Pharmacological inhibition of PTEN rescues dopaminergic neurons by attenuating apoptotic and neuroinflammatory signalling events

Aishwarya Mary Johnson (aishwaryajohnson15@gmail.com)
UAE University: United Arab Emirates University https://orcid.org/0000-0002-9030-9954
Sara Jose
University Of Queensland UQCCR: The University of Queensland Centre for Clinical Research
Abdul Rasheed Palakkot
UAE University: United Arab Emirates University
Farheen Badrealam Khan
UAE University: United Arab Emirates University
Jaleel Kizhakkayil
UAE University: United Arab Emirates University
Shamma Ali Abdulla AlNaqbi
UAE University: United Arab Emirates University
Mark GH Scott
INSERM U1016: Institut Cochin
Mohammed Akhli Ayoub
UAE University: United Arab Emirates University
Richard Gordon
University Of Queensland UQCCR: The University of Queensland Centre for Clinical Research
Hariharan Saminathan
UAE University: United Arab Emirates University

Research Article

Keywords: Neurodegeneration, Neuroinflammation, Parkinson's Disease, PTEN, FYN Kinase, VO-OHpic

Posted Date: July 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1785484/v1

Version of Record: A version of this preprint was published at Journal of Neuroimmune Pharmacology on August 17th, 2023. See the published version at https://doi.org/10.1007/s11481-023-10077-8.

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a selective degeneration of dopaminergic neurons in the ventral mid-brain of humans called substantia nigra. This results in an irreversible and debilitating motor dysfunction. Though both genetic and idiopathic factors are implicated in the disease etiology, idiopathic PD comprise the majority of clinical cases and is caused due to environmental toxicants and oxidative stress. Activation of Fyn kinase has been implicated to be an early signalling event that primes both neuroinflammatory and neurodegenerative events associated with dopaminergic cell death. Fyn kinase is activated by dephosphorylation at the negatively regulating tyrosine site by tyrosine phosphatases. However, the tyrosine phosphatase that dephosphorylates and activates Fyn kinase is unidentified. One of the tyrosine phosphatases - PTEN (Phosphatase and Tensin homolog deleted on chromosome 10) a lipid and protein tyrosine phosphatase pathological roles in causing Parkinson's disease has been previously studied in experimental models. We sought to study if PTEN would be the upstream regulator of Fyn activation in PD models. Our findings demonstrate for the first time that PTEN is a very early stress-sensor in response to oxidative stress and neurodegenerative toxicants in *in vitro* models of PD. Pharmacological inhibition of PTEN attenuates Fyn kinase and rescues dopaminergic neurons from neurotoxicants induced cytotoxicity. Our findings also identify PTEN's additional and novel roles in contributing to mitochondrial dysfunction and neuroinflammatory pathways, both of which contribute to neurodegenerative processes. Taken together, we have identified PTEN as a disease course altering pharmacological target that may be further validated for the development of novel therapeutic strategies.

Introduction

Parkinson's Disease (PD) is the fastest growing neurodegenerative disorder, and its disease pathology is characterized by the selective degeneration and irreversible loss of dopaminergic neurons in the *substantia nigra* pars *compacta* (SNpc) region of the brain. This pathological event results in significant depletion of the neurotransmitter - dopamine levels in the SNpc projection region, anatomically known as the *caudate putamen*. The loss of innervation precedes and contributes to widely recognized motor symptoms of PD *(viz.)* - akinesia, bradykinesia, rigidity and postural instability. As we continue to unravel the key molecular mechanisms that contribute to cellular events causing the degeneration of dopaminergic neurons, the disease pathology still remains an enigma. Despite, the chronic and progressive nature of the disease pathology, we do not fully understand the early molecular events that initiate the selective loss of neurons. From a therapeutic standpoint of view, this is particularly challenging to devise therapeutic strategies as multiple molecular pathways may be active at tandem resulting in dopaminergic degeneration (Hariz and Obeso 2017, Przedborski 2017).

The etiology of idiopathic PD consists of a myriad of environmental, genetic, epigenetic components, oxidative stress, neuroinflammation, apoptosis and more recently upheavals in gut microbiome to be among the most widely recognized mechanistic underpinnings that lead to clinical PD (Saminathan, Asaithambi et al. 2011, Ghosh, Kanthasamy et al. 2012, Lin, Chandramani-Shivalingappa et al. 2012, Song, Charli et al. 2019). While most of the market-approved drugs for the clinical management of PD, including the dopamine biosynthesis precursor levodopa and other dopamine receptor agonists, provide temporary symptomatic relief, they cannot halt or slow-down the neurodegenerative processes (Olanow and Obeso 2011, Olanow 2014). Currently, treatments that can potentially impede or reverse the degenerative process in PD are not available because, our understanding of the molecular neurodegenerative mechanisms contributing to the gradual loss of dopaminergic neurons in the *substantia nigra* is vastly limited (Korczyn and Hassin-Baer 2015, Lungu, Cedarbaum et al. 2021). There is therefore an unmet medical need which seeks to identify early events of neurodegeneration that may be targeted during progressive phases of Parkinson's disease. While the scientific community is in hot pursuit to develop strategies that will alter the course of disease, quest remains unmet.

Fyn kinase belongs to the Src family of kinases and has been widely investigated in experimental models of PD as an upstream activator of neuroinflammatory and neurodegenerative signalling events. Fyn activity is regulated by post-translational modification via phosphorylation/dephosphorylation on amino acid residues - Tyr 529 (Y-529), located six residues upstream to the C-terminus and Tyr 417 (Y-417) in the kinase domain. Similar to other members of Src family of kinases, phosphorylation of Fyn at Tyr 529 renders it enzymatically inactive by binding to the SH-2 domains. At basal levels, 90–95% of the Src in cells are in inactive state (Roskoski 2005, Roskoski 2015, Zheng, Li et al. 2017). Whereas phosphorylation of Tyr 417 residue is the key to the signaling events involving SFKs, since phosphorylation renders it to achieve remarkable levels of kinase activities (Roskoski 2005, Roskoski 2015, Zheng, Li et al. 2017). It is therefore reasoned that a phosphatase may lie upstream and activate Fyn kinase by dephosphorylating the negatively regulating Tyr 530 residue, leaving Fyn susceptible to activation by auto-phosphorylation at Tyr 417.

Among the several protein tyrosine phosphatases known, PTEN has been reported to be associated with PD pathology (Kim and Mak 2006, Domanskyi, Geissler et al. 2011). Also, the roles that PTEN plays in apoptosis have been widely studied (Gary and Mattson 2002, Zhu, Hoell et al. 2006). It was initially thought that most, if not all of PTEN's target effects were brought about through its lipid phosphatase activity; (viz.) the dephosphorylation of the lipid secondary messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PTEN negatively regulates the phosphoinositide 3 kinase (PI3K)-Akt signaling pathway that mediates various pro-survival actions (Carracedo and Pandolfi 2008). Interestingly recent investigations about PTEN biology have studied its protein phosphatase activity in mediating excitotoxicity (Ning, Drepper et al. 2010, He, Long et al. 2017). Additionally, phosphatase independent role of PTEN in carrying out various cell functions such, as maintaining chromosomal integrity has also been recently identified (Guan, Yang et al. 2015, Hou, Ouyang et al. 2017).

The requirement of tyrosine phosphorylation dependent activation of Fyn and previous findings implicating PTEN to contribute to neurodegenerative pathways underlying PD led us to hypothesize if PTEN activation is the early signalling event that primes neuroinflammatory and neurodegenerative processes. Using multiple triggers that cause dopaminergic degeneration and the collateral inflammatory events that accentuate neurodegeneration, we show for the first time that PTEN is a key sensitizing molecule that mediates Fyn activation and associated molecular events underlying PD progression.

Materials And Methods

Chemicals and Biological Agents:

(OC-6-45)-Aqua(3-hydroxy-2-pyridinecarboxylato-κ-N1,κO2)[3-(hydroxy-κO)-2-pyridinecarboxylato(2-)-κO2]oxovanadate(1-),hydrogen (VO-OHpic) was purchased from Tocris Bioscience (Bristol, UK). Chemicals such as N-Methyl-4-Phenylpyridinium Iodide (MPP^+ iodide), 6-hydroxydopamine hydrobromide (6-OHDA) and hydrogen peroxide (H_2O_2) was purchased from Sigma-Aldrich (St. Louis, MO). MitoTracker Red CMXRos and SYTOX® Green nucleic acid stain was purchased from Molecular probes (Eugene, US). The primary antibodies used in this study were PTEN Native (Rabbit monoclonal, Abcam), Phospho-PTEN Ser380/Thr382/383(Rabbit polyclonal, CST), phospho-PTEN 370 (Rabbit polyclonal, Abcam), phospho-Fyn 530 (Rabbit polyclonal, Abcam) and β-actin (mouse monoclonal, Sigma). HRP-conjugated secondary antibody against rabbit and mouse were purchased from CST. Pierce BCA Protein Assay Kit, RIPA lysis buffer, enhanced chemiluminescence (ECL), Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute Medium (RPMI 1640), heat-inactivated fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA). The plasmid coding for Rluc-PTEN-YFP sensor was a gift from Dr. Mark Scott (Cochin Institute, Paris, France). Coelenterazine was purchased from Promega (Madison, WI). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA) and Opti-MEM was purchased from GIBCO (Carlsbad, CA).

