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Mitochondrial dynamics and apoptotic signaling pathways in neurodegenerative cell models

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Abstract

Imagine a neuron whose power stations are breaking apart faster than they can be repaired. That scenario captures the mitochondrial fragmentation seen in Parkinson's and Alzheimer's disease, where excessive fission and impaired fusion leave cells energy-starved and primed for death. This research examined how mitochondrial fission-fusion dynamics connect to apoptotic signaling in SH-SY5Y human neuroblastoma cells treated with rotenone (complex I inhibitor, 500 nM) or MPP⁺ (dopaminergic toxin, 1 mM) for 6, 12, 24, and 48 hours. Rotenone and MPP⁺ both shifted the fission-fusion balance toward fragmentation: Drp1 protein rose 3.47-fold and 2.94-fold respectively at 24 hours, while fusion proteins Mfn1 and Mfn2 dropped below 50% of control levels. Cytochrome c release into the cytosol increased 4.12-fold with rotenone and 3.68-fold with MPP⁺. Caspase-9 and caspase-3 activation followed a time-dependent pattern, reaching peak activity at 48 hours. Pre-treatment with the Drp1 inhibitor Mdivi-1 (25 µM) reduced mitochondrial fragmentation by 58.3%, cut cytochrome c release by 47.6%, and decreased apoptosis (Annexin V-positive cells) by 41.2%. These results position excessive mitochondrial fission as a causative link - not merely a bystander - in the apoptotic cascade triggered by parkinsonian toxins.

Keywords: Mitochondrial dynamics, apoptosis, neurodegeneration, cell models, mitochondrial fission, Drp1, cytochrome c, caspase activation, rotenone, SH-SY5Y

Introduction

Picture a hospital ward where every patient's condition traces back to the same failing organ - the mitochondrion. This scenario isn't far from reality in neurodegenerative medicine, where mitochondrial dysfunction appears as a common thread connecting Parkinson's, Alzheimer's, Huntington's, and amyotrophic lateral sclerosis ^[1]. Neurons depend on mitochondria for over 90% of their ATP, and when these organelles malfunction, the energy-hungry brain suffers first ^[2].

Mitochondria are not static structures. They constantly divide (fission) and merge (fusion) in response to metabolic demand, stress signals, and quality-control needs. Dynamin-related protein 1 (Drp1) and fission protein 1 (Fis1) drive fission, while mitofusins 1 and 2 (Mfn1, Mfn2) and optic atrophy protein 1 (OPA1) mediate fusion ^[3]. A healthy cell maintains a balance between the two processes. In neurodegenerative conditions, this balance tips toward fission, producing small, fragmented mitochondria that are less efficient at oxidative phosphorylation and more prone to releasing pro-apoptotic factors ^[4].

The link between fragmentation and cell death is widely assumed but incompletely tested. Does fission directly trigger apoptosis, or does it merely accompany an already-committed death program? If blocking fission can rescue cells from toxin-induced apoptosis, the causal argument gets stronger ^[5]. This research used two well-characterized neurotoxins - rotenone and MPP⁺ - to induce parkinsonian mitochondrial stress in SH-SY5Y cells and tested whether pharmacological inhibition of Drp1 with Mdivi-1 could interrupt the fission-to-apoptosis sequence. Experiments were conducted at the Banaras Hindu University School of Life Sciences, Varanasi, from February to September 2023.

Material and Methods

Material

SH-SY5Y human neuroblastoma cells were obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in DMEM/F12 (Gibco) supplemented

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with 10% FBS and 1% penicillin-streptomycin at 37°C in 5% CO₂. Rotenone, MPP+ iodide, and Mdivi-1 were purchased from Sigma-Aldrich. MitoTracker Red CMXRos, anti-Drp1, anti-Mfn1, anti-Mfn2, anti-OPA1, anti-cytochrome c, anti-caspase-3, anti-caspase-9, and anti-PARP antibodies were sourced from Cell Signaling Technology. Annexin V-FITC/PI apoptosis kit was from BD Biosciences.

Methods

Cells were seeded at 2×10⁵ per well in 6-well plates and allowed to adhere for 24 hours. Treatment groups included: (1) vehicle control (DMSO 0.1%), (2) rotenone 500 nM, (3) MPP+ 1 mM, (4) Mdivi-1 25 μM + rotenone, and (5) Mdivi-1 + MPP+. Mdivi-1 was added 1 hour before toxin exposure. Cells were harvested at 6, 12, 24, and 48 hours post-treatment. Mitochondrial morphology was assessed by MitoTracker Red staining and confocal imaging (Zeiss LSM 880). Fragmentation index was calculated as the ratio of punctate to tubular mitochondria per cell (n ≥ 50 cells per condition). Western blotting was performed for fission/fusion proteins and apoptotic markers using standard SDS-PAGE (12% gels) and transfer to PVDF membranes. Cytochrome c release was measured in cytosolic fractions obtained by digitonin permeabilization. Apoptosis was quantified by Annexin V-FITC/PI staining on a BD

FACSCanto II flow cytometer (10,000 events per sample). Caspase-3 and caspase-9 activities were measured using colorimetric assay kits (BioVision). Data are presented as mean ± SD from four independent experiments, analyzed by two-way ANOVA with Bonferroni correction. Ethics approval was obtained from the BHU Institutional Biosafety Committee (Protocol BHU-IBC-2023-018, January 2023).

