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Mitochondrial function in Parkinson's disease cybrids containing an nt2 neuron-like nuclear background

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Abstract

Mitochondria likely play a role in Parkinson's disease (PD) neurodegeneration. We modelled PD by creating cytoplasmic hybrid (cybrid) cell lines in which endogenous mitochondrial DNA (mtDNA) from PD or control subject platelets was expressed within human teratocarcinoma (NT2) cells previously depleted of endogenous mtDNA. Complex I activity was reduced in both PD cybrid lines and in the platelet mitochondria used to generate them. Under basal conditions PD cybrids had less ATP, more LDH release, depolarized mitochondria, less mitochondrial cytochrome *c*, and higher caspase 3 activity. Equivalent MPP^+ exposures are more likely to trigger programmed cell death in PD cybrid cells than in control cybrid cells. Our data support a relatively upstream role for mitochondrial dysfunction in idiopathic PD.

Keywords: Cybrids; MPP^+; Mitochondria; Cytochrome *c*; Apoptosis

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder. It is characterized by extensive loss of nigrostriatal dopaminergic neurons. Residual nigral neurons contain intraneuronal inclusions called Lewy bodies (LBs). Five to 10% of cases are postulated to have a genetic component, while the majority is categorized as idiopathic and sporadic. Sporadic PD prevalence is age-associated, with approximately 1% of the population affected at 65 years and 4–5% at 85 years of age (Reviewed in Cardoso et al., 2005).

While the etiology of the disease for most affected people remains unclear, mitochondrial dysfunction likely plays a key role in PD pathogenesis. Mitochondria are central not only to cell bioenergetics but also to apoptotic cell death (Shults, 2004). They are believed to play a fundamental role in ageing, and interact with specific proteins previously implicated in genetic forms of this neurodegenerative disease.

Several lines of evidence link sporadic PD to mitochondrial dysfunction. Complex I activity is reduced in both autopsy brain and platelets of sporadic PD subjects (Parker et al., 1989; Shapira et al., 1989, 1990; Mizuno et al., 1989). Recently, Keeney and co-workers observed complex I is oxidatively damaged in PD brain (Keeney et al., 2006). While the mechanisms underlying this are not entirely clear, in sporadic PD mutated or oxidatively damaged mitochondrial DNA (mtDNA) may play a contributory role (Ibeke et al., 1995; Cortopassi and Wang, 1995).

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Mitochondrial dysfunction in sporadic PD appears to arise at least in part from mtDNA (Swerdlow et al., 1996, 1998; Gu et al., 1998; Shults and Miller, 1998). Interestingly, relative to other brain areas the substantia nigra contains particularly high levels of large mtDNA deletions (Soong et al., 1992). These mtDNA deletions likely cause functional impairment in aged human substantia nigra neurons (Kraytsberg et al., 2006). PD brains also have discrete microheteroplasmic mutations in the ND5 gene that distinguish them from control brains (Smigrodzki et al., 2004; Parker and Parks 2005). Together, these studies suggest mtDNA may mediate mitochondrial dysfunction in PD and therefore contribute to PD neurodegeneration.

To address the relevance of mitochondrial function to PD, we used a cytoplasmic hybrid (cybrid) approach to generate a disease-specific, *ex-vivo* model of mitochondrial dysfunction. This approach has previously been used to produce cell culture models of PD mitochondrial dysfunction; successful studies utilizing SH-SY5Y and A549 human cell line nuclear backgrounds are reported (Swerdlow et al., 1996, 1998; Gu et al., 1998; Shults and Miller, 1998). This technique involves the transfer of platelet mitochondria from either PD or control subjects to mtDNA-depleted recipient cells (rho0 cells). The resulting cybrid lines express the nuclear genes of the recipient rho0 cell line and the mitochondrial genes of the platelet donor. If mitochondrial defects found in transferred platelet mitochondria persist in culture, it is hypothesized mtDNA should account for those persistent defects. Regardless of the role of mtDNA, though, cybrid models facilitate the study of downstream consequences of mitochondrial dysfunction in PD (Swerdlow et al., 1996; Ghosh et al., 1999).