N27 Dopaminergic Neuronal Cell Culture

Immortalized rat mesencephalic N27 Dopaminergic neuronal cells were purchased from Millipore. They represent a homogenous tyrosine hydroxylase-positive (TH+) cell line, which has been widely used as an *in vitro* model for PD (Kaul, Kanthasamy et al. 2003, Kaul, Anantharam et al. 2005, Peng, Stevenson et al. 2005). N27 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 50 units of penicillin and 50 µg/ml of streptomycin. The cells were maintained in a humidified atmosphere with 5% CO2 at 37°C as described previously (Saminathan et al., 2011).

HEK293 cells and SH-SY5Y human dopaminergic neuroblastoma cell line

Human dopaminergic neuroblastoma SH-SY5Y cell line (ATCC Cat# CRL-2266 RRID: CVCL_0019) was obtained from the American Type Culture Collection (ATCC). HEK293 cells were generous gift from Dr. Mohammed Ayoub Akhli. Both HEK293 cells and SH-SY5Y cells were grown in DMEM supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 50 U of penicillin, and 50 µg/ml of streptomycin.

Raw-blue reporter cells and treatments

Raw-Blue™ cells are derived from murine RAW 264.7 cells and have been engineered to stably express secreted embryonic alkaline phosphatase (SEAP) reporter gene, which is induced via NF-κB and activator protein-1 activation. The cells were grown in DMEM containing 4.5 g/litre glucose, 10% heat-inactivated FBS, 2mM L-glutamine,100µg/ml Normocin, 1% Pen-Strep and 200 µg/ml Zeocin at 37 ^{0}C in 5% CO2. For the test media, the above same media was used without Zeocin added. The stimulation of the Raw-Blue™ cells by ligands results in secretion of SEAP that could be measured by colorimetric enzyme assay using Quanti-Blue solution, In-vivogen (San Diego, USA). Raw-blue reporter cells at a concentration of 100,000 cells per well were plated on a 96 well plate. The cells were pre-treated with 30nM VO-OHpic for 1hour and stimulated with 10ng/ml LPS-EP (LPS O111:B4, Sigma Aldrich, St. Louis, MO) for 20-24 hrs

Primary cell cultures and treatments

Peripheral blood mononuclear cells (PBMCs)

PBMCs were obtained using the Ficoll-Paque centrifugation according to the manufacturer's instructions. The cells were grown in RPMI-1640 media with 10% heat-inactivated FBS, 10mM Penicillin Streptomycin and 10mM L-glutamine. To investigate the secretion of IL-1β from PBMCs, the cells were plated at a concentration of 300,000-500,000 cells/well in 96 well plate, primed using 200ng/ml ultrapure LPS for 3hrs. The cells were pre-treated with 30nM VO-OHpic for at least 1 hour and activated using 5mM ATP (Sigma Aldrich, St. Louis, MO) for 1 hour. The supernatant was used for IL-1β ELISA.

To check if the non-inflammasome pathway is inhibited by VO-OHpic, the PBMC's were pre-treated with the 30nM VO-OHpic for 1 hour and stimulated with 10ng/ml LPS-EP (LPS O111:B4, Sigma Aldrich, St. Louis, MO) for 20-24 hrs. The supernatant was used to measure TNF-α using ELISA.

Primary microglia

Primary microglia were obtained from brains of C57BL/6J WT mouse pups (P0-P2 postnatal day). The magnetic separation of microglia was carried as described in (Gordon, Hogan et al. 2011). The cells were grown in Dulbecco's modified Eagle's medium/F-12 nutrient mixture (DMEM- F12, Gibco Carlsbad, CA) containing 10% heat-inactivated FBS (Lonza, Basel, Switzerland), 1% 2 mM L-glutamine (Sigma Aldrich St. Louis, MO), 1% 100mM non-essential amino acids (Gibco Carlsbad, CA), 1% 1 mM sodium pyruvate (Gibco Carlsbad, CA) and 100 mM penicillin streptomycin (Sigma Aldrich, St. Louis, MO). After magnetic separation of primary microglia, the following day cells were serum starved by replacing media containing 10% heat-inactivated FBS with media containing no FBS and kept overnight in the serum-free media. After serum starvation, the next day, the cells were primed with 200 ng/mL of ultrapure LPS (E. coli, InvivoGen, San Diego, CA) and incubated for 3 hours. After priming, cells were washed twice with serum-free media and incubated with 30nM VO-OHpic at 37°C for 1 hour. Following VO-OHpic treatment, cells were activated with 5 mM ATP (Sigma Aldrich, St. Louis, MO) and incubated at 37°C. Supernatant was collected 1 hour after ATP treatment.

Cell Culture and Transfection

HEK293 cells were maintained as described above and transient transfections were carried out using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) as previously described (Ayoub et al., 2015a; Ayoub et al., 2015b). For BRET assays, cells were directly transfected in 96-well white plates. Briefly, for each 96-well

25 ng of Rluc-PTEN-YFP coding plasmid was dissolved in 25 µl of Opti-MEM. In parallel, 0.5 µl per well of Lipofectamine™ 2000 was mixed in 25 µl of Opti-MEM. After incubation for 5 min at room temperature (RT), the plasmid solution was mixed with Lipofectamine solution and incubated for 20 min at RT. Then, the transfection mix (50 µl) was directly added to the cells (5 x 10^4 cells per well) in a final volume of 200 µl of culture medium to each well in a 96-well white plate. BRET assays were carried out 48 h post-transfection.

BRET assay in live cells

BRET assay in live cellsHEK293 cells transiently expressing Rluc-PTEN-YFP sensor were seeded in 96-well white plates. After 48 h of post-transfection, cells were treated with 100µM H_2O_2 and 100µM 6-OHDA separately for different timepoints and washed with 50 µl/well of PBS. Then, cells were resuspended in 30 µl of PBS followed by addition of 10 µl of coelenterazine h (2.5 µM), and BRET signals were measured in live cells using a Tristar 2 multi-label plate reader (Berthold Technologies GmbH & Co.), allowing luminescence recording and signal integration at 480nm and 540 nm. The data were presented as H2O2-induced or 6-OHDA-induced BRET changes within Rluc-PTEN-YFP sensor by subtracting the 540 nm/480 nm ratio of H2O2 or 6-OHDA treated cells from the corresponding ratio of untreated cells (Control).

Western Blotting

N27 dopaminergic cells cell lysates were prepared using RIPA lysis buffer and normalized for equal amounts of protein using the Bradford protein assay kit. Equal amounts of protein (10–50 µg) were loaded for each sample and separated on either 12% or 15% SDS-PAGE gels based on the molecular weight of the target protein. After separation, proteins were transferred to a nitrocellulose membrane and the nonspecific binding sites were blocked for 1 h using a 5% non-fat milk (BIORAD, Hercules, CA). Membranes were then probed overnight at 4°C with the respective primary antibodies PTEN native- 1:1000; PTEN phospho 380– 1:1000; PTEN phospho 370 – 1:1000; FYN phospho 530 – 1:2000. HRP tagged secondary anti- mouse or anti-rabbit secondary antibodies were added at a dilution of 1:2000 and for loading control, β-Actin – 1:3000 was added to the membranes. After incubation, the membranes were washed 3 times with PBS containing 0.05% Tween-20 and the membranes visualized on the BIORAD molecular imager gel doc XR for reading chemiluminescence.

Sytox Cell Death Assay

The N27 dopaminergic cells were seeded in a 96-well plate (1.5 x 10^4cells/well) and incubated with 300 µM MPP^+ iodide for up to 24 h in the presence or absence of 30nM VO-OHpic. Following incubation, cell death was determined using cell impermeable Sytox green (Molecular Probes, Eugene, US). Sytox green intercalates with the DNA in the membrane-compromised cells to produce a green fluorescence that is quantifiable at 485/538 nm using the fluorescence microplate system (Synergy 4, Biotek, Winooski, VT).

Mitotracker Assay

MitoTracker Red CMXRos was used according to previous publications (Charli et al., 2016; Gordon, Richard et al., 2016). Briefly, SH-SY5Y dopaminergic cells were plated in black 96-well plate (4 x 10^4 cells/well) and treated separately with 500 µM MPP^+ iodide, 100 µM 6-OHDA, 20 µM H_2O_2 for up to 24 h in the presence or absence of 30nM VO-OHpic for each parkinsonian toxin. After the treatment the cells were washed and incubated with MitoTracker Red CMXRos dye (200 nM) for 15 min. Following incubation, cells were washed twice with 1XPBS to remove phenol-red containing medium. MitoTracker Red CMXRos dye stains mitochondria in live cells and its accumulation is dependent upon membrane potential. The fluorescence was read at 569/599nm using the fluorescence microplate system (Synergy 4, Biotek, Winooski, VT).

