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Poster Abstracts

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1

Cellular, molecular, and pharmacological mechanisms underlying neuropathic pain in an alpha-synucleinopathy model

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Parkinson's disease (PD) is the most common α-synucleinopathy and the leading motor neurodegenerative disorder worldwide. It is pathologically characterized by the misfolding and aggregation of α-synuclein into Lewy bodies, which spread in a prion-like manner across interconnected brain regions. This propagation is associated not only with motor dysfunction but also with early and persistent non-motor symptoms such as chronic pain, which can precede motor signs by decades. However, the underlying cellular mechanisms of pain in PD remain poorly understood, but it is currently hypothesized that pathological α-synuclein could be directly involved in the origin of neuropathic pain wich is been associated with spinal immune activation and the downregulation of the potassium-chloride cotransporter KCC2, this downregulation could lead to chloride accumulation and a depolarizing shift in the chloride reversal potential (ECl-), transforming GABAergic neurotransmission from inhibitory to excitatory via GABAA receptor activation. PD animal model induced by intranigral administration of β-sitosterol β-D-glucoside (BSSG), a neurotoxin known to replicate key features of α-synucleinopathy. Mechanical allodynia and hyperalgesia were assessed using von Frey filaments. Spinal cord tissue was analyzed histologically to detect α-synuclein aggregates and evaluate KCC2 expression. To examine the role of GABAergic signaling, we pharmacologically blocked GABAAα6 receptors using furosemide and evaluated analgesic responses. Our results demonstrated that BSSG-treated rats developed significant reductions in paw withdrawal thresholds, indicative of neuropathic pain. Histological analysis evidenced the presence of misfolded α-synuclein aggregates in the dorsal horn of the spinal cord and a marked downregulation of KCC2 expression in both dorsal and ventral horns. Pharmacological blockade of GABAAα6 receptors with furosemide resulted in analgesic effects, suggesting a pronociceptive role for these receptors in the context of altered GABAergic signaling. Our findings support the hypothesis that pathological α-synuclein contributes to neuropathic pain in PD by disrupting chloride homeostasis and transforming GABAergic transmission from inhibitory to excitatory. This study highlights a novel mechanistic link between α-synucleinopathy and chronic pain, suggesting new therapeutic targets to alleviate non-motor symptoms in PD patients.

2

Neuroprotective effects of pioglitazone in a model of alpha-synucleinopathy: Evidence from behavioral and histopathological analyses

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Parkinson's disease (PD), the most common α-synucleinopathy and motor disorder, is characterized by chronic and progressive accumulation of α-synuclein and degeneration of the nigrostriatal pathway, which gives rise to the classic symptoms. Pioglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist, has been associated with reduced PD incidence in epidemiological studies and has shown neuroprotective properties in multiple animal models of neurodegeneration. However, its dosing regimen and therapeutic impact have not yet been evaluated in an α-synucleinopathy model. In this study, we assessed the safety and neuroprotective efficacy of four pioglitazone treatment regimens in a murine model of α-synucleinopathy induced by a single supranigral administration of β-sitosterol β-D-glucoside (BSSG), which replicates key pathological and behavioral features of human PD. Adult male Wistar rats were randomly assigned to Untreated, Mock, αSN, and four PGL treatment groups with distinct dosing schedules. Behavioral evaluations targeting sensorimotor function, motor performance, anxiety-like behavior, learning, and memory were conducted at three time points. Histological analyses included the quantification of tyrosine hydroxylase (TH)-positive dopaminergic neurons, Nissl-stained viable neurons, and α-synuclein aggregates in the substantia nigra and striatum. BSSG-exposed rats exhibited motor and sensorimotor impairments, increased α-synuclein accumulation, and significant loss of TH-positive neurons. Pioglitazone treatment, particularly the regimen combining prophylactic administration with twice-weekly post-induction doses, was well tolerated, ameliorated behavioral deficits, and attenuated both α-synuclein pathology and dopaminergic neurodegeneration. Our findings suggest that pioglitazone exerts robust neuroprotective effects in a progressive α-synucleinopathy model, especially when administered under a prophylactic and sustained dosing scheme. These results support the potential of pioglitazone as a disease-modifying therapeutic candidate for Parkinson's disease and related synucleinopathies.

3

Maneb-induced oxidative stress drives alpha-synuclein upregulation and glial resolution responses

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Maneb (MB) is a dithiocarbamate pesticide whose prolonged use is considered an environmental risk factor for Parkinson's disease (PD). Previous studies from our group and others have demonstrated that MB induces the upregulation of α-synuclein. The pathogenesis of PD is not only linked to α-synuclein accumulation but also to neuroinflammation. Chronic neuroinflammation likely arises from impaired resolution mechanisms that fail to limit early pro-inflammatory responses, ultimately leading to neuronal death. Although resolution pathways in the central nervous system remain poorly characterized, glial cells are considered key candidates to mediate these processes.

