

The role of mitochondrial dysfunction in Parkinson's disease

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INTRODUCTION

Parkinson's disease (PD) is a prevalent neurodegenerative movement disorder, affecting 1% of those aged over 60 [reviewed in Abou-Sleiman et al., (1)]. As substantia nigra (SN) dopamine neurons degenerate, patients begin to struggle with initiation and maintenance of movement. Rigidity, postural instability and a characteristic resting tremor ensue. Associated with areas of neuronal degeneration are intra-cytoplasmic inclusions containing alpha-synuclein protein (Lewy bodies) (2). Degeneration of nigral projections to the striatum is gradual and patients become symptomatic when striatal dopamine (DA) levels get down to about 30% of normal (3). Sporadic idiopathic disease accounts for 95% of cases and tends to be of late-onset, while familial cases have been linked to mutations in the genes for alpha-synuclein, parkin, PINK1, DJ1 and others (1).

The pathogenesis of idiopathic PD appears to involve multiple processes. Neuroinflammation, excitotoxicity, oxidative stress, environmental toxins and accumulation of misfolded proteins from proteasomal impairment are likely responsible (4,5). This review will present evidence for the involvement of mitochondria in PD and discuss how mitochondrial dysfunction leads to neuronal dysfunction and death.

Mitochondria modulate many cellular processes. Adenosine triphosphate (ATP) is generated as electrons are passed along the protein complexes of the mitochondrial electron transport chain. Mitochondria sequester surplus cytoplasmic Ca^{2+} and play a role in neutralisation of free radicals. However, mitochondrial processes themselves produce much of the cell's free radical load, while release of certain mitochondrial factors into the cytoplasm is enough to trigger apoptosis.

EVIDENCE FOR THE ROLE OF MITOCHONDRIA IN PARKINSON'S DISEASE

Dysfunction of Complex I of the mitochondrial electron transport chain leads to a Parkinsonian phenotype

Mitochondrial dysfunction became a suspect in Parkinson's aetiology after a case of illicit drug use. A dose of 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) injected intravenously created parkinsonism in humans (6). It was later discovered that MPTP's active metabolite, 1-methyl-4-phenylpyridinium ion (MPP⁺), was able to selectively accumulate in dopamine neurons after uptake by the dopamine transporter. In the neuron MPP⁺ inhibited complex I of the mitochondrial electron transport chain (1). In fact, MPTP complex I inhibition is now used to induce PD in animal models.

Indeed, further investigation demonstrated a significant decrease in complex I activity in post-mortem human PD brains (7), as well in skeletal muscle and platelets of the patients (8,9). This systemic defect in complex I is perhaps a result of a genetic flaw or exposure to a mitochondrial toxin during life.

Mitochondrial DNA deletions and particular polymorphisms accompany Parkinson's disease

Mitochondrial DNA codes for many proteins of the electron transport chain. Acquired mitochondrial DNA (mtDNA) deletions that can expand clonally are observed in individual PD involved neurons, although they are also found at high levels in normal aged neurons (10). There does not seem to be a difference in the number of acquired point mutations in mtDNA between PD and controls (11, 12). Instead, mitochondrial polymorphisms that increase relative uncoupling of mitochondria have been found to decrease the risk of developing PD (13). Uncoupling refers to the dissipation of the proton gradient across the inner mitochondrial membrane, which keeps oxidative phosphorylation induced free radical production in check. Uncoupling protein-2 (UCP-2) knock-out mice are predisposed to nigral neurodegeneration, probably from changes in reactive oxygen species buffering ability, while UCP-2 overexpressing animals are resistant (14). Polymorphisms in mtDNA can explain why PD is so strongly associated with ageing (15), as they lead to small changes in the efficiency of mitochondrial oxidative phosphorylation and free radical generation, which accumulate over the lifetime of the person and account for the degree of neuronal cellular ageing and therefore the risk of developing PD.

Mutations in nuclear DNA affect mitochondrial function and account for familial Parkinson's disease

Nuclear DNA codes for most mitochondrial proteins. PTEN induced putative kinase-1 (PINK1) is a mitochondrial kinase that normally protects against cell death by controlling cytochrome c release from mitochondria (16). Loss of function mutations in the PINK1 gene can be rescued by wild type parkin (ubiquitin E3 ligase and component of the ubiquitin-proteasome system that performs ATP-dependent protein degradation) acting downstream of PINK1 to prevent mitochondrial swelling and cytochrome c release (13). PINK1 or parkin loss of function mutations can lead to autosomal recessive juvenile PD (17, 18).