Quantiblue SEAP assay

The cell culture supernatant (20µl) from each well of the raw-blue cell treatment (as described above) was collected and transferred to a transparent 96-well plate. 180µl of Quanti-blue solution In-vivogen (San Diego, USA) was added into each well and incubated at 37^0C for 1 hour or till the color of the media changes purple. The optical density was measured using a plate reader at an absorbance of 620nm.

ELISA

Enzyme-linked immunosorbent analysis (ELISA) was used to measure the cytokines IL-1β and TNF-α from the supernatant obtained from the above cell treatment experiments. The ELISA kit from R&D systems was used following the manufacturer's instructions. The absorbance was measured at 570nm using a Spark microplate reader (Tecan). The concentration of cytokines in samples were determined from the standard curve.

Microglial nitric oxide detection

Griess reagent (Sigma Aldrich St Louis, MO) was used to determine nitric oxide production from the VO-OHpic treated microglia. The microglial cells were treated with 1 µg/mL of LPS in pretreated cells with VO-OHpic for 1 hour. After 48hrs, 80 µl of supernatant was collected from the well and an equal volume of the Griess reagent (Sigma Aldrich, St Louis, MO) was added. The samples were incubated at room temperature for 15 mins and absorbance was measured at 540nM using a Tecan plate reader. The nitrite concentration was determined using a sodium nitrite standard curve.

Statistical analysis:

All in vitro data were determined from three to eight biological replicates. GraphPad 5.0 was used for statistical analysis with $p \leq 0.05$ considered statistically significant. Two-way ANOVA was used for comparing multiple groups and ordinary-one-way ANOVA was used for the rest. In most cases, Bonferroni post

analysis was applied. For comparing 2 groups, Student's *t*-test was used. Where the normality assumption was violated, we conducted nonparametric tests, however, in no case did the nonparametric results change the overall interpretation of parametric results.

Results

Parkinsonian toxicant MPP^+ induces concomitant PTEN activity

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a phosphatase that suppresses many tumour types and plays numerous roles during the development of nervous system (Maehama and Dixon 1999, Lee, Chen et al. 2018). PTEN is associated with neurological disorders such as autism (Zhou and Parada 2012, Spina Nagy, Kawamoto et al. 2021). Interestingly, genetic ablation of PTEN has been previously shown to afford neuroprotection in experimental models of PD (Domanskyi, Geissler et al. 2011, Ogino, Ichimura et al. 2016). Previously, Src family kinase – Fyn kinase has been identified as a key signalling molecule that led to molecular events underlying neuroinflammation and dopaminergic degeneration in experimental models of PD (Saminathan, Asaithambi et al. 2011, Panicker, Sarkar et al. 2019, Saminathan, Charli et al. 2020, Saminathan, Ghosh et al. 2021). In this study, we sought to understand that the activation profile of PTEN using *in vitro* models of PD. Treatment of N27 dopaminergic cells with 300μM of MPP^+ iodide induced dephosphorylation of Fyn kinase at Tyr-530 (Y-530) from 30 minutes until 90 minutes, indicative of Fyn activation (Fig 1(a)). This finding was interestingly paralleled by dephosphorylation of PTEN specifically, at Ser-370 (S-370) *(viz.)* in our study we found that PTEN is specifically dephosphorylated at S-370 by MPP^+ iodide. Dephosphorylation of PTEN at S-370 is indicative of its activation (Gericke, Munson et al. 2006) was observed as early as 15 minutes and was sustained until 90 minutes (Fig 1(b)). Taken together, these findings suggest that PTEN activation precedes Fyn kinase activation. We were intrigued by these findings and set to investigate the ramifications of these molecular events in relation to dopaminergic degeneration.

PTEN is an early stress sensor in live cells

Biosensors based on intramolecular resonance energy transfer (RET) detect molecular proximity between donor and acceptor molecules at nanometre scale using either bioluminescence or fluorescence resonance energy transfer (BRET and FRET, respectively) technologies (Lohse, Nuber et al. 2012). Changes in the intramolecular RET, depending on the relative orientation of the donor and acceptor proteins in the fusion, permit the monitoring of conformational changes in the 'test' protein sandwiched between the donor/acceptor couple (Charest, Terrillon et al. 2005, Lohse, Nuber et al. 2012). To determine changes in PTEN conformation, which is indicative of PTEN involvement and activation upon inducing stress, we used an intramolecular BRET-based biosensor (named Rluc-PTEN-YFP), comprising the energy donor, *Renilla* luciferase (Rluc), fused to the amino terminus of PTEN and the energy acceptor, the yellow fluorescent protein (YFP), fused to the carboxy terminus of PTEN as previously described (Lima-Fernandes, Misticone et al. 2014). The Rluc-PTEN-YFP biosensor was used to rapidly detect conformational changes within PTEN in intact cells and in real-time upon its transient expression in HEK293, exposed to 100μM of pro-oxidant H_2O_2 and 100 μM 6-OHDA separately at different time (0, 5, 15…..min).. We observed an increase in BRET signals as early as 5 min after both treatments (Fig 2(a, b)). The change in BRET signals were observed at 5 min and 90 min during H_2O_2 treatment and 6-OHDA induced BRET signals were observed at 5 min and 45 min. This indicates changes in PTEN conformation upon H_2O_2-induced and 6-OHDA-induced cell stress suggesting the implication of PTEN in such a pathway. Taken together with our earlier findings where we observed dephosphorylation of PTEN at S-370 site, these results suggest of PTEN activation as an early sensor of stress signals in *in vitro* models of PD.

Pharmacological inhibition of PTEN reverses Parkinsonian toxicants induced Fyn activation

Several studies have previously implicated Fyn activation as an early signalling event during dopaminergic neuronal cell death (Panicker, Kanthasamy et al. 2019, Saminathan, Charli et al. 2020, Saminathan, Ghosh et al. 2021). However, the upstream signaling event in the signalling cascade that leads to Fyn kinase activation during dopaminergic cell death remains uninvestigated. Earlier findings in this study shows that PTEN activation precedes Fyn kinase activation. Several researchers have shown previously that Fyn attains an open conformation following dephosphorylation at the negatively regulating Y-530 site at the C-terminus paralleled by several folds of increase in Fyn enzymatic activity (Palacios-Moreno, Foltz et al. 2015, Mkaddem, Murua et al. 2017). This molecular event that leads to Fyn activation has to be effected by a tyrosine phosphatase that lies upstream in the signalling cascade. While we identified concomitant activation of PTEN and Fyn in *in vitro* models of PD, and we sought to know how pharmacological inhibition of PTEN modulates Fyn activation. Among the few of commercially available pharmacological inhibitors of tyrosine phosphatases, VO-OHpic is the most widely used tool compound to research on PTEN in both *in vitro* and *in vivo* studies (Johnson and Singla 2018, Wang, Wang et al. 2020, Yao, Yu et al. 2020). Previous studies show that VO-OHpic inhibits PTEN at 30 nM concentration in a protein-free system. Because we are unaware of the protein binding properties of VO-OHpic in our system, we chose to use 30 nM for our pharmacological studies (Rosivatz, Matthews et al. 2006).

We treated N27 dopaminergic neuronal cells with 300 μM MPP^+ in the presence or absence of PTEN inhibitor - VO-OHpic and assayed for Fyn activation by immunoblotting. As anticipated, we observed that pharmacological inhibition of PTEN attenuated MPP^+ induced Fyn dephosphorylation suggesting that PTEN is the tyrosine phosphatase that induces Fyn activation in *in vitro* models of PD (Fig 3).

Rescue of mitochondrial dysfunction by pharmacological inhibition of PTEN

Classical Parkinsonian toxicants, as well as oxidative stress inducers are well known to induce dopaminergic cell death by causing mitochondrial dysfunction (Heales, Menzes et al. 2011, Schapira and Patel 2014).
Previously, PTEN has been implicated in hippocampal neuronal apoptosis by causing mitochondrial dysfunction (Zhu, Hoell et al. 2006). To understand pharmacological inhibition of PTEN in experimental models of PD, we exposed SHSY- 5Y dopaminergic cells to Parkinsonian toxicants – MPP^+ iodide and 6-OHDA and pro-oxidant H_2O_2 for a duration of 24 hours in the presence or absence of 30nM VO-OHpic. At the end of treatment, the cells were assayed for mitochondrial functionality by the addition of *MitoTracker*

Red *CMXRos*, a fluorescent dye that binds to mitochondria when it is intact, irrespective of mitochondrial potential. The signal from this fluorescent dye when excited at 569nm is indicative of overall health of mitochondria and loss of signals indicates loss of mitochondrial function.