In this study, we aimed to describe the biochemical events associated with pesticide-induced toxicity, α-synuclein upregulation, neuroinflammation, and neuron–glia crosstalk. For this purpose, we used the astrocytic C6 cell line, primary glial cultures, and dopaminergic neuronal cell lines (IMR-32 and N27) exposed to MB. Their secretomes were collected and used as conditioned media to investigate the biochemical mechanisms underlying resolution responses between neurons and glia.

In dopaminergic neurons, α-synuclein upregulation was accompanied by increased reactive oxygen species (ROS), lipid peroxidation, and mitochondrial alterations consistent with ferroptosis. Treatment with the antioxidant N-acetylcysteine and the ferroptosis inhibitor ferrostatin-1 reversed both α-synuclein overexpression and MB-induced mitochondrial disruption.

MB exposure also triggered pro-inflammatory signaling, including cyclooxygenase-2 upregulation and nuclear translocation of NF-κB, alongside a reduction in ALOX15 mRNA expression. In astrocytes, MB treatment increased glial fibrillary acidic protein (GFAP) expression. Despite the pro-inflammatory environment, astrocyte viability was only mildly affected, and their secretome was capable of protecting neurons from MB-induced cell death. Furthermore, the secretome from MB-exposed neurons upregulated ALOX15 expression and promoted a proliferative, A2 anti-inflammatory phenotype in astrocytes. In this context, we further explored the role of the pro-resolving lipid mediator lipoxin A4 as a key component of neuron–astrocyte resolution signaling, acting via the G-protein–coupled receptor FPR2/ALX. Our results suggest that, in the context of pesticide-induced neurotoxicity, α-synuclein upregulation is driven by oxidative stress, associated with ferroptosis, and linked to both pro- and anti-inflammatory signaling during the early stages of neurodegeneration.

4

Neuronal alpha-synuclein overexpression shapes astrocyte lipid metabolism

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The accumulation of the presynaptic protein α-synuclein (A-Syn) in dopaminergic neurons leads to the formation of Lewy bodies, which are considered pathognomonic markers of Parkinson's disease (PD). A-Syn has the ability to interact with membranes of a different lipid composition as well as with fatty acids. Moreover, the discovery of several genes linked to lipid metabolism that are also associated with an increased risk of PD has prompted a shift in perspective: from viewing the disease as a proteinopathy to considering it, at least in part, a lipidopathy.

In line with this hypothesis, the aim of this study was to investigate how neuronal A-Syn overexpression modulates lipid metabolism in astrocytes. To this end, we examined various aspects of neutral lipid metabolism and lipid signaling using a rat C6 glioma astrocytic cell line and primary glial cultures exposed to the conditioned medium derived from dopaminergic neurons stably overexpressing the wild-type form of human A-Syn (WT-A-Syn neurons).

We observed an increase in the accumulation of lipid droplets, along with elevated DGAT2 (diacylglycerol acyltransferase 2) mRNA expression in astrocytes exposed to the conditioned medium of WT-A-Syn neurons compared to those treated with control medium. In addition, glial cells exposed to the WT-A-Syn secretome showed increased filipin staining, indicating upregulated free cholesterol levels. Astrocytes treated with the WT-A-Syn secretome also displayed higher expression of phospholipase D1 (PLD1), which is a key enzyme involved in the regulation of phosphatidic acid, a key phospholipid that modulates both lipid metabolism and signaling pathways. Notably, our lab had previously shown that PLD1 is downregulated in neurons overexpressing A-Syn. This contrasting regulation between neurons and astrocytes suggests a cell-type specific modulation of phosphatidic acid levels mediated by A-Syn.

Additionally, astrocytes exposed to WT-A-Syn conditioned medium exhibited reduced lactate release compared to controls. This reduction may indicate a shift in carbon flux toward triacylglycerol synthesis, likely stored in lipid droplets under these conditions. Based on these findings, we propose that the observed increase in neutral lipid content and PLD1 upregulation are key indicators of a metabolic shift in astrocytes in response to neuronal A-Syn overexpression.

