doi:10.1017/S1431927611001851
Microsc. Microanal. 17 (Suppl 2), 2011

Mitochondrial Fragmentation Mediates MPP^+ Toxicity in Neurons: Implications for Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by loss of nigrostriatal dopaminergic neurons. Familial genetically-cased PD accounts for less than 10% of all cases, with the majority of PD cases being sporadic. While the etiology of sporadic PD has not been completely elucidated, there is growing concerns that environmental factors, such as exposure to neurotoxins, increase PD risk [1]. In this regard, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), via its active metabolite 1-methyl-4-phenylpyridinium ion (MPP^+), may be a contributing issue in the etiopathogenesis of the disease. As such, both MPTP and MPP^+ have been extensively used in a variety of *in vivo* mammalian species and *in vitro* paradigms as experimental models of PD, respectively[2]. Given the parallels with PD, further understanding the mechanisms by which MPTP/MPP^+ lead to dopaminergic neuronal death could provide insights into therapeutic targets for PD.

Mitochondria play a pivotal role in neuronal energy supply and calcium buffering. Compelling evidence suggest that mitochondrial dysfunction could represent a critical event in the pathogenesis of PD [3]. However, the mechanisms underlying mitochondrial dysfunction in PD remain elusive. Mitochondria are dynamic organelles that undergo continual fission and fusion events which control not only mitochondrial number and morphology but also mitochondrial distribution and function [4]. Mutations in PINK-1, Parkin and DJ-1 cause autosomal recessive PD and recent studies suggested that PINK/Parkin/DJ-1 are all involved in pathways regulating mitochondrial dynamics or that an altered mitochondrial dynamics is a common pathogenic pathway [5]. However, to date, the impact of MPP^+ on mitochondrial dynamics remains unknown. To address this, in this study, we determined whether MPP^+ induced abnormal changes in mitochondrial dynamics and determined the temporal and causal relationship between changes in mitochondrial dynamics and MPP^+-induced mitochondrial abnormalities and neuronal death.

By using fluorescence time lapse microscopy, we demonstrated that 2.5 mM MPP^+ induces a biphasic decrease in mitochondrial length in SH-SY5Y neuroblastoma cells with a very rapid initial decrease within 1 hr followed by a second wave of dramatic decrease around 4 hr after MPP^+ treatment, suggesting significant mitochondrial fragmentation. Notably, this is accompanied by a rapid increase in mitochondrial recruitment of DLP1 and a more prolonged dramatic increase in total levels of DLP1. DLP1 knockdown could almost completely block MPP^+-induced mitochondrial fragmentation, confirming the critical role of DLP1 in MPP^+-induced mitochondrial fragmentation. Most importantly, we demonstrated that MPP^+-induced mitochondrial fragmentation is not only a temporally-early event, it also mediates the toxic effects of MPP^+ on mitochondrial properties including ATP production, mitochondrial membrane potential, calcium handling, ROS generation and mitophagy as well as eventual cell death since blockage of mitochondrial fragmentation by DLP1 knockdown could alleviate or

even completely prevent these deficits induced by MPP^+, suggesting that prevention of mitochondrial fragmentation is an attractive site of intervention to prevent MPP^+-induced deficits which may have profound implications in PD treatment. Mechanistically, we also show that increases in ROS and elevations in intracellular calcium contribute to MPP^+-induced mitochondrial fragmentation, suggesting the presence of a complex crosstalk between mitochondrial fragmentation, ROS production and calcium disturbances.

To determine whether MPP^+ exerts similar effects on mitochondria in differentiated primary dopaminergic neuronal cells, primary rat E18 midbrain neurons (DIV=6) were treated with 0.5 μM or 5 μM MPP^+. Interestingly, double-label immunofluoresence for tyrosine hydroxylase (TH) and COX-IV (marker of mitochondria) revealed that although 5 μM MPP^+ caused mitochondrial fragmentation in both TH-positive and TH-negative neurons, 0.5 μM MPP^+ only elicited the appearance of small round mitochondria in both soma and neurites in TH-positive neurons, indicative of mitochondrial fragmentation, whereas 0.5 μM MPP^+-treated TH-negative neurons retained a tubular and filamentous morphology similar to non-treated control cultures. This finding not only confirms that dopaminergic neurons are more sensitive to the effect of MPP^+, but also suggests that alterations of mitochondrial morphology are specific intracellular effects of MPP^+ in TH-positive neurons. Our time course study confirmed that, similar to SH-SY5Y cells, mitochondrial fragmentation is a very early event, which suggest that it likely also mediates toxic effects induced by MPP^+ in primary TH-positive neurons. Interestingly, in addition to the rapid mitochondrial fragmentation, we also found that MPP^+ induces a biphasic decrease in mitochondrial coverage in neurites (i.e., decreased neurite mitochondrial index), which is likely due to a combined effect of reduced mitochondrial size and changes in mitochondrial numbers in neurites.

Overall, our findings indicate that MPP^+-induced DLP1-dependent mitochondrial fragmentation is an early and upstream event and plays a critical role in mediating downstream adverse effects such as reduced ATP generation, increased ROS production, decreased MMP, calcium disturbance, increased mitophagy and cell death in neuronal cells. A crosstalk between ROS and calcium disturbance and mitochondrial fragmentation form a mitochondrial fission-initiated downward spiral that augments these adverse effects.

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[6] This research was supported by the NIH (R21NS071184)

https://doi.org/10.1017/S1431927611001851 Published online by Cambridge University Press