For the first time, as shown in Fig 4(a, b, c), we identify that PTEN plays a key role in maintaining mitochondrial health and function in dopaminergic cells as pharmacological inhibition of PTEN attenuates mitochondrial damage caused by classical parkinsonian toxicants. This novel finding ne of PTEN in maintaining mitochondrial health during dopaminergic degeneration.

Pharmacological inhibition of PTEN rescues dopaminergic neuronal cells from MPP^+ induced cytotoxicity

Previously, we observed that pharmacological inhibition of PTEN attenuated Fyn activation by maintaining the phosphorylated status of Y530 site on the C-terminus, thereby keeping it inactive. Researchers in the past have shown that genetic ablation of PTEN protects nigral dopaminergic neurons in *in vivo* models of PD (Diaz-Ruiz, Zapata et al. 2009, Domanskyi, Geiβler et al. 2011).
We next sought to examine the effect of pharmacological inhibition of PTEN on Parkinsonian toxicant MPP^+ iodide induced cytotoxicity. N27 dopaminergic cells were treated with MPP^+ iodide in the presence or absence of PTEN inhibitor - VO-OHpic and cytotoxicity was measured by cell-permeable Sytox green, fluorescent dye at 24 h post-
treatment. As anticipated, we found that pharmacological inhibition of PTEN protected dopaminergic cells from MPP^+ induced cytotoxicity (Fig 5(a,b,c). Our findings using pharmacological inhibitor of PTEN further validates this finding and more importantly, we have identified a potentially viable strategy for devising future pharmacological interventions targeting PD.

PTEN inhibitor significantly reduces transcription of NF-κB and IL-1β induced by LPS

The undeniable connection between system & chronic inflammatory conditions that induces a sustained release of pro-inflammatory mediators and dopaminergic degeneration has been retold by several researchers (Reale, Iarlori et al. 2009, Madeira, Boia et al. 2015). Recently, the involvement of neuroinflammation in the degeneration of dopaminergic neurons has been increasingly in the limelight for PD and is thought to be a major concerning cause in neurodegeneration.(Stojkovska, Wagner et al. 2015, Gordon, Singh et al. 2016, Pajares, I Rojo et al. 2020, Saminathan, Charli et al. 2020). Inflammasome, an NLR-based multiprotein responsible for caspase-1 activation in immune cells has been implicated to be a main component in neuroinflammation (Albornoz, Woodruff et al. 2018, Deora, Lee et al. 2020, Zheng, Liwinski et al. 2020, Johnson, Ou et al. 2022). Members of the NLR family (NLR proteins –NLRP) and the adaptor ASC form multiprotein complexes which are required for the activation of the pro-inflammatory caspase-1 and subsequent processing of pro-interleukin (IL)-1β and pro-IL-18 into the mature forms that are released from the cell (Martinon and Tschopp 2007, Takeuchi and Akira 2010). Also, the priming of NLRP activation and the release of IL-1β is tightly regulated by NF-κB (Bauernfeind, Horvath et al. 2009). Here, we wanted to understand PTEN role in neuroinflammation in PD. Moreover,(Wu, Huang et al. 2019, Sarn, Jaini et al. 2021, Zhao, Gao et al. 2021) PTEN is implicated to be a driving factor in many disorders such as ASD, epilepsy and cancer. We pharmacologically inhibited PTEN and checked the NLRP3 inflammasome activation, NF-kB levels, TNF-a and nitrite levels. The concentration of LPS and time for incubation was standardised during the development of the reporter cell assay. The cells were pre-treated with various concentrations of the drug (10nM - 30μM) for 1 hour followed by LPS activation (500 pg/ml) for 20-24hrs. The PTEN inhibitor VO-OH p i c was found to inhibit transcription of NF-κB/AP-1 significantly at 30nM.

Treatment with PTEN inhibitor significantly reduces production of IL-1β induced by LPS primed PBMC cells

To determine PTEN had inhibitory effects within *in-vitro* models of inflammasome pathway, we used human Peripheral Blood Mononuclear Cells (PBMCs) that were pre-treated with VO-OHpic then primed with LPS and activated with ATP. As we anticipated the pharmacological inhibition of PTEN using VO-OHpic significantly blocked NLRP3 activation and IL-1β release (Fig 6a). However, with the non-inflammasome pathway, no significant change in the levels of TNF-a were detected with the treatment of VO-OHpic. This result showed that PTEN plays a crucial role in regulating neuroinflammation (Fig 6b).

Treatment with PTEN inhibitor significantly reduces production of IL-1β induced by LPS primed microglial cells

Next microglia, primed with UP LPS and activated with ATP were utilised to study effect of the drug in inflammasome inhibition. Interestingly, like the PBMC's the PTEN inhibition appears to significantly block NLRP3 activation and IL-1β release as shown in Fig 7a. Other pro-inflammatory mediators of microglia such as nitric oxide also showed substantial reduction when measured using Griess assay (Fig 7b).

Treatment with PTEN inhibitor significantly reduces transcription of NF-κB induced by LPS

To further elucidate the role of PTEN in inflammasome activation, we checked the levels of NF-κB using Raw blue reporter cells. The cells were pre-treated with PTEN inhibitor- VO-OHpic for 1 hour led by LPS activation (500pg/ml) for 20-24hrs. We observed a significant inhibition of transcription of NF-κB/AP-1 using the Quantiblue SEAP assay (Fig 8a) and no change in the TNF-α levels (Fig 8b).

Discussion

In this study we have shown in *in vitro* models of Parkinson's Disease that PTEN regulates Fyn kinase activation and is a key sensitizing molecule that modulates neuroinflammatory events and dopaminergic cell death. We have also identified a crucial role of PTEN in the inflammasome activation regulated by microglia using pharmacologically inhibitors of PTEN. Using multiple cell-culture models of PD treated with classical Parkinsonian toxicants - MPP^+ iodide and 6-OHDA, we show that PTEN activation by post-translational modification as an early event that precedes Fyn activation. Additionally, we also demonstrate undeniable role to PTEN in causing mitochondrial dysfunction that result in dopaminergic cell death. Previously, Fyn has been widely investigated and has been shown to cause dopaminergic degeneration in various models of in PD by regulating the PKCδ signaling pathway (Saminathan,

Ghosh et al. 2021). However, the upstream activator of Fyn kinase is remained unidentified. In this study, we have shown that PTEN, a dual specific phosphatase lies upstream in the Fyn signalling cascade during dopaminergic cell death.

PTEN has been drawing attention among several researchers investigating neurological disorders including Alzheimer's disease (Cui, Wang et al. 2017). Physiologically, PTEN is an important molecule that is known to regulate crucial cellular processes such as cell proliferation and survival (Lee, Chen et al. 2018). Interestingly, recent evidences have also implicated pathological roles to PTEN using PD models disease (Domanskyi, Geiβler et al. 2011, Wang, Lu et al. 2020). In this investigation, we have unravelled deeper insights into molecular underpinnings of PTEN activation and that PTEN lies upstream to Fyn activation, both of which are very early molecular events that cause dopaminergic cell death. First, we reconfirmed and established the dynamics of Fyn activation profile in our laboratory conditions and our findings were in agreement with earlier findings (Saminathan, Ghosh et al. 2021). More importantly, we demonstrate that PTEN activation precedes the Fyn kinase activation in the cell culture models of PD suggesting that PTEN may be upstream phosphatase that regulated of Fyn activation. While BRET assay demonstrates changes to the molecular configuration of PTEN to parallel with its activation as shown by immunoblotting indicating its implication in the stress pathway.

To understand if pharmacological inhibition of PTEN modulates Fyn activation, we utilized the commercially available pharmacological inhibitor - VO-OHpic for further experimentation. As anticipated, pharmacological inhibition of PTEN attenuated Fyn activation. Strikingly, for the first time, we have demonstrated that attenuation of PTEN activity reversed mitochondrial dysfunction and dopaminergic cell death. Taken together these observations suggest that PTEN is the upstream sensitizer of stress signals, activator of Fyn kinase and also contributes to dopaminergic cell death.

Evidences from experimental models and human PD postmortem studies strongly implicate the microglia-mediated inflammatory response as a major driver in the progression of PD. However, the key upstream cell signaling mechanisms that govern the neuroinflammatory processes are yet to be elaborately elucidated. In this study, we have shown that PTEN plays a significant role in influencing the proinflammatory mediators following an insult. Previously PTEN has been noted to be involved in the inflammatory pathway in pathogenesis of tumor and as well as several neurological diseases (Wu, Huang et al. 2019, Sarn, Jaini et al. 2021, Zhao, Gao et al. 2021, Zeng, Jiang et al. 2022). Classical activation of microglia by TLR and TNFR1 agonists produces proinflammatory cytokines and chemokines, which mediate the downstream effects of microglial activation. It is showed that TNFα directly induces dopaminergic neuronal apoptosis (Gordon, Anantharam et al. 2012). Previous investigators have undeniably argued that prolonged activation of inflammasome is shown having a causal relationship in the pathogenesis of Parkinson's disease and several other neurological disorders, our novel findings demonstrate that PTEN plays a major role in promoting inflammasome activation when an insult triggers microglia (Albornoz, Woodruff et al. 2018, Gordon, Albornoz et al. 2018, Johnson, Ou et al. 2022).