Mutations in the alpha-synuclein gene lead to aggregation of the protein and autosomal dominant PD (19). Alpha-synuclein knock-out mice are resistant to MPTP induced neurotoxicity, suggesting that the sequelae of complex I inhibition may include derangements in protein handling. The development of mitochondrial pathology with overexpression of mutant alpha-synuclein (20) demonstrates the connection between abnormal nuclear DNA derived mitochondrial proteins and mitochondrial dysfunction.

DJ-1 is a peroxiredoxin-like peroxidase that scavenges excess H_2O_2 (21), therefore DJ-1 loss of function mutations lead to decreased protection against apoptosis. Leucine-rich repeat kinase-2 (LRRK2) mutations account for 1-2% of late-onset sporadic PD cases (13). Protein phosphorylation of mutated LRRK2 and PINK1 contributes to protein mishandling that is a key part of PD pathogenesis (22). Proteolytic stress and abnormal phosphorylation of mitochondrial proteins likely affect the function of mitochondria themselves (23).

DOWNSTREAM CONSEQUENCES OF MITOCHONDRIAL DYSFUNCTION

Mitochondrial reactive oxygen species lead to oxidative stress and altered cell signalling

Mitochondria are central to the generation of reactive oxygen and nitrogen species and integration of pro- and anti-apoptotic signals in the cell (24). Mitochondrial ROS generation and maintenance of antioxidant defences are dependent upon the redox and energetic state of the cell (13). Complex I dysfunction increases ROS production by interrupting the flow of electrons along the electron transport chain. Complex I dysfunction likely also lowers the threshold for apoptosis in PD (23, 25).

Indeed, post-mortem analysis of PD affected brains showcases oxidative damage to lipids, proteins and DNA (26). Mitochondria themselves can become susceptible to the ROS they generate if their pool of reduced glutathione becomes depleted (27). Glutathione normally donates electrons to ROS in order to achieve neutralisation; however one of the earliest changes in PD is a marked decrease in nigral glutathione (28).

Production of ROS by mitochondria can also have an effect on signal transduction in the cell (24). Mitochondria generate most of the cell's superoxide radicals as electrons escape to join molecular oxygen prematurely (28). Because of the high activity of Manganese-superoxide dismutase within mitochondria, much of the superoxide is converted to H_2O_2 , however H_2O_2 can permeate through the mitochondrial membrane into the cytoplasm (25). There it can regulate dopamine transmission of the nigro-striatal pathway via the activation of ATP-sensitive K^+ channels (29).

Dopamine release, neuronal death and K_{ATP} channels

K_{ATP} channels are downstream of complex I, and are controlled by mitochondrial metabolism. Activation depends on the degree of mitochondrial uncoupling and ROS generation (30) and is mediated by H_2O_2 . Activation of glutamatergic AMPA receptors on medium spiny neurons of the dorsal striatum enhances mitochondrial generation of H_2O_2 , which then diffuses to DA terminals to open K_{ATP} dependent channels and inhibit DA release (29). K_{ATP} channels are selectively activated in response to complex I inhibition in the SN but not in ventral tegmental area (VTA) dopamine neurons, likely accounting for the preferential degeneration of SN neurons in PD (30). K_{ATP} channel subunit $Kir_{6.2}$ knockout mice were protected against MPTP induced neurodegeneration in the SN (30), showing that the activation of this channel perhaps mediates cell death of DA neurones in PD.

Therefore, overproduction of H_2O_2 by mitochondria can lead not only to oxidative damage but also to decreased release of DA in the striatum. Depleting levels of DA will result in an increased suppression of the ventral lateral nucleus of the thalamus and subsequent decrease in the excitatory input to the motor cortex, leading to the physical symptoms of PD. Differential degeneration of SN and not VTA dopamine neurons in PD, can be explained by activation of K_{ATP} channels.

Mitochondrial K_{ATP} channels and mitochondrial Ca^{2+} regulation

Mitochondria are important in Ca^{2+} homeostasis as they sequester excess cytoplasmic Ca^{2+} (27). Cytoplasmic Ca^{2+} is implicated in activation of Ca^{2+} dependent enzymes and apoptosis. Ca^{2+} accumulation in mitochondria can affect ATP synthesis and decreased ATP synthesis will in turn decrease the removal of Ca^{2+} from the cytoplasm by ATP requiring ion pumps (27). Oxidative stress together with Ca^{2+} and phosphate is thought to open the mitochondrial transition pore, leading to the release of cytochrome c into the cytoplasm and also to mitochondrial swelling (31).