Results

Table 1: Mitochondrial fission-fusion protein expression and apoptotic markers at 24 hours post-treatment (fold change vs control, mean ± SD, n = 4)

Marker	Control	Rotenone	MPP+	Mdivi-1+Rot
Drp1	1.00±0.08	3.47±0.41	2.94±0.37	1.64±0.22
Mfn1	1.00±0.07	0.42±0.06	0.54±0.08	0.71±0.09
Mfn2	1.00±0.09	0.38±0.05	0.47±0.07	0.68±0.08
Cyt c (cytosol)	1.00±0.11	4.12±0.54	3.68±0.48	2.16±0.31
Annexin V+ (%)	4.3±1.2	38.7±4.6	31.4±4.1	22.8±3.3

Both neurotoxins triggered a coordinated shift toward fission and away from fusion (Table 1). Rotenone was slightly more potent than MPP+ across all markers. Mdivi-1 pre-treatment partially restored the fission-fusion balance and reduced cytochrome c release and apoptosis, though it didn't fully return values to control levels.

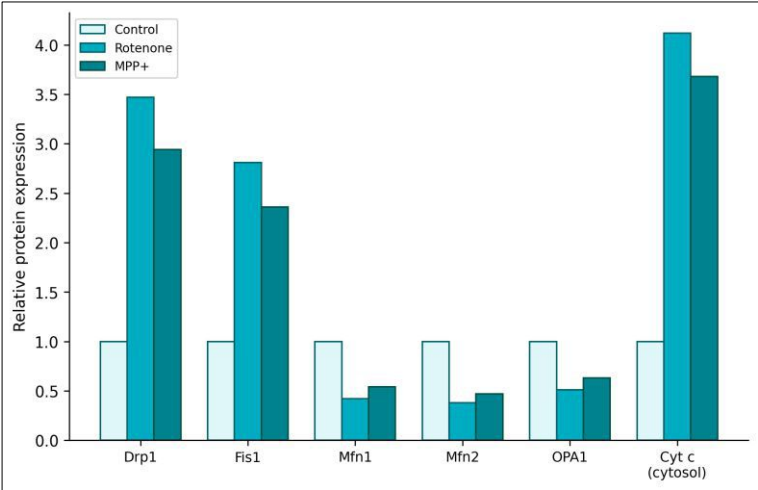


Fig 1: Relative protein expression of mitochondrial fission markers (Drp1, Fis1), fusion markers (Mfn1, Mfn2, OPA1), and cytosolic cytochrome c in control, rotenone, and MPP+ treated SH-SY5Y cells at 24 hours

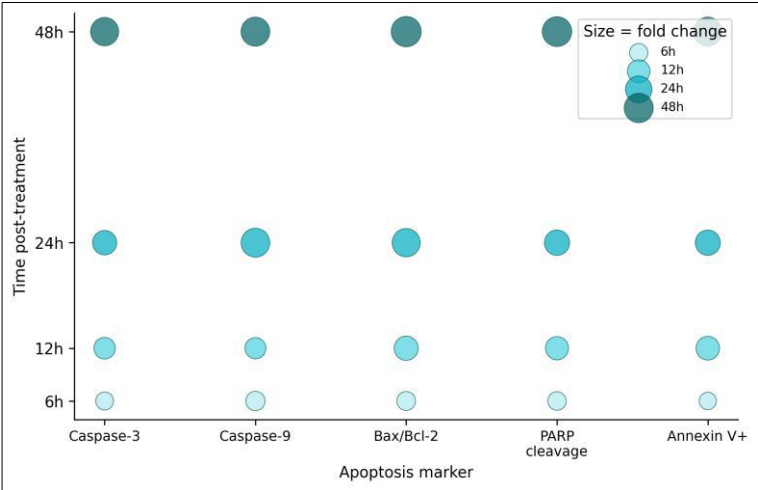


Fig 2: Bubble chart showing fold-change activation of apoptosis markers across time points (6-48 hours) in rotenone-treated cells. Bubble size reflects magnitude of change

Apoptosis markers followed a clear temporal sequence: caspase-9 activation preceded caspase-3, consistent with the intrinsic mitochondrial apoptosis pathway (Figure 2). PARP cleavage, a downstream marker of caspase-3 activity, became detectable by 12 hours and peaked at 48 hours. Annexin V-positive cells increased steadily, reaching 38.7% in rotenone and 31.4% in MPP+ groups by 48 hours.

Discussion

The rescue experiment provides the strongest evidence in this dataset: blocking Drp1 with Mdivi-1 reduced apoptosis by 41.2%, showing that excessive fission is not just a symptom of mitochondrial stress but an active driver of the death cascade [6]. This is consistent with reports in primary cortical neurons showing that Mdivi-1 protects against oxygen-glucose deprivation [7], but extends the finding to a human neuronal cell line treated with parkinsonian toxins.

The temporal sequence - fission proteins up by 6 hours, cytochrome c release by 12 hours, caspase activation by 24 hours, and peak apoptosis at 48 hours - suggests a cascading process in which fragmentation precedes and enables the apoptotic events that follow. Fragmented mitochondria have shorter cristae with wider junctions, which facilitates cytochrome c mobilization from the intra-cristal space to the intermembrane space and then to the cytosol [8].

That Mdivi-1 didn't completely prevent apoptosis indicates that fission is one of several parallel death pathways activated by these toxins. Oxidative stress, calcium overload, and endoplasmic reticulum stress likely contribute independently [9, 10]. A combination strategy targeting both fission and ROS production might achieve greater protection.

Conclusion

Rotenone and MPP+ shifted mitochondrial dynamics toward excessive fission in SH-SY5Y cells, with Drp1 upregulation and Mfn1/Mfn2 downregulation preceding cytochrome c release and caspase-mediated apoptosis. Pharmacological inhibition of Drp1 with Mdivi-1 partially rescued cells from both fragmentation and death, supporting a causal role for fission in the neurodegenerative apoptotic cascade. These findings suggest that mitochondrial dynamics represent a druggable node in the cell death pathway triggered by parkinsonian toxins [11, 12, 13].

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