To summarize, we have shown that PTEN is an early sensitizer of molecular events that cause dopaminergic cell death and activate neuroinflammatory pathways that culminate in PD. By using pharmacological inhibitor, we have also shown that targeting PTEN could be a potentially viable and a novel interventional strategy to alter the course of PD that necessitates further investigation.

Declarations

Author's information

Contribution

AMJ and SJ are co-first authors in the study, and they have contributed equally. HS, RG and MAA conceptualized the hypothesis. ARP, FBK, SAAA and JK performed some experiments. AMJ and SJ performed majority of the experiments and data analysis under the supervision of HS, RG, MAA, MGS. HS, RG, MAA, AMJ, SJ contributed to the drafting of the manuscript. All authors have read and approved the final version of the manuscript.

Corresponding author

Correspondence to Aishwarya Mary Johnson (email – aishwaryajohnson15@gmail.com, aishwarya.mary@uaeu.ac.ae)

Competing Interest

The authors have no competing financial or non-financial interests to declare that are relevant to this manuscript.

References

1. Albornoz EA, Woodruff TM, Gordon R (2018) Inflammasomes in CNS diseases. Inflammasomes: Clinical and Therapeutic Implications, Springer: 41–60 https://doi.org/10.1007/978-3-319-89390-7_3
2. Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA (2009) "Cutting edge: NF-κB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression". J Immunol 183(2):787–791. https://www.jimmunol.org/content/jimmunol/183/2/787.full.pdf
3. Carracedo A, Pandolfi PP (2008) "The PTEN–PI3K pathway: of feedbacks and cross-talks. " Oncogene 27(41):5527–5541. https://doi.org/10.1038/onc.2008.247
4. Charest PG, Terrillon S, Bouvier M (2005) "Monitoring agonist-promoted conformational changes of beta-arrestin in living cells by intramolecular BRET". EMBO Rep 6(4):334–340. https://doi.org/10.1038/sj.embor.7400373

5. Cui W, Wang S, Wang Z, Wang Z, Sun C, Zhang Y (2017) "Inhibition of PTEN Attenuates Endoplasmic Reticulum Stress and Apoptosis via Activation of PI3K/AKT Pathway in Alzheimer's Disease". Neurochem Res 42(11):3052–3060. https://doi.org/10.1007/s11064-017-2338-1
6. Deora V, Lee JD, Albornoz EA, McAlary L, Jagaraj CJ, Robertson AA, Atkin JD, Cooper MA, Schroder K, Yerbury JJ (2020) "The microglial NLRP3 inflammasome is activated by amyotrophic lateral sclerosis proteins. " Glia 68(2):407–421. https://doi.org/10.1002/glia.23728
7. Diaz-Ruiz O, Zapata A, Shan L, Zhang Y, Tomac AC, Malik N, de la Cruz F, Bäckman CM (2009) "Selective deletion of PTEN in dopamine neurons leads to trophic effects and adaptation of striatal medium spiny projecting neurons". PLoS ONE 4(9):e7027–e7027. https://pubmed.ncbi.nlm.nih.gov/19750226
8. Domanskyi A, Geissler C, Vinnikov IA, Alter H, Schober A, Vogt MA, Gass P, Parlato R, Schutz G (2011) "Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models". FASEB J 25(9):2898–2910. https://www.ncbi.nlm.nih.gov/pubmed/21593433
9. Domanskyi A, Geißler C, Vinnikov IA, Alter H, Schober A, Vogt MA, Gass P, Parlato R, Schütz G (2011) "Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models". FASEB J 25(9):2898–2910. https://faseb.onlinelibrary.wiley.com/doi/abs/ 10.1096/fj.11-181958
10. Gary DS, Mattson MP (2002) "PTEN regulates Akt kinase activity in hippocampal neurons and increases their sensitivity to glutamate and apoptosis". Neuromol Med 2(3):261–269. https://doi.org/10.1385/NMM:2:3:261
11. Gericke A, Munson M, Ross AH (2006) "Regulation of the PTEN phosphatase". Gene 374:1–9. https://doi.org/10.1016/j.gene.2006.02.024
12. Ghosh A, Kanthasamy A, Joseph J, Anantharam V, Srivastava P, Dranka BP, Kalyanaraman B, Kanthasamy AG (2012) Anti-inflammatory and neuroprotective effects of an orally active apocynin derivative in pre-clinical models of Parkinson's disease. J Neuroinflammation 9:241. https://www.ncbi.nlm.nih.gov/pubmed/23092448
13. Gordon R, Albornoz EA, Christie DC, Langley MR, Kumar V, Mantovani S, Robertson AAB, Butler MS, Rowe DB, O'Neill LA, Kanthasamy AG, Schroder K, Cooper MA, Woodruff TM (2018) "Inflammasome inhibition prevents α-synuclein pathology and dopaminergic neurodegeneration in mice". Sci Transl Med 10(465). https://www.science.org/doi/10.1126/scitranslmed.aah4066
14. Gordon R, Anantharam V, Kanthasamy AG, Kanthasamy A (2012) Proteolytic activation of proapoptotic kinase protein kinase Cδ by tumor necrosis factor α death receptor signaling in dopaminergic neurons during neuroinflammation. J Neuroinflammation 9:82. https://doi.org/10.1186/1742-2094-9-82
15. Gordon R, Singh N, Lawana V, Ghosh A, Harischandra DS, Jin H, Hogan C, Sarkar S, Rokad D, Panicker N, Anantharam V, Kanthasamy AG, Kanthasamy A (2016) Protein kinase Cδ upregulation in microglia drives neuroinflammatory responses and dopaminergic neurodegeneration in experimental models of Parkinson's disease. Neurobiol Dis 93:96–114. https://www.sciencedirect.com/science/article/pii/S0969996116300900
16. Guan Y, Yang F, Yao Q, Shi J, Wang G, Gu Z, Zhou F, Shen J (2015) Impacts of phosphatase and tensin homology deleted on chromosome ten (PTEN)-inhibiting chitosan scaffold on growth and differentiation of neural stem cells. Int J Clin Exp Med 8(8):14308–14315. https://www.ncbi.nlm.nih.gov/pubmed/26550415
17. Hariz M, Obeso JA (2017) "What Would Dr. James Parkinson Think Today? I. The Role of Functional Neurosurgery for Parkinson's Disease. " Mov Disord 32(1):2–4. https://www.ncbi.nlm.nih.gov/pubmed/28124429
18. He J, Long C, Huang Z, Zhou X, Kuang X, Liu L, Liu H, Tang Y, Fan Y, Ning J, Ma X, Zhang Q, Shen H (2017) "PTEN Reduced UVB-Mediated Apoptosis in Retinal Pigment Epithelium Cells". Biomed Res Int 2017:3681707. https://www.ncbi.nlm.nih.gov/pubmed/28321407
19. Heales SJ, Menzes A, Davey GP (2011) Depletion of glutathione does not affect electron transport chain complex activity in brain mitochondria: Implications for Parkinson disease and postmortem studies. Free Radic Biol Med 50(7):899–902. https://doi.org/10.1016/j.freeradbiomed.2010.11.032
20. Hou SQ, Ouyang M, Brandmaier A, Hao H, Shen WH (2017) "PTEN in the maintenance of genome integrity: From DNA replication to chromosome segregation."Bioessays**39**(10)https://www.ncbi.nlm.nih.gov/pubmed/28891157
21. Johnson AM, Ou Z-YA, Gordon R, Saminathan H (2022) Environmental neurotoxicants and inflammasome activation in Parkinson's disease–a focus on the gut-brain axis. Int J Biochem Cell Biol 142:106113. https://doi.org/10.1016/j.biocel.2021.106113
22. Johnson TA, Singla DK (2018) PTEN inhibitor VO-OHpic attenuates inflammatory M1 macrophages and cardiac remodeling in doxorubicin-induced cardiomyopathy. Am J Physiol Heart Circ Physiol 315(5):H1236–H1249. https://doi.org/10.1152/ajpheart.00121.2018
23. Kaul S, Anantharam V, Yang Y, Choi CJ, Kanthasamy A, Kanthasamy AG (2005) Tyrosine phosphorylation regulates the proteolytic activation of protein kinase Cδ in dopaminergic neuronal cells. J Biol Chem 280(31):28721–28730. https://doi.org/10.1074/jbc.M501092200
24. Kaul S, Kanthasamy A, Kitazawa M, Anantharam V, Kanthasamy AG (2003) Caspase-3 dependent proteolytic activation of protein kinase Cδ mediates and regulates 1-methyl-4-phenylpyridinium (MPP+)-induced apoptotic cell death in dopaminergic cells: relevance to oxidative stress in dopaminergic degeneration. Eur J Neurosci 18(6):1387–1401. https://doi.org/10.1046/j.1460-9568.2003.02864.x
25. Kim RH, Mak TW (2006) "Tumours and tremors: how PTEN regulation underlies both". Br J Cancer 94(5):620–624. https://www.ncbi.nlm.nih.gov/pubmed/16495927
26. Korczyn AD, Hassin-Baer S (2015) "Can the disease course in Parkinson's disease be slowed? " BMC Med 13:295. https://www.ncbi.nlm.nih.gov/pubmed/26653056
27. Lee Y-R, Chen M, Pandolfi PP (2018) The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol 19(9):547–562. https://doi.org/10.1038/s41580-018-0015-0
28. Lima-Fernandes E, Misticone S, Boularan C, Paradis JS, Enslen H, Roux PP, Bouvier M, Baillie GS, Marullo S, Scott MG (2014) "A biosensor to monitor dynamic regulation and function of tumour suppressor PTEN in living cells". Nat Commun 5. https://doi.org/10.1038/ncomms5431
29. Lin M, Chandramani-Shivalingappa P, Jin H, Ghosh A, Anantharam V, Ali S, Kanthasamy AG, Kanthasamy A (2012) "Methamphetamine-induced neurotoxicity linked to ubiquitin-proteasome system dysfunction and autophagy-related changes that can be modulated by protein kinase C delta in dopaminergic neuronal cells. " Neurosci 210:308–332. https://www.ncbi.nlm.nih.gov/pubmed/22445524