5

Effect of amazonian *banisteriopsis caapi* extract on the disaggregation of the amyloidogenic protein alpha-synuclein involved in Parkinson's Disease

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INTRODUCTION: The accumulation of protein aggregates is a hallmark of several neurodegenerative diseases, including Parkinson's Disease (PD), where intracellular inclusions of α-Synuclein (aSyn), known as Lewy bodies, are a defining pathological feature. In this context, amyloid deposits contribute to dopaminergic neuronal loss and disease progression. As there is currently no curative treatment for PD, aSyn aggregates have emerged as a promising therapeutic target. Small molecules able to both inhibit fibril formation and promote the disaggregation of β-sheet-rich structures are considered strong candidates for disease-modifying interventions. Inspired by Brazil's rich biodiversity, we investigated the effects of *Banisteriopsis caapi* (B. caapi), a plant endemic to the Amazon and traditionally used in religious rituals, on aSyn aggregation. Our findings demonstrate that B. caapi extract not only inhibits aSyn fibrillization but also promotes the disaggregation of preformed amyloid fibers, highlighting its therapeutic potential in synucleinopathies. **OBJECTIVE:** To evaluate the disaggregation potential of B. caapi extract and its major alkaloids, Harmine (HMN) and Harmaline (HML), on aSyn aggregates. **METHODS:** Recombinant aSyn was heterologously expressed in *E. coli* and purified. Disaggregation was assessed using ThT fluorescence kinetics in the presence of *B. caapi* extract, HMN, and HML. Endpoints were further analyzed using Transmission Electron Microscopy (TEM), Circular Dichroism (CD), and Light Scattering. Additionally, the effects of the extract and its major components were tested in an intracellular aSyn aggregation model using H4 human neuroglioma cells transfected with aSyn. Intracellular inclusions were quantified with and without treatment. **RESULTS AND DISCUSSION:** Our results show that *Banisteriopsis caapi* extract (0.1–1 mg/mL) effectively disaggregates amyloid fibers, significantly reducing their abundance. Additionally, the alkaloids HMN and HML (70–700 μM) promoted fiber remodeling into amorphous structures, suggesting a direct effect on the conformational stability of aSyn aggregates. In a cellular model, transfected cells with pre-existing intracellular inclusions treated with the extract or isolated alkaloids exhibited a marked reduction in puncta, indicating the potential of these compounds to functionally modulate intracellular aggregation. **CONCLUSION:** These findings suggest that *B. caapi* extract and its major alkaloids are promising candidates for disaggregating and remodeling aSyn aggregates and hold potential for the development of novel therapeutic strategies for Parkinson's Disease.

6

Minimally invasive detection of alpha-synuclein using seed amplification assay for Parkinson's Disease diagnosis in chilean patients

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Background: The pathological accumulation of α-synuclein (α-syn) aggregates is a hallmark biomarker of Parkinson's disease (PD). Seed amplification assays (SAAs) offer a highly sensitive approach for detecting these aggregates, enabling early and minimally invasive diagnosis. **Methods:** We aimed to assess the diagnostic accuracy, reliability, and reproducibility of SAAs, as well as to explore their correlation with clinical markers of PD. Skin punch biopsies (3 mm diameter; 24 PD, 17 healthy controls), bilateral nasal swabs (31 PD, 25 healthy controls), and tear fluid samples (25 µL per eye; 7 PD, 11 healthy controls) from Chilean participants were analyzed. Each sample was tested in quadruplicate, and a sample was considered positive when at least three out of four replicates crossed the background fluorescence threshold. **Results:** SAA reactions seeded with PD samples produced fibrillary α-syn aggregates, whereas control samples did not induce conversion of the recombinant α-syn substrate. SAA confirmed the clinical diagnosis of PD, with the highest diagnostic accuracy and sensitivity observed in skin samples, which was significantly greater than that of olfactory mucosa and tear fluid. Across all sample types, the lag phase was significantly shorter in PD samples compared to controls ($p < 0.01$), indicating faster seeding kinetics in the disease state. Although no consistent association was found between disease progression and seeding activity across all sample types, increased α-syn seeding was observed in skin biopsies from patients with longer disease duration and advanced PD stages. **Conclusions:** This study is the first to evaluate SAA technology for PD diagnosis in a Chilean cohort, utilizing multiple sample types. Our findings validate the feasibility of detecting pathological α-syn from minimally invasive samples using SAA, supporting its potential for early diagnosis and monitoring of PD. Further studies are needed to refine assay conditions and validate the clinical utility of SAAs as a non-invasive, cost-effective diagnostic tool.