Mitochondrial K_{ATP} channels sit in the inner mitochondrial membrane and lead to the depolarisation of the mitochondria, unlike plasma membrane K_{ATP} channels that hyperpolarise the cell (31). Depolarisation leads to protein kinase C activation, which activates ROS scavengers and promotes the synthesis of other anti-ROS proteins (31). Preventing swelling of the mitochondria limits Ca^{2+} influx and thus preserves efficiency of respiration (31). ROS can activate mitochondrial K_{ATP} channels (31); however uncontrolled ROS production may modify the channel so that its

function is compromised. Agents that control Ca^{2+} entry into the cytosol (dihydropyridines), as well as agents that activate mitochondrial K_{ATP} channels to limit Ca^{2+} accumulation in mitochondria, are neuroprotective (15, 31).

Mitochondria-dependent apoptosis in PD

Cytochrome c and apoptosis-inducing factor (AIF) can be released from mitochondria into the cytoplasm to trigger cell death (27). Translocation of AIF to the nucleus leads to nuclear alterations such as chromatin condensation and large scale DNA fragmentation (27). Cytochrome c interacts with apoptotic protease activating factor (Apaf-1), encouraging Apaf-1 to bind procaspase-9, which initiates procaspase-9 cleavage to its active protease form. Caspase-9 goes on to cleave and activate caspase-3 (27).

It is believed that the death of dopamine neurons in PD is mediated via the cytoplasmic actions of mitochondrial cytochrome c. Cytochrome c is normally bound by anionic phospholipids, primarily cardiolipin, to the inner mitochondrial membrane (32). Complex I induced disruption of mitochondrial respiration blocks electron flow along the mitochondrial transport chain and produces ROS. These oxidatively modify cardiolipin and increase the soluble pool of cytochrome c in the mitochondrial intermembrane space (32). Discharge of accumulated cytochrome c into the cytoplasm is dependent upon proapoptotic Bcl-2 family member Bax (32). Bax permeabilises the outer mitochondrial membrane allowing cytochrome c to leak into the cytosol. DNA damage from ROS activates transcription factor p53 and c-Jun N-terminal kinase (JNK) pathways, acting through BH3-only protein Bim (26). P53 is responsible for inducing transcription of Bax, while Bim mediates translocation of Bax from the cytosol to the mitochondrion.

Therefore a self-amplifying cascade is set up, starting with mitochondrial dysfunction and increased ROS production and ending at mitochondrial liberation of cytochrome c and activation of apoptotic mediators such as caspases. Attenuation of mitochondria-dependent apoptosis can be achieved by targeting cytochrome c or Bax, however it is important to note that neurons have separate self-destruct programmes for cell bodies and axons (33). Axonal degeneration is caspase independent and likely begins before soma degeneration (33). The contribution of axonal degeneration to PD symptom development needs elucidating.

CONCLUSION AND THERAPEUTIC IMPLICATIONS

Dopamine neurons are under significantly more stress than other neurons because the metabolism of dopamine is in itself associated with reactive oxygen species generation (4). Reliance on L-type Ca^{2+} channels for pacemaker activity leads to Ca^{2+} accumulation in mitochondria which comes at an energetic cost (15). It is not surprising that SN neurons are lost at a much higher rate even with normal aging (15). PD may represent an acceleration of the normal aging process due to complex I dysfunction and resultant increase in ROS production that ultimately leads to mitochondrial DNA and protein damage, and later, to cell injury and death. Most toxins used to model PD such as MPTP, 6-hydroxydopamine (6-OHDA), paraquat and rotenone achieve nigro-striatal degeneration by means of overproducing ROS and increasing oxidative stress (25).

Genetic causes of PD supports the notion that mitochondrial dysfunction, oxidative stress and proteasomal system dysfunction are interdependent and interact to influence each other and to contribute to the parkinsonian phenotype. Genetic mutations, polymorphisms and environmental toxins likely contribute to oxidative stress and mitochondrial dysfunction. Mitochondria-dependent apoptosis seems to be the end point of all insults. Targeting mitochondria to modify the course of PD is currently being explored.

MitoQ aims to mimic endogenous mitochondrial co-enzyme Q10 to achieve an anti-apoptosis and anti-oxidative stress effect (34). The efficacy of this orally active antioxidant in slowing the progression of Parkinson's is being tested in Phase III clinical trials. Creatine for enhancement of mitochondrial energy production is also in trials. Although some investigators

document no improvement of the Unified Parkinson's Disease Rating Scale score (35, 36), Neuroprotection Exploratory Trials in Parkinson Disease (NET-PD) have deemed both compounds worthy of further study (37, 38). This lack of clear success, however, has led to some questioning the primary importance of mitochondrial impairment, particularly oxidative stress, in the pathogenesis of Parkinson's disease (39). It is likely that individuals do vary in the relative contribution of mitochondrial dysfunction to their disease onset and progression. Identification of those in whom mitochondrial derangement plays the greatest role may represent the next stage of Parkinson's treatment, as these are the people who may benefit most from pharmacological mitochondrial agents.

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