30. Lohse MJ, Nuber S, Hoffmann C (2012) "Fluorescence/bioluminescence resonance energy transfer techniques to study G-protein-coupled receptor activation and signaling". Pharmacol Rev 64(2):299–336. https://pubmed.ncbi.nlm.nih.gov/22407612/
31. Lungu C, Cedarbaum JM, Dawson TM, Dorsey ER, Faraco C, Federoff HJ, Fiske B, Fox R, Goldfine AM, Kieburtz K, Macklin EA, Matthews H, Rafaloff G, Saunders-Pullman R, Schor NF, Schwarzschild MA, Sieber BA, Simuni T, Surmeier DJ, Tamiz A, Werner MH, Wright CB, Wyse R (2021) "Seeking progress in disease modification in Parkinson disease". Parkinsonism Relat Disord 90:134–141. https://www.ncbi.nlm.nih.gov/pubmed/34561166
32. Madeira MH, Boia R, Santos PF, Ambrósio AF, Santiago AR (2015) "Contribution of Microglia-Mediated Neuroinflammation to Retinal Degenerative Diseases." Mediators of Inflammation **2015**: 673090https://doi.org/10.1155/2015/673090
33. Maehama T, Dixon JE (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. Trends Cell Biol 9(4):125–128. https://www.sciencedirect.com/science/article/pii/S0962892499015196
34. Martinon F, Tschopp J (2007) Inflammatory caspases and inflammasomes: master switches of inflammation. Cell Death & Differentiation 14(1):10–22. https://doi.org/10.1038/sj.cdd.4402038
35. Mkaddem SB, Murua A, Flament H, Titeca-Beauport D, Bounaix C, Danelli L, Launay P, Benhamou M, Blank U, Daugas E, Charles N, Monteiro RC (2017) "Lyn and Fyn function as molecular switches that control immunoreceptors to direct homeostasis or inflammation". Nat Commun 8(1):246. https://doi.org/10.1038/s41467-017-00294-0
36. Ning K, Drepper C, Valori CF, Ahsan M, Wyles M, Higginbottom A, Herrmann T, Shaw P, Azzouz M, Sendtner M (2010) "PTEN depletion rescues axonal growth defect and improves survival in SMN-deficient motor neurons". Hum Mol Genet 19(16):3159–3168. https://www.ncbi.nlm.nih.gov/pubmed/20525971
37. Ogino M, Ichimura M, Nakano N, Minami A, Kitagishi Y, Matsuda S (2016) Roles of PTEN with DNA Repair in Parkinson's Disease. Int J Mol Sci 17(6):954. https://pubmed.ncbi.nlm.nih.gov/27314344
38. Olanow CW (2014) "Parkinson disease: Gene therapy for Parkinson disease–a hope, or a dream?". Nat Rev Neurol 10(4):186–187. https://www.ncbi.nlm.nih.gov/pubmed/24662536
39. Olanow CW, Obeso JA (2011) "Levodopa toxicity and Parkinson disease: still a need for equipoise. " Neurol 77(15):1416–1417. https://www.ncbi.nlm.nih.gov/pubmed/21917774
40. Pajares M, Rojo AI, Manda G, Boscá L, Cuadrado A (2020) "Inflammation in Parkinson's Disease. Mech Therapeutic Implications " Cells 9(7):1687. https://pubmed.ncbi.nlm.nih.gov/32674367
41. Palacios-Moreno J, Foltz L, Guo A, Stokes MP, Kuehn ED, George L, Comb M, Grimes ML (2015) "Neuroblastoma tyrosine kinase signaling networks involve FYN and LYN in endosomes and lipid rafts". PLoS Comput Biol 11(4):e1004130. https://doi.org/10.1371/journal.pcbi.1004130
42. Panicker N, Sarkar S, Harischandra DS, Neal M, Kam T-I, Jin H, Saminathan H, Langley M, Charli A, Samidurai M, Rokad D, Ghaisas S, Pletnikova O, Dawson VL, Dawson TM, Anantharam V, Kanthasamy AG, Kanthasamy A (2019) "Fyn kinase regulates misfolded α-synuclein uptake and NLRP3 inflammasome activation in microglia". J Exp Med 216(6):1411–1430. https://pubmed.ncbi.nlm.nih.gov/31036561
43. Peng J, Stevenson FF, Doctrow SR, Andersen JK (2005) Superoxide dismutase/catalase mimetics are neuroprotective against selective paraquat-mediated dopaminergic neuron death in the substantial nigra: implications for Parkinson disease. J Biol Chem 280(32):29194–29198. https://doi.org/10.1074/jbc.M500984200
44. Przedborski S (2017) The two-century journey of Parkinson disease research. Nat Rev Neurosci 18(4):251–259. https://www.ncbi.nlm.nih.gov/pubmed/28303016
45. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M (2009) Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun 23(1):55–63. https://doi.org/10.1016/j.bbi.2008.07.003
46. Rosivatz E, Matthews JG, McDonald NQ, Mulet X, Ho KK, Lossi N, Schmid AC, Mirabelli M, Pomeranz KM, Erneux C, Lam EWF, Vilar R, Woscholski R (2006) "A Small-Molecule Inhibitor for Phosphatase and Tensin Homologue Deleted on Chromosome 10 (PTEN)". ACS Chem Biol 1(12):780–790. https://doi.org/10.1021/cb600352f
47. Roskoski R Jr (2005) "Src kinase regulation by phosphorylation and dephosphorylation". Biochem Biophys Res Commun 331(1):1–14. https://www.ncbi.nlm.nih.gov/pubmed/15845350
48. Roskoski R Jr (2015) "Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors". Pharmacol Res 94:9–25. https://www.ncbi.nlm.nih.gov/pubmed/25662515
49. Saminathan H, Asaithambi A, Anantharam V, Kanthasamy AG, Kanthasamy A (2011) "Environmental neurotoxic pesticide dieldrin activates a non receptor tyrosine kinase to promote PKCdelta-mediated dopaminergic apoptosis in a dopaminergic neuronal cell model. " Neurotoxicology 32(5):567–577. https://www.ncbi.nlm.nih.gov/pubmed/21801747
50. Saminathan H, Charli A, Luo J, Panicker N, Gordon R, Hostetter JM, Jin H, Anantharam V, Kanthasamy AG, Kanthasamy A (2020) "Fyn kinase mediates pro-inflammatory response in a mouse model of endotoxemia: Relevance to translational research". Eur J Pharmacol 881:173259. https://www.sciencedirect.com/science/article/pii/S0014299920303514
51. Saminathan H, Ghosh A, Zhang D, Song C, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2021) Fyn Kinase-Mediated PKCδ Y311 Phosphorylation Induces Dopaminergic Degeneration in Cell Culture and Animal Models: Implications for the Identification of a New Pharmacological Target for Parkinson's Disease. Front Pharmacol 12. https://www.frontiersin.org/article/ 10.3389/fphar.2021.631375
52. Sarn N, Jaini R, Thacker S, Lee H, Dutta R, Eng C (2021) Cytoplasmic-predominant Pten increases microglial activation and synaptic pruning in a murine model with autism-like phenotype. Mol Psychiatry 26(5):1458–1471. https://doi.org/10.1038/s41380-020-0681-0