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FFPrime: A computational tool for the design of new regio-selective alpha-synuclein aggregation inhibitor

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The origins of Parkinson's disease can be traced back to the aggregation of alphasynuclein (αS), a 140 aminoacids intrinsically disordered protein. Three different regions in the αS sequence are defined: the positively charged N terminal region (1-60), the hydrophobic non-amyloid component (61-95) and the very acidic negatively charged C-terminal region (96-140)(1). The development of new molecules capable of specifically binding to αS to inhibit aggregation have to consider the varying chemical nature of the governing interaction in each targeted region. Recent NMR spectroscopic studies have shown that the negatively charged aromatic compound, phthalocyanine tetrasulfonate (PcTS), interacts with aromatic residues in the N-terminal region of αS, modulating its aggregation behavior (2). In contrast, the positively charged aromatic compound meso-Tetra(N-Methyl-4-Pyridyl) Porphine (PmTP) has shown selective interaction to aromatic residues at the C-terminal region of the protein.

Atomistic molecular dynamics (MD) simulations offer an alternative strategy to computationally guided screening for studying the regioselective binding of aggregation inhibitors to distinct regions of α-synuclein (αS). MD acts as a "computational microscope," enabling the extraction of detailed structural, dynamic, and thermodynamic properties at atomic resolution, often in agreement with experimental data. However, it is imperative to have an accurate description of molecular interactions to accurately reproduce the real physics of the system. In MD, the force fields are a set of mathematical functions that quantify interactions. For protein interactions, there is a vast amount of force fields available with high accuracy. For drug-like molecules, however, the availability and accuracy of force fields are lower, mainly due to the high dimension of chemical space treated.

Here, we present FFprime, a Python package for deriving force field parameters directly from electronic structure calculations. Invoking the theory of Atoms-In-Molecules (AIM), FFprime offers an alternative approach to parameterizing organic molecules without relying on experimental data. Our recent work demonstrates that FFprime-derived parameters outperform general-purpose force fields such as AMBER and CHARMM in accurately modeling noncovalent interactions, providing a robust framework for simulating protein–ligand complexes3,4. This molecular modeling approach is used to derive new force fields for PcTS and PmTP to study their interactions with αS through MD.

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8

Modulation of alpha-synuclein-induced microglial activation by food-derived bioactives

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Microglia are the resident immune cells of the central nervous system, responsible for the uptake of α-synuclein (α-syn) species released from neurons via different mechanisms. Upon α-syn exposure, microglia initiate an inflammatory and oxidative response that alters their phenotype.

Our recent work focuses on understanding the modulatory effects of food-derived bioactive compounds on microglial activation, particularly their potential to attenuate the pro-inflammatory profile induced by α-syn. Bioactive compounds are secondary plant metabolites with physiological functions beyond nutritional ones, providing significant health benefits. Among these bioactives, we are particularly interested in urolithins, microbial metabolites derived from ellagic acid, a complex polyphenol abundant in pomegranates, berries, and nuts.

Urolithin A (UA) is produced in the gut through the transformation of dietary ellagitannins and ellagic acid by microbiota. Notably, UA has been shown to cross the blood–brain barrier after oral intake and to exert neuroprotective and anti-inflammatory effects in both in vitro and in vivo models.

Using the human microglial cell line HMC3, we investigated the activation of microglia in response to different species and concentrations of α-syn (ranging from 0.02 to 2.0 mg/mL), including both monomeric and fibrillar forms. Cell viability was assessed under these conditions to determine the toxicity of the different α-syn species.

Microglial activation was evaluated by quantifying the expression of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α) using q-PCR. We observed that exposure to 0.04 mg/mL of monomeric α-syn for 24 hours significantly increased cytokine expression in HMC3 cells, indicating an activated pro-inflammatory state. However, pretreatment with 2 μM UA for 6 hours led to a two-fold decrease in IL-6 expression.

Given that sustained microglial activation and chronic inflammation are implicated in the pathogenesis of neurodegenerative disorders, our results support the potential of UA as a dietary-derived compound capable of attenuating microglial-driven inflammation in the context of synucleinopathies.

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A novel chalcone derivative as a promising multitarget candidate for neurodegenerative diseases

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Chalcones are simple compounds belonging to the flavonoid family. Scientific evidence suggests that flavonoid-rich diets are associated with a lower incidence of neurodegenerative diseases. Our goal is to develop chalcone-derived compounds capable of simultaneously modulating multiple pharmacological targets relevant to these disorders.

From a library of 2'-hydroxychalcones synthesized in our lab, we identified chalcone 1 (3-chloro-4',5'-dimethyl-2'-hydroxychalcone). This compound showed good blood–brain barrier permeability in the PAMPA-BBB assay and was non-cytotoxic in SH-SY5Y cells up to 10 μM. It also met Lipinski's rule of five, suggesting favorable pharmacokinetic properties and good drug-likeness.