We now report for the first time an analysis of PD cybrids prepared using a human teratocarcinoma (NT2) nuclear background. We further used these cell lines to study how PD and control (CT) cybrid cell lines respond to a mitochondrial toxin, 1-methyl-4-phenylpyridinium ion (MPP^+). MPP^+, a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), induces Parkinsonism in humans, primates, and mice (Langston et al., 1983; Beal, 2001). It causes anatomically specific degeneration of substantia nigra pars compacta and locus coeruleus catecholaminergic neurons by inhibiting complex I of the mitochondrial respiratory chain (Przedborski et al., 2004; Mochizuki et al., 1996). In addition to finding the complex I defect of PD platelets perpetuates in NT2 PD cybrids, we describe for the first time that MPP^+ treated CT cybrids showed mitochondrial impairments similar to untreated PD cybrids. Furthermore, mitochondrial dysfunction observed in untreated PD cybrids renders them more susceptible to MPP^+ induced mitochondrial dependent apoptosis.

2. Materials and methods

Subject participation was approved by the Institutional Review Board of the University Hospital of Coimbra. PD subjects were recruited from the Neurology Service at the University Hospital of Coimbra and met the Gelb and co-workers (1999) criteria for probable PD. They did not manifest signs or symptoms of an alternative neurodegenerative disease. The mean age of the CT group ($n = 3$) was 64.3 ± 8.4, and for the PD group ($n = 2$) was 65.0 ± 5.

2.1. Preparation of platelet mitochondria

Following informed consent, 60 ml of blood was collected through venipuncture in tubes containing acid–citrate–dextrose as an anticoagulant. Mitochondria were obtained from human platelets according to previously described methods (Krige et al., 1992). Platelet mitochondria protein concentrations were measured by the Bradford protein assay (Bradford, 1976), in which bovine serum albumin was used as the standard.

2.2. Cell culture

These experiments utilized NT2 human teratocarcinoma cells (Stratagene, La Jolla, CA) depleted of endogenous mtDNA (rho0 cells) via long-term ethidium bromide exposure (Swerdlow et al., 1997; Binder et al., 2005). Platelet mitochondria from either PD or CT subjects were used to repopulate NT2 rho0 cells with mtDNA as previously described (Swerdlow et al., 1997; Binder et al., 2005). Untransformed cells were removed by withdrawal of pyruvate and uridine from the culture medium and substitution of dialyzed, heat inactivated fetal calf serum for non-dialyzed, heat inactivated fetal calf serum (Swerdlow et al., 1996; Miller et al., 1996). Specifically, the selection medium consisted of Optimen (Gibco Life Technologies) supplemented with 10% dialyzed, heat inactivated fetal calf serum, penicillin (50 U/mL), and streptomycin (50 μg/mL). Cells were grown in 75 cm^2 tissue culture flasks maintained in a humidified incubator at 37 °C and 5% CO_2.

For the MTT assay, cells were plated in 24-well plates at a density of 0.1×10^6 cells/well. For rhodamine 123 measurements, cells were plated in 24-well plates at a density of 0.05×10^6 cells/well. For ATP and LDH determinations, cells were plated in 12-well plates at a density of 0.2×10^6 cells/well. For measurements of caspase enzyme activities, Western blot analysis of α-spectrin, and Western blot analysis of poly (ADP-ribose) polymerase (PARP) cells were plated in 6-well plates at a density of 0.5×10^6 cells/well. For Western blot analysis of cytochrome *c* and determinations of electron transport chain (ETC) enzyme activities, cells were plated in petri dishes (10 cm) at a density of 2.5×10^6 cells/dish.

2.3. MPP^+ treatment

MPP^+ was purchased from Sigma. Twenty-four hours after plating, cybrid cell lines were treated with 100, 500 or 1 mM MPP^+ (water diluted) and maintained at 37 °C for another 24 h. For each experimental parameter, a con-