53. Schapira AHV, Patel S (2014) "Targeting Mitochondria for Neuroprotection in Parkinson Disease". JAMA Neurol 71(5):537–538. https://doi.org/10.1001/jamaneurol.2014.64
54. Song C, Charli A, Luo J, Riaz Z, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2019) "Mechanistic Interplay Between Autophagy and Apoptotic Signaling in Endosulfan-Induced Dopaminergic Neurotoxicity: Relevance to the Adverse Outcome Pathway in Pesticide Neurotoxicity. " Toxicol Sci 169(2):333–352. https://www.ncbi.nlm.nih.gov/pubmed/30796443
55. Spina Nagy G, Kawamoto EM, Bridi JC (2021) The role of PTEN signaling in synaptic function: Implications in autism spectrum disorder. Neurosci Lett 759:136015. https://www.sciencedirect.com/science/article/pii/S0304394021003931
56. Stojkovska I, Wagner BM, Morrison BE (2015) "Parkinson's disease and enhanced inflammatory response." Experimental biology and medicine. (Maywood N J) 240(11):1387–1395. https://pubmed.ncbi.nlm.nih.gov/25769314
57. Takeuchi O, Akira S (2010) "Pattern Recognit receptors Inflamm " Cell 140(6):805–820
58. Wang L, Lu G, Shen H-M (2020) The Long and the Short of PTEN in the Regulation of Mitophagy. Front Cell Dev Biology 8. https://www.frontiersin.org/article/ 10.3389/fcell.2020.00299
59. Wang W, Wang X, Guo H, Cai Y, Zhang Y, Li H (2020) PTEN inhibitor VO-OHpic suppresses TSC2(-) (/) (-) MEFs proliferation by excessively inhibiting autophagy via the PTEN/PRAS40 pathway. Exp Ther Med 19(6):3565–3570. https://doi.org/10.3892/etm.2020.8629
60. Wu Z-S, Huang W-L, Gong S-J (2019) "Effect of adenovirus-mediated overexpression of PTEN on brain oxidative damage and neuroinflammation in a rat kindling model of epilepsy". Chin Med J 132(21):2628–2635. https://journals.lww.com/cmj/Fulltext/2019/11050/Effect_of_adenovirus_mediated_overexpression_of.15.aspx
61. Yao X, Yu S, Jing X, Guo J, Sun K, Guo F, Ye Y (2020) PTEN inhibitor VO-OHpic attenuates GC-associated endothelial progenitor cell dysfunction and osteonecrosis of the femoral head via activating Nrf2 signaling and inhibiting mitochondrial apoptosis pathway. Stem Cell Res Ther 11(1):1–18. https://doi.org/10.1186/s13287-020-01658-y
62. Zeng L, Jiang H, Ashraf GM, Liu J, Wang L, Zhao K, Liu M, Li Z, Liu R (2022) Implications of miR-148a-3p/p35/PTEN signaling in tau hyperphosphorylation and autoregulatory feedforward of Akt/CREB in Alzheimer's disease. Mol Therapy-Nucleic Acids 27:256–275. https://doi.org/10.1016/j.omtn.2021.11.019
63. Zhao M, Gao J, Cui C, Zhang Y, Jiang X, Cui J (2021) "Inhibition of PTEN Ameliorates Secondary Hippocampal Injury and Cognitive Deficits after Intracerebral Hemorrhage: Involvement of AKT/FoxO3a/ATG-Mediated Autophagy." Oxidative Medicine and Cellular Longevity **2021**: 5472605https://doi.org/10.1155/2021/5472605
64. Zheng D, Liwinski T, Elinav E (2020) "Inflammasome activation and regulation: Toward a better understanding of complex mechanisms". Cell discovery 6(1):1–22. https://doi.org/10.1038/s41421-020-0167-x
65. Zheng J, Li H, Xu D, Zhu H (2017) Upregulation of Tyrosine Kinase FYN in Human Thyroid Carcinoma: Role in Modulating Tumor Cell Proliferation, Invasion, and Migration. Cancer Biother Radiopharm 32(9):320–326. https://www.ncbi.nlm.nih.gov/pubmed/29140740
66. Zhou J, Parada LF (2012) "PTEN signaling in autism spectrum disorders". Curr Opin Neurobiol 22(5):873–879. https://www.ncbi.nlm.nih.gov/pubmed/22664040
67. Zhu Y, Hoell P, Ahlemeyer B, Krieglstein J (2006) PTEN: A crucial mediator of mitochondria-dependent apoptosis. Apoptosis 11(2):197–207. https://doi.org/10.1007/s10495-006-3714-5

Figures

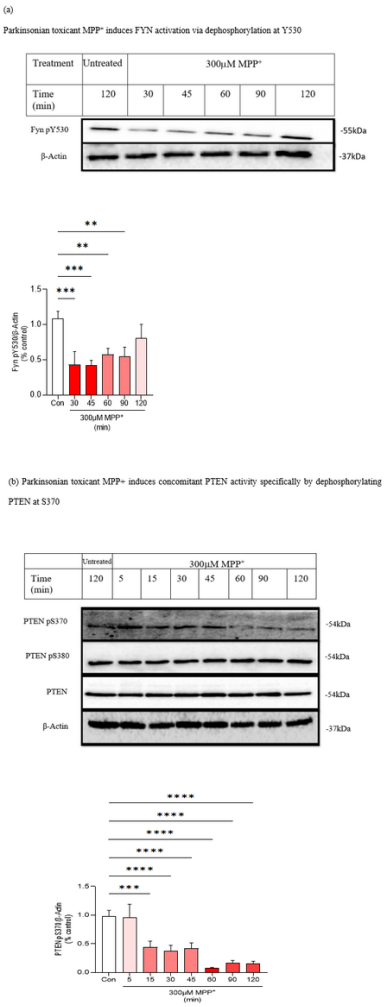

Figure 1

PTEN and Fyn activation Profile in N27 cells (a) Representative western blot image for Fyn pY530 during treatment with 300μM of MPP^+ for different time points. Loss of signal(dephosphorylation) at Y530 corresponds to Fyn kinase activation and the corresponding densitometric analysis of Fyn pY530 using ImageJ software, analysed by a multiple comparison one-way ANOVA with ordinary ANOVA tests (b) Representative western blot image for PTEN pS370 during treatment with 300μM of MPP^+ for different timepoints. N27 cells showed a PTEN activation in later timepoints at S370. Loss of signal (dephosphorylation) at S370 corresponds to PTEN activation. and densitometric analysis of PTEN S370 using ImageJ software. Analysed by a multiple comparison one-way ANOVA with ordinary ANOVA test****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns = not significant ($P > 0.05$)

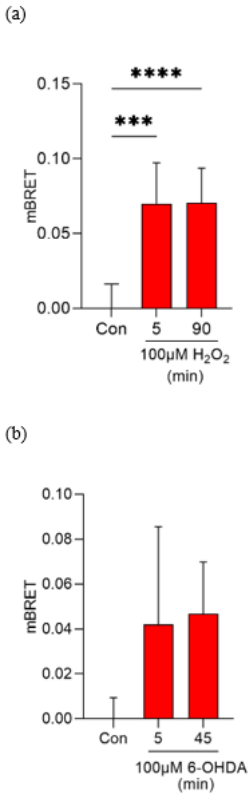

Figure 2

PTEN activation Profile of PTEN assayed by bioluminescence resonance energy transfer (BRET) assay in HEK293 cells. HEK293 cells were transiently transfected with Rluc-PTEN-YFP plasmid for 48hrs. PTEN activation profile when measured as BRET signal changes upon cell treatment with (A)100 μM H_2O_2 (B)100μM 6-OHDA at the indicated time points. Analysed by a multiple comparison one-way ANOVA with ordinary ANOVA tests. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns = not significant ($P > 0.05$)