Chalcone 1 demonstrated in vitro activity on targets associated with Alzheimer's disease (AD). It inhibited mouse brain acetylcholinesterase (IC_{50} = 4.37 ± 0.83 μM) with low inhibition of human butyrylcholinesterase, and significantly reduced Aβ peptide in vitroaggregationat 10 μM (51.6 ± 11.3%).

In behavioral studies in Swiss albino mice, acute administration of chalcone 1 (3 mg/kg, i.p.) improved spatial working memory (Y-maze) and long-term memory (novel object recognition), with no anxiolytic, sedative, or motor effects observed (evaluated in elevated plus maze and hole-board tests).

Moreover, chalcone 1 selectively and reversibly inhibited human monoamine oxidase B (hMAO-B) (IC_{50} = 0.354 ± 0.084 μM), a validated therapeutic target in Parkinson's disease (PD), with insignificant effect on hMAO-A.

In a rotenone-induced mouse model of PD, 7-day administration of chalcone 1 (3 mg/kg, i.p.) reversed motor deficits (cylinder test), reduced brain oxidative stress (TBARS), and prevented rotenone-induced decreases in rearing and grooming behaviors, without altering these parameters in control animals.

Additionally, in vitro ThT-based RT-QuIC assays showed that chalcone 1(10 and 100 μM) reduced α-synuclein aggregation as evidenced by a significant decrease(35.2 ± 7.9% and 58.2 ± 5.8%, respectively) in the maximum ThT fluorescence reached compared to the control (DMSO); while showing similar $t_1/_2$ values.

Overall, these results position chalcone 1 as a promising multitarget lead compound for the treatment of neurodegenerative disease such as PD and AD, combining reversible MAO-B inhibition, anti-cholinesterase, anti-aggregation (Aβ and α-synuclein), antioxidant, neuroprotective, and cognitive-enhancing properties.

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Alpha-synuclein biology in Schwann cells

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Alpha-synuclein (aS) is a moonlighting protein widely studied in the central nervous system (CNS) mainly due to its role on neurodegeneration associated with Parkinson's disease (PD). Interestingly, aS is also expressed in the peripheral nervous system (PNS), specifically in Schwann cells (SCs). These glial cells are essential for the myelination of peripheral nerves. In both healthy and parkinsonian PNS tissue, aS localization follows an expression pattern similar to S100β, a marker of mature SCs. While its role in SCs remains unclear, emerging evidence suggests aS may be linked to myelination processes.

To explore this possibility, we analyzed transcriptomic datasets from purified differentiated human and rat SCs. We also explored transcriptomic and proteomic data obtained from sural nerve biopsies from a nerve transplantation trial (NCT02369003). Differentiation of SCs was accompanied by a rapid increase in *SNCA* expression, coinciding with upregulation of myelin-associated genes such as *MPZ* and *MBP*. Conversely, donor-matched nerves revealed reduced aS mRNA and protein levels after two weeks of transection compared with the naïve ones. Results obtained in transected nerves were associated with dedifferentiated SCs, paralleling with the downregulation of myelin genes.

We then performed interactome analyses focused on lipid metabolism-associated genes and found that aS interacts with gene clusters enriched in Gene Ontology (GO) biological processes related to myelination and glial cell differentiation. Clustering methods (k-means and MCL) positioned aS within modules regulating cholesterol transport and myelination, suggesting a role as a positive regulator of lipid metabolism enzymes.

Proteomics data of naïve and injured human nerves from the same trial further supported the above-mentioned findings. GO enrichment analysis linked aS to biological processes as protein polymerization downregulation and its localization in axonal compartments.

These transcriptomic and proteomic data together with their interactomes support the notion that aS is a novel biomarker of the differentiated SC phenotype and may act as a positive modulator of lipid metabolism and myelination in the PNS. These findings expand our knowledge of aS beyond its canonical role in neurodegeneration and open new avenues for understanding its biology in PNS glial cells.

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Behavioral and molecular insights of neuroprotection mediated by yerba mate in a drosophila melanogaster Parkinson's model

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Yerba mate (YM) is a traditional herbal infusion widely consumed in South America. It has been inversely associated with Parkinson's disease (PD) in two independent epidemiological studies, and previous work from our lab showed that YM extract promotes dopaminergic neuron survival in vitro. However, the mechanisms underlying these effects remain largely unknown. Here, we studied the behavioral and molecular effects of chronic YM intake in a Drosophila melanogaster model of PD expressing wild-type human alpha-synuclein (aSyn). Flies were fed YM extract for up to 30 days. We evaluated motor behavior and climbing performance, assessed aSyn levels via western blot, and examined synaptic connectivity using the GRASP technique. We also analyzed the mRNA expression of metabolic regulators, including AMPK and downstream autophagy-related genes, by qRT-PCR. Our results show that flies fed YM exhibited mild motor improvement, and YM enhanced neuronal connectivity between dopaminergic and circadian neurons in wild-type aged flies. On the molecular level, YM treatment led to upregulation of AMPK signaling and genes involved in autophagy, suggesting enhanced metabolic regulation and proteostasis. Taken together, these findings provide novel evidence that YM can modulate key pathways involved in neuronal health. By improving energy metabolism and reducing pathological protein accumulation, YM may exert neuroprotective effects relevant for PD and related disorders. While further studies are still necessary, our results support the idea that dietary factors can influence neuronal function and contribute to slowing down neurodegenerative processes.

