neuron against neurotoxic metabolites in PD.

1. Introduction

Environmental factors such as exposure to pesticides have been reported to cause PD (Fitzmaurice et al., 2013; Hatcher et al., 2008). Benomyl, one of the widely used fungicides, at nanomolar concentrations induces dopaminergic neuronal loss by inhibiting aldehyde dehydrogenase activity, and resulted in accumulation of the toxic aldehyde, DOPAL (Fitzmaurice et al., 2013).

Oxidative stress and free radicals from both reactive oxygen species and reactive aldehyde species induce dopaminergic cell death due to an accumulated high level of toxic aldehydes that causes protein modification and enzyme inhibition (Koutsilieri et al., 2002). The increased ratio of DOPAL to DOPAC in the post-mortem brain of PD patients suggests the reduced detoxification of DOPAL by aldehyde reductase (Goldstein et al., 2011). Decreased ratio of GSH (reduced glutathione) to GSSH (oxidized glutathione), increased iron concentration, and lipid peroxidation have been shown in PD patients (Sofic et al., 1988). In addition, the accumulation of reactive oxygen species (ROS) and reactive nitrogen species contribute to the degeneration of dopaminergic neurons (Metodiewa and Koska, 2000). Several studies show that BH_4,

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which is responsible for the biosynthesis of dopamine, contributes to oxidative stress-induced cell death (Anastasiadis et al., 2001; Kim et al., 2005). Aldose reductase (AR), one of the enzymes involves in biosynthesis of BH_4, is present in many tissues (Milstien and Kaufman, 1989; Srivastava et al., 2005). Under hyperglycemic condition, AR reduces glucose to sorbitol in pancreatic β-cells by using NADPH as a cofactor and induces oxidative stress responsible for the diabetic complications (Chung et al., 2003; Ho et al., 2006). Also, AR is also responsible for reducing toxic aldehydes that are by-products of metabolism. Therefore, AR plays a dual role in diabetic animals, that is, damages the cells by increasing oxidative stress and protects the cells by reducing the toxic aldehydes.

The fungicide benomyl interferes with aldehyde dehydrogenase leading to neurotoxic DOPAL accumulation that causes the degeneration of dopaminergic neurons (Fitzmaurice et al., 2013). Also, elevation of DOPAL level contributes to complex I inhibition and induces AR activation to form nontoxic DOPET against oxidative stress (Manna et al., 1997).

However, to date, there are no studies on the role of AR in PD using in vivo model. In cerebellar tissue of PD patients, the level of AR is significantly lower than that of cerebellar tissue of non-PD patients (Tobin et al., 2007). If AR contributes to the biosynthesis of BH_4, this may account for the low level of BH_4 observed in CSF of PD patients. Since AR is also involved in the neutralization of toxic aldehydes, the overall consequences of the reduced level of AR in the brains of PD patients is not clear. Here, we investigated possible role of AR in PD condition by comparing $AR^{+/+}$ and $AR^{-/-}$ mice that were treated with MPTP to develop PD-like symptoms (Ho et al., 2000). We found that AR deficiency led to an increase in severe neurological deficits. However, contrary to expectation, AR deficiency led to increased BH_4 and dopamine levels in the midbrain of vehicle- and MPTP-treated mice, suggesting that the increase in severe neurological deficits in MPTP-treated $AR^{-/-}$ mice were not due to depletion of BH_4, but more likely due to increased oxidative stress.

2. Materials and methods

2.1. Animals

Age-matched (10 weeks old) male littermate $AR^{+/+}$ and $AR^{-/-}$ mice in C57BL/6 genetic background were used in this study. Mice were housed in a humidity and temperature-controlled room under a 12:12-hour light:dark cycle with *ad libitum* access to chow and water. The animal experimental protocols were approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong (ID: 1763-08).

2.2. Experimental PD in $AR^{+/+}$ and $AR^{-/-}$ mice

1-methyl-4-phenyl-1,2,3,6-tetrahydopyridine (MPTP HCl, Research Biochemicals International, MA USA, Cat no. D-045) was dissolved in saline and administered subcutaneously to $AR^{+/+}$ and $AR^{-/-}$ mice with concentration of 40 mg/kg, single injection on Day 1 (Mejias et al., 2006). An equivalent volume of 0.9% saline was applied to $AR^{+/+}$ and $AR^{-/-}$ mice as control.