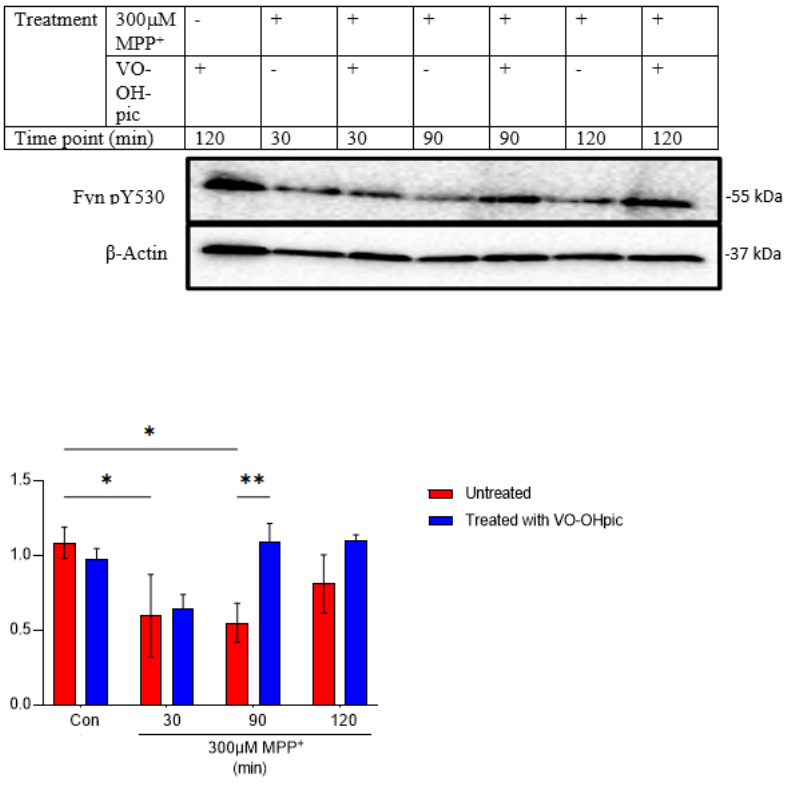

Figure 3

PTEN inhibitor VO-OHpic significantly reverses activation of Fyn. When N27 cells were co- treated with 300μM MPP^+ and 30nM of VO-OH-pic for different points showed a significant reversal of phosphorylation of Fyn at Y530 at 90min. Loss of signal of Fyn at Y530 corresponds to Fyn activation. Representative Western blot images. Densitometric analyses of Fyn activation profile at Y530 and co-treatment of VO-OHpic was done using ImageJ software. Statistical analysis by a multiple comparison one-way ANOVA with ordinary ANOVA tests. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns = not significant ($P > 0.05$)

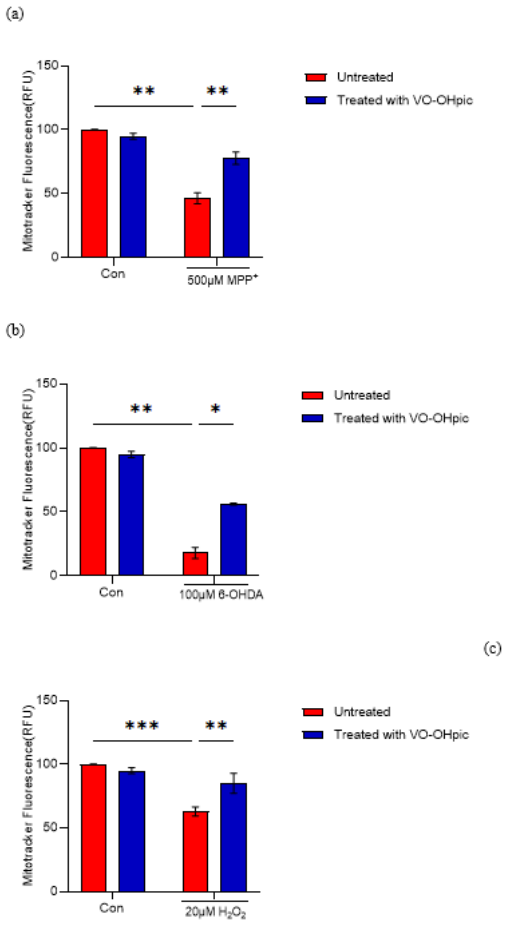

Figure 4

PTEN inhibitor – VO-OHpic reverses Parkinsonian toxicants and pro-oxidant stressor induced mitochondrial dysfunction. Rescue of viable mitochondria when treated with 30nM VO- OHpic in SH-SY 5Y dopaminergic cells. 200nM of MitoTracker Red CMXRos assay was used to detect viable mitochondria. The fluorescence was read at 569/599nm (A) 500μM MPP^+, (B) 100μM 6-OHDA, (C) 20μM H_2O_2 respectively was co-treated with 30nM PTEN inhibitor. Statistical analysis was performed by a multiple comparison one-way ANOVA with ordinary ANOVA tests. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns = not significant ($P > 0.05$)

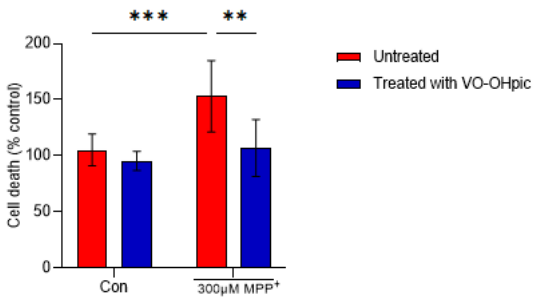

Figure 5

PTEN inhibitor – VO-OHpic rescues dopaminergic cells from Parkinsonian toxins induced cell death. Rescue of cells were observed when treated with 30nM VO-OHpic. Sytox cell viability assay was performed to detect the dead cells. The fluorescence was measure at 480/560nm. 300μM of MPP^+ was used as the trigger with a co-treatment of 30nM VO-OHpic. Analysed by a multiple comparison one-way ANOVA with Brown-Forsythe and Welch ANOVA tests. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, ns = not significant (P > 0.05)

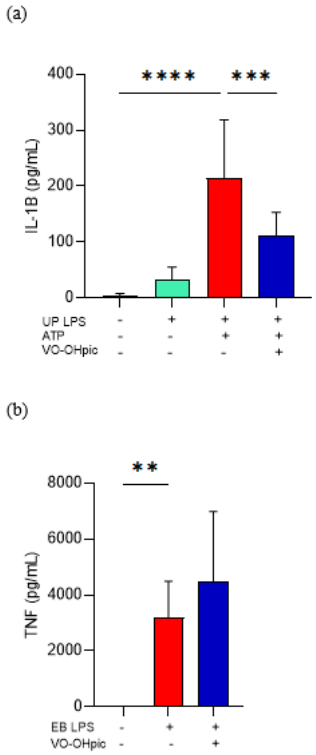

Figure 6

Effect of VO-OHpic on PBMCs in inflammasome and non- inflammasome pathway. (A) IL-1β secretion in PBMCs primed with ultrapure LPS (UP) and activated with 5 mM ATP following treatment with 30nM VO-OHpic (B) TNF-α secretion in PBMC's treated with LPS and pre-treated with VO-OHpic (n=8 per treatment). Data represented as mean ± SEM. **P<0.01, ****P<0.0001 analysed by a multiple comparison one-way ANOVA with Bonferroni's post hoc test

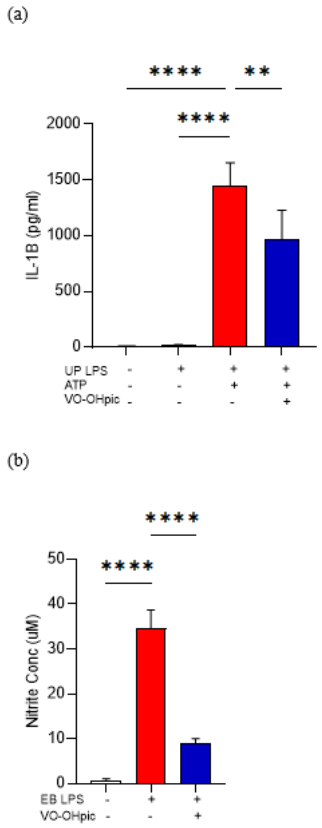

Figure 7

Pharmacological inhibition of PTEN with VO-OHpic blocks inflammasome activation and neuroinflammatory responses in microglia- (A) IL-1β secretion in microglia. Primary microglia primed with ultrapure LPS (UP) and activated with 5 mM ATP following treatment with 30nM concentration of VO-OHpic (B) Nitrate concentration in the supernatants of LPS-EB activated microglia in the absence or presence of VO-OHpic (n = 4 per treatment) Data represented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 analysed by a multiple comparison one-way ANOVA with Bonferroni's post hoc test

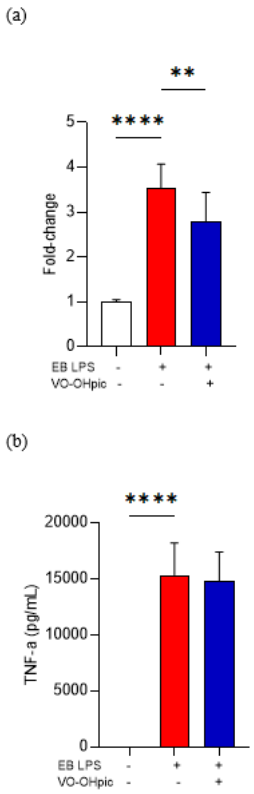

Figure 8

Pharmacological inhibition of PTEN with VO-OHpic significantly inhibits NF-κB/AP-1 release using Raw-blue reporter cell assay. Raw blue-reporter cells that stably express secreted embryonic alkaline phosphatase (SEAP) reporter gene, shows (A) NF-κB and activator protein-1 activation with LPS treatment (500pg/ml) and treatment with the VO- OHpic (n=12 per treatment) (B) TNF-α levels with VO-OHpic treatment (n=11 per treatment). Data represented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 analysed by a multiple comparison one-way ANOVA with Bonferroni's post hoc test

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- graphicalabstract.png