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# PPARγ activation rescues mitochondrial function from inhibition of complex I and loss of PINK1

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ABSTRACT

Parkinson's disease has long been associated with impaired mitochondrial complex I activity, while several gene defects associated with familial Parkinson's involve defects in mitochondrial function or 'quality control' pathways, causing an imbalance between mitochondrial biogenesis and removal of dysfunctional mitochondria by autophagy. Amongst these are mutations of the gene for PTEN-induced kinase 1 (PINK1) in which mitochondrial function is abnormal. Peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor and ligand-dependent transcription factor, regulates pathways of inflammation, lipid and carbohydrate metabolism, antioxidant defences and mitochondrial biogenesis. We have found that inhibition of complex I in human differentiated SHSY-5Y cells by the complex I inhibitor rotenone irreversibly decrease mitochondrial mass, membrane potential and oxygen consumption, while increasing free radical generation and autophagy. Similar changes are seen in PINK1 knockdown cells, in which potential, oxygen consumption and mitochondrial mass are all decreased. In both models, all these changes were reversed by pre-treatment of the cells with the PPARγ agonist, rosiglitazone, which increased mitochondrial biogenesis, increased oxygen consumption and suppressed free radical generation and autophagy. Thus, rosiglitazone is neuroprotective in two different models of mitochondrial dysfunction associated with Parkinson's disease through a direct impact on mitochondrial function.



## Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder of ageing, affecting some 1:500 people, representing an incalculable challenge to an ageing population. It is characterised by the progressive loss of dopaminergic neurons in the substantia nigra leading to a dopamine deficit in the striatum. Currently there is no effective treatment that slows the progression of the disease, and management remains symptomatic. PD has long been associated with mitochondrial dysfunction, and particularly with impaired activity of mitochondrial complex I (CI) (Schapira et al., 1990). The neurotoxins rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or 1-Methyl-4-phenylpyridinium ion ($MPP^+$), inhibitors of CI, have been widely used in vivo and in vitro to model PD due to their capacity to produce neurochemical, neurological, and pathological changes analogous to those observed in PD (Alam and Schmidt, 2002; Betarbet et al., 2000; Corona et al., 2010; Langston et al., 1983). Partial inhibition of CI has a number of downstream effects on cellular function, including the generation of oxidative stress, which can initiate a spiral that results in neuronal death (Betarbet et al., 2000; Schapira et al., 1990).

More recently it has become clear that several genes associated with familial hereditary forms of PD map to pathways of mitochondrial quality control, in particular influencing the clearance of damaged mitochondria by autophagy, resulting in an accumulation of dysfunctional mitochondria with a damaged respiratory chain (Osellame et al., 2013). Thus mutations in PINK1 (PTEN-induced putative kinase 1), cause an autosomal recessive early onset form of PD (Valente et al., 2004a). The clinical phenotype of PINK1 mutant PD patients is often indistinguishable from idiopathic, sporadic PD (Tan et al., 2006; Valente et al., 2004b). The PINK1 protein is targeted to mitochondria and is thought to be involved in protection against free radical generation (Gautier et al., 2008) and in the orchestration of macroautophagy through the recruitment of parkin to dysfunctional mitochondria (Narendra et al., 2008). Thus, PINK1 gene mutations or PINK1 silencing

*Abbreviations:* PD, Parkinson's disease; PPARγ, peroxisome proliferator-activated receptor gamma; TZDs, thiazolidinediones; RGS, Rosiglitazone; $\Delta\Psi_m$, Mitochondrial membrane potential; ROS, reactive oxygen species; PI, propidium Iodide; TMRM, Tetramethylrhodamine methyl ester; DHE, Dihydroethidium; $DiOC_6(3)$, 3,3′-dihexyloxacarbocyanine iodide; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; SOD1, superoxide dismutase 1; PGC1, peroxisome proliferator-activated receptor gamma coactivator 1; mtTFA, mitochondrial transcription factor A; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $MPP^+$, 1-Methyl-4-phenylpyridinium ion; COX-1, subunit I of complex IV; SDHA, subunit of Complex II; CI, complex I; PINK1, PTEN-induced putative kinase 1.

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results in impaired mitochondrial function including a reduced membrane potential ($\Delta\Psi_m$) and impaired oxidative phosphorylation, excessive vulnerability to raised mitochondrial calcium, reduced mtDNA copy number and increased free radical generation (Gandhi et al., 2009; Gegg et al., 2009; Gispert et al., 2009; Hoepken et al., 2007; Piccoli et al., 2008).

The concept of mitochondrial quality control has recently emerged as a key theme in cellular energy homeostasis (for review see (Tatsuta and Langer, 2008)). The term refers broadly to the maintenance of a healthy mitochondrial population through the balance between removal of dysfunctional mitochondria by autophagy, their renewal by biogenesis and the maintenance of mitochondrial protein quality by intramitochondrial chaperones and proteolytic enzymes. Accumulating evidence points to a disturbance in these pathways as a contributor to the pathogenesis of PD (McCoy and Cookson, 2012). At present links between impaired quality control mechanisms and impaired CI activity in PD remain obscure, but it seems plausible that the accumulation of mitochondria with a damaged respiratory chain as a result of impaired turnover could account for the age dependence of the disease.

Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily, and are intimately involved in the regulation of mitochondrial biogenesis. The PPAR receptors include PPARγ, PPARα, and PPARβ/δ. These nuclear receptors when activated directly bind to DNA and regulate gene expression through transcriptional co-activation (also called master regulators of transcriptional cascades). PPARγ is activated by the thiazolidinediones (TZDs), a group of drugs widely used in patients in the management of type II diabetes, as they regulate glucose metabolism, adipogenesis, differentiation, and the expression of several genes including antioxidant defences and inflammatory cytokines such as TNFα, COX, and iNOS (Chung et al., 2008; Dello Russo et al., 2003; Jung et al., 2007; Lehrke and Lazar, 2005; Nolan et al., 1994; Tontonoz et al., 1994). Activation of PPARγ receptors has a major impact on mitochondrial function. PPARγ agonists increase mitochondrial biogenesis, increasing the expression of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α), nuclear respiratory factors (NRF1-2), and mitochondrial transcription factor A (TFAM) with a consequent increased mitochondrial DNA (mtDNA) copy number, increased oxygen consumption and an increased capacity for oxidative phosphorylation (Bogacka et al., 2005; Ghosh et al., 2007; Miglio et al., 2009; Strum et al., 2007). In the central nervous system (CNS), PPARγ is widely expressed in basal ganglia, piriform cortex, and dentate gyrus and it has been detected both in neurons and glia (Cristiano et al., 2001; Cullingford et al., 1998; Moreno et al., 2004; Woods et al., 2003). There is also compelling evidence suggesting that PPARγ agonists are neuroprotective in several animal models of neurodegeneration (Abdelrahman et al., 2005; Schintu et al., 2009; Schutz et al., 2005; Watson et al., 2005). Nevertheless, it is not clear which cellular mechanisms mediate this activity, as TZDs can also influence inflammation, glucose metabolism and antioxidant status. Understanding the relative importance of the underlying mechanisms of protection is important if we are to refine treatments to target the primary protective pathways.

We have therefore asked whether activation of pathways of mitochondrial biogenesis by TZDs can rescue mitochondrial function in differentiated neuroblastoma SH-SY5Y cells associated with chronic partial inhibition of CI with rotenone and with loss of PINK1 function. Our data show that chronic partial inhibition of CI in these cells by rotenone causes irreversible decreases of mitochondrial mass, membrane potential and oxygen consumption, while increasing free radical generation and autophagy. Similar changes are seen in PINK1 KD cells, in which there is a significant decreased capacity for oxidative phosphorylation. In both models, rosiglitazone (RGS) reversed the mitochondrial pathology, and rescued mitochondrial membrane potential, mitochondrial mass and oxygen consumption towards control values. These data strongly suggest that these pathways represent a valuable therapeutic target for the management of PD.

## Materials and methods

### *Antibodies and reagents*

Matrigel basement membrane matrix was obtained from BD Biosciences (Bedford, MA). DMEM, F12 and foetal bovine serum (FBS) were obtained from Invitrogen Life Technologies (UK). Rosiglitazone and GW9662 were obtained from Cayman Chemical, MI, USA. Retinoic acid, rotenone, 3,3′-dihexyloxacarbocyanine iodide [$DiOC_6(3)$] and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich, UK. The antibodies against PPARγ (1:200), PGC-1(1:200), mtTFA (1:1000), SOD1 (1:1000), Nrf2 (1:1000) and β-actin (1:1000) were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The antibodies against LC3B and NQO1 (1:1000) were obtained from Cell Signalling Technology (UK). Biogenesis Western blot cocktail (COX-1, SHDA and β-actin) and β-III tubulin (1:1000) were obtained from Abcam (Cambridge, MA). Propidium iodide (PI), Tetramethylrhodamine methyl ester (TMRM), Calcein-AM and Dihydroethidium (DHE) were obtained from Molecular Probes-Invitrogen (UK).

### *Cell culture*

All the experiments in this study were carried out using human neuroblastoma SH-SY5Y cells and PINK1 KD cells, grown in DMEM/F12 medium supplemented with 10% FBS, containing penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively), in a humidified incubator at 37 °C and 5% $CO_2$. The cells were differentiated into human neuron-like cells, as follows. $6 \times 10^3$ cells/cm$^2$ were seeded on Matrigel basement membrane matrix-coated culture dishes and allowed to attach overnight. The FBS content of the culture medium was then reduced to 2% and cells exposed to 10 μM retinoic acid. The cells were kept under these conditions for 7 days, changing the culture medium every 2 days. The cells were pre-treated for 7 days with rosiglitazone 10 μM (RGS was added every 2 days at the same time that retinoic acid). After 7 days of differentiation (control cells and cells treated with RGS), the growth medium was supplemented with rotenone 200 nM for 24 h.

### *PINK1 knockdown (KD) SH-SY5Y cells*

Generation of stable PINK1 KD in SH-SY5Y cells has been previously described (Muqit et al., 2006). Briefly, mammalian expression vectors expressing shRNA targeted to PINK1 mRNA sequence were packaged into retroviral particles, and used to infect SH-SY5Y cells. Stable cell lines expressing PINK1 shRNA were screened for PINK1 expression using semi-quantitative real time PCR. Clones with >90% reduction in gene expression were selected. Clones expressing empty vector were used as controls.

### *Western blot*

Cells were processed for the determination of protein content using standard protocols. Proteins were transferred onto activated polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA, USA). The membranes were blocked with 10% nonfat dried milk in PBS, 0.2% Tween-20 (PBST). Blocked membranes were incubated overnight with primary antibodies diluted in albumin solution at 4 °C. The membranes were then rinsed three times in PBST and incubated with the corresponding peroxidase-conjugated secondary antibody for 1 h at room temperature (RT). Peroxidase conjugated secondary antibodies were enhanced by chemiluminescence (ECL-Advanced; Amersham Biosciences) and detected by ChemiDoc XRS + System (Bio-Rad).