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Ayahuasca alkaloids as novel inhibitors of alpha-synuclein amyloid fibril formation

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INTRODUCTION: The accumulation of protein aggregates rich in β-sheet structures, known as amyloid fibrils (AFs), is associated with cellular toxicity in various pathologies, including Parkinson's Disease (PD), associated with the aggregation and formation of AFs by the α-synuclein (αSyn) protein. In this context, small molecules capable of modulating αSyn aggregation have been explored as potential therapeutic strategies, this is the case of polyphenols, alkaloids and many other compounds. Therefore, in this study, we used an aqueous extract of *Banisteriopsis caapi* (BC), an Amazonian endemic plant and a key component of the Ayahuasca ritualistic brew, as a source for prospecting novel plant-derived molecules capable of inhibiting αSyn aggregation into AFs. **OBJECTIVE:** To evaluate whether the extract of BC and its major alkaloids components, harmine (HMN) and harmaline (HML), can inhibit the formation of αSyn AFs. **METHODS:** αSyn was heterologously expressed, and its aggregation was assessed in the presence of BC, HMN, and HML using Thioflavin T (ThT) fluorescence kinetics, Transmission Electron Microscopy (TEM) and Circular Dichroism (CD). The effects of the alkaloids were also evaluated H4 cells transfected with αSyn and in an *in vivo* PD model in C. *elegans*. RESULTS/DISCUSSION: Our results show that BC (0.1 – 1.0 mg/mL), HMN, and HML (70 – 700 μM) inhibit the formation of αSyn (70 μM) AFs in a dose-dependent manner, as demonstrated by ThT kinetics and TEM images. Also, CD analysis shows that αSyn maintains its intrinsic disordered structure in presence of either BC, HMN and HML, indicating the maintenance of the monomeric, soluble state. Moreover, we observed that BC treatment reduced the number of αSyn aggregates in H4 cells transfected with αSyn. Finally, the treatment of C. *elegans* expressing αSyn with BC showed a marked reduction in αSyn aggregates withing the muscular cells. **CONCLUSION:** Altogether, our data suggest that BC and its major alkaloids components are potential therapeutic candidates for hindering αSyn AF formation associated with PD.

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Development of an experimental model that replicates the disease specific structures of alpha-synuclein filaments in brain's patients with multiple system atrophy

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A plethora of evidences associates structural dysfunction of the protein alpha-synuclein (αS) and self-assembly into filaments with the neuropathology of Synucleinopathies such as Parkinson disease (PD) and Multiple System Atrophy (MSA). The protein exhibits a high potential to form polymorphic fibrils. Consistently, high-resolution structural determination of αS fibrils has unveiled a variety of polymorphic structures of either in vitro fibrils or ex vivo fibrils extracted from the brain of patients. Recently, structural polymorphism of αS fibrils has been associated with distinct Synucleinopathies. Interestingly, a characteristic shared by all post-mortem αS filament structures is the presence of non-proteinaceous molecules in assembled αS, indicating that chemical ligands may be involved in the assembly of αS fibrils in patient's brain. These evidences highlight the complexity of the αS aggregation process and emphasizes the importance of developing conditions that lead to a better understanding of the structural and molecular basis behind αS assembly. In this work we set-up an experimental model that replicates the disease specific structures of αS filaments extracted from patients with MSA. These kind of experimental models will be invaluable for gaining a better understanding of disease, and thus for developing safe and effective mechanism-based therapies.

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Metallobiology of Parkinson's Disease: Reshaping copper catalyzed oxidation in alpha-synuclein

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Amyloid aggregation of α-synuclein (αS) has been linked to the pathological effects associated with Parkinson's disease (PD). Protein-metal interactions play an important role in αS aggregation and might represent a link between the pathological processes of protein aggregation, oxidative damage in the brain and neuronal cell loss. Indeed, the role of copper ions in αS amyloid assembly and neurodegeneration became a central question in the pathophysiology of PD. Cu(II) binds specifically at the N-terminus of αS and triggers its aggregation, whereas the formation of an αS-Cu(I) complex at the N-terminal region stabilizes local conformations with α-helical secondary structure and restricted motility. In addition, site-specific Cu(I)-catalyzed oxidation of AS has been proposed as a plausible mechanism for metal-enhanced αS amyloid formation. Our previous findings support a mechanism where the interaction of αS with copper ions leads to site-specific metal-catalyzed oxidation in the protein under physiologically relevant conditions. In those conditions, the Met-1 and Met-5 residues involved in high-affinity Cu(I) binding could be oxidized rapidly after air exposure of the αS-Cu(I) complex, whereas Met-116 and Met-127 in the C-terminal region remain unaffected. Met-1 displays higher susceptibility to oxidative damage compared to Met-5 because it is directly involved in both Cu(II) and Cu(I) coordination, resulting in closer exposure to the reactive oxygen species that may be generated by the redox cycling of copper. Recently, abundant evidence revealed that the protein AS undergoes N-terminal acetylation in vivo (AcAS). In that direction, it was reported recently that N-terminal acetylation of AS abolishes Cu(II) binding at the high-affinity Met-1 site present in the non-acetylated protein. In this scenario, the His site becomes the only motif capable of binding the copper ion in its two oxidation states with relatively high affinity. In this work, we have explored this new scenario by using NMR spectroscopyin combination withsite-directed mutagenesis andsynthetic peptide models. Our findings open new avenues of investigations into the metallobiology of PD, reshaping the consideration of copper mediated pathology in vivo.

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Targeting alpha-synuclein aggregation via hHep1-mediated phase separation: Mechanistic insights into the cochaperone alpha-synuclein interaction

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder and is characterized by the progressive loss of dopaminergic neurons in the substantia nigra. A major pathological hallmark of PD is the intracellular aggregation of α-synuclein (α-Syn), a 14 kDa presynaptic protein abundantly expressed in neuronal and non-neuronal tissues. α-Syn contains three regions: an N-terminal amphipathic domain that mediates membrane binding, a central non-amyloid-β component (NAC) region that drives aggregation, and an acidic C-terminal domain involved in calcium binding and chaperone interactions. Under pathological conditions, α-Syn adopts β-sheet-rich conformations, initiating the formation of toxic oligomers and fibrils. Peptides containing the GKNEE motif, such as in the bacterial chaperone Csg-C, have been shown to impair α-Syn aggregation. Human Hsp70-escort protein 1 (hHEP1), a ~20 kDa cochaperone composed of 124 amino acids, is known to stabilize mitochondrial HSPA9 and stimulate Hsp70 ATPase activity. hHEP1 features a disordered proline-rich N-terminal region, a structured central zinc-finger-like domain with an exposed W115 residue, and an acidic, disordered C-terminal tail containing potential aggregation-inhibitory sequences. We investigated the potential of hHEP1 to modulate α-Syn aggregation. Recombinant α-Syn was incubated with increasing molar ratios of hHEP1 (1:0.25 to 1:2). Thioflavin T fluorescence assays revealed that hHEP1 delayed aggregation onset at sub stoichiometric ratios and completely inhibited fibril formation at equimolar concentrations. Transmission electron microscopy (TEM) confirmed the presence of small oligomers at a 1:1 ratio, but no fibrillar structures were observed at 1:2. When hHEP1 was added at various time points after aggregation initiation (1, 3, 5, 7, and 21 hours), no inhibitory effect was detected, indicating that hHEP1 acts primarily on monomeric α-Syn. To elucidate the mechanism, we explored liquid-liquid phase separation (LLPS) under crowding conditions (30% PEG 4000). hHEP1 underwent LLPS and formed condensates, while α-Syn alone did not. Fluorescence microscopy showed that hHEP1 droplets recruited α-Syn, suggesting a sequestration-based mechanism. These results indicate that hHEP1 inhibits α-Syn aggregation by capturing monomeric species into LLPS compartments, thereby preventing nucleation and fibril propagation.

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Cannabidiol induces autophagy via CB1 receptor and reduces alpha-synuclein cytosolic levels

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The pathological feature of Parkinson's disease is the misfolding and cytosolic accumulation of α-synuclein, which leads to its aggregation and formation of Lewy bodies in dopaminergic neurons. Autophagy plays a central role in the clearance and degradation of misfolded proteins, dysfunctional organelles and protein aggregates. Many reports demonstrate the impairment of autophagy in both in vivo and in vitro models of Parkinson's disease; in fact, the overexpression of α-synuclein leads to autophagy impairment. Numerous studies have explored the role of cannabinoids in neurodegenerative diseases and neurological conditions. The restoration of autophagy has been proposed as a potential target for the treatment of neurodegenerative diseases, and cannabidiol has been demonstrated to induce a clinical improvement of signal and symptoms of Parkinson's disease. In our study, we used a neuroblastoma cell line that overexpresses wild-type α-synuclein to investigate the effects of cannabidiol on autophagy modulation and consequently the reduction of the cytosolic level of α-synuclein. Additionally, we also investigated the cannabinoid receptors involved in the modulation of autophagy. Our results demonstrated that cannabidiol enhances autophagy flux, observed by the accumulation of LC3-II- and GFP-LC3-positive vesicles. In addition, cells treated with cannabidiol showed a reduction in cytosolic levels of α-synuclein. These effects were inhibited when the cells were treated with AM251, a CB1 receptor-selective antagonist, indicating that the effects of cannabidiol on autophagic flux are mediated by its interaction with CB1 receptors. Additionally, the autophagic flux was also evaluated after treatments with selective agonists of CB1, CB2 and TRPV1 receptors. We observed the increase of autophagy and reduction of α-synuclein cytosolic levels in cells that were treated with cannabinoid compounds that interact selectively with the CB1 receptor. Taken together, our data suggest that cannabidiol can induce autophagy and promote the reduction of α-synuclein cytosolic levels. These biological effects are mediated preferentially through the interaction of cannabidiol with CB1 receptors. Selective CB1 agonists could represent a new approach for autophagy modulation and degradation of protein aggregates.

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Disruption of Tyr-39 aromaticity weakens PcTS interaction with alpha-synuclein's Master Regulator Sequence: Insights from NMR and MD simulations

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Recent studies have demonstrated that molecular events involved in both the physiological and pathological roles of α-synuclein (αS) may be regulated by specific sequence motifs within its primary structure1–3. One of the most important of these motifs, known as the master regulator sequence, encompasses residues 36–42, where the aromatic character of the Tyr39 plays a particularly crucial role in promoting αS aggregation1–4. NMR spectroscopy studies have revealed that the compound phthalocyanine tetrasulfonate (PcTS) can specifically interact with the monomeric form of αS, specifically around the aromatic residues Phe4 and Tyr39 based on pi-stacking interactions, thereby modulating αS aggregation5,6. These properties make PcTS an excellent molecular probe for exploring the structural mechanisms through which the master regulator sequence controls αS aggregation. In this study, we prepared a series of αS mutants in which the aromatic residues Phe4 and Tyr39 were substituted to eliminate their aromatic character (Phe4Ala, Tyr39Ala, Tyr39Leu, and the double mutant Phe4Ala/Tyr39Ala). Using NMR spectroscopy and molecular dynamics simulations, we investigated the details of the interaction between PcTS and these αS variants. Our NMR results indicate that the removal of aromatic residues in these mutants significantly reduces or nearly abolishes PcTS binding in the mutated regions, highlighting the importance of aromaticity in mediating the interaction. Molecular dynamics simulations of the PcTS-αS variants demonstrated that PcTS residence time is modulated by the nature of the aminoacid's side chain in excellent agreement with NMR results. Together, these complementary findings highlight the power of combining NMR spectroscopy with molecular dynamics simulations to elucidate the molecular basis of αS interactions, paving the way for rational design of modulators targeting specific sequences of the protein in order to well-known and modulate its aggregation process.

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Chemical and mechanistic analysis of photodynamic inhibition of alpha-synuclein amyloid fibril assembly

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Aggregation of α-synuclein (αS) into amyloid is the pathological hallmark of several neurodegenerative disorders, including Parkinson disease, dementia with Lewy bodies, and multiple system atrophy. It is widely accepted that αS aggregation is associated with neurodegeneration; therefore, the inhibition of αS aggregation is a potential therapeutic approach against these diseases. Medical application of photoactive chemicals is a promising therapeutic strategy for treating various diseases owing to its temporal and spatial controllability and reduced side effects. Recently, light-induced treatment using organic photosensitizers was shown to be an attractive option for inhibition of Aβ aggregation and its cytotoxicity. Photodynamic prion-strain-specific inactivation was also reported recently. Here, we explore a light-induced suppression of αS amyloid assembly by phthalocyanine molecules. We present here biophysical studies that have enabled us to characterize the interaction of a metal-substituted, tetrasulfonated phthalocyanineand its free-base with αS. Our results demonstrate conclusively that the inhibitory activity and molecular mechanism of the assayed compounds on amyloid fibril formation are determined by their physicochemical properties; particularly, its relative ability to self-associate through p-p interactions. Data from NMR spectroscopy and enzymatic digestion followed by mass spectrometry demonstrate that light-excited