2.3. Behavioral assessments

Body weight was measured and several behavioral assessments, including hanging wire test, traction test and pole test were performed according to the published standard procedures every day for 7 days (Cryns et al., 2008; Rommelfanger et al., 2007; Sanberg et al., 1988; Sedelis et al., 2001). In all experiment, the person who performed the behavioral assessments was blinded to the genotypes of the mice. The hanging wire test measures mouse's grip strength. The mouse was placed on the top of a wire cage lid. After the mouse gripped onto the wire, the lid was turned upside down and held approximately 20 cm above the ground. The time the mouse could hold onto the wire was recorded. Maximum testing time was 120 seconds. Traction test measures the mouse's muscle strength and coordination. The mouse was allowed to hang onto a horizontal pole with its forepaws. Score was given depending on the mouse's coordination ability of gripping its hind legs onto the pole (3 = both hind legs, 2 = one hind leg and 1 = none of the leg). The pole test evaluates the movement disorder, and motor coordination of a mouse. The mouse was placed head-upward on the top of a vertical rough-surfaced pole of 1 cm diameter. The time it takes to orient downwards (T-turn) and the time it takes to reach the floor (locomotion activity time; T-LA) were recorded. Maximum testing time was 120 seconds.

2.4. Immunocytochemical analysis

After the behavioral assessments, the brains of mice were removed and fixed with 4% paraformaldehyde, then processed for immunocytochemical analysis. Coronal brain slices at −2.0 mm from bregma were used for immunocytochemical study. Brain sections were incubated with antibodies against tyrosine hydroxylase (1:5000, IMG-689, Imgenex, USA). Signals were visualized by Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) with 3,3′-diaminobenzidine tetrahydrochloride (Zymed, South San Francisco, CA, USA).

2.5. Western blot analysis

The midbrains (striatum and ventral mid-brain) were isolated at 6 hours and day 7 after MPTP treatment and homogenized in lysis buffer (50 mM Tris-HCL, pH 6.8, 150 mM NaCl, 5 mM EDTA, 0.5% sodium deoxycholate, 0.5% NP-40, plus proteinase inhibitor cocktail). Homogenate was centrifuged (3000 × g, 5 minutes, 4 °C) and the supernatant was used for the Western blot analysis. Blots were incubated with antibodies against TH (~60 kDa, 1:5000, IMG-689, Imgenex, USA), phospho-ERK1/2 (p-ERK1/2) (42 and 44 kDa, 1:1000, #9101, Cell Signaling, USA), Cu/Zn superoxide dismutase (16 kDa, 1:1000, 574597, Calbiochem, USA), peroxiredoxin 2 (20–30 kDa, 1: 2000, LF-MA0144, LabFrontier, Korea), transferrin (79 kDa, 1:200, sc-22579, Santa Cruz), nitrotyrosine (16, 66 and 215 kDa, 1:500, #06-284, Merck Millipore, USA), beclin-1 (60 kDa, 1:1000, #3738, Cell Signaling, USA), LC3B (14 and 16 kDa, 1:1000, #2775, Cell Signaling, USA), inducible nitric oxide synthase (iNOS; 130 kDa, 1:1000, n-20, Santa Cruz, USA), CRIF1 (24 kDa, 1:500, M-222, Santa Cruz, USA), COX2 (70–72 kDa, 1:500, sc-M19, Santa Cruz, USA), oxidative phosphorylation (OXPHOS; CI-CV subunits, 1:1000, ab110413, abcam), and α-tubulin (~50 kDa, 1:5000, sc-5286, Santa Cruz, USA). Signals were visualized by ECL (Amersham) and quantitated using PhotoImager (Molecular Dynamics). Values for protein levels were given as a percentage of $AR^{+/+}$ saline-treated group after normalization with α-tubulin level.

2.6. Determination of tissue dopamine and BH_4 levels

To measure contents of dopamine, the reversed phase HPLC with electrochemical detection was performed by a modified method that was described previously (Kim et al., 2003). After decapitation, the mouse brains were collected at 6 hours after MPTP injection and immediately frozen in liquid nitrogen. The striatal tissue was excised in a brain matrix, according to the mouse brain atlas (MacKenzie-Graham et al., 2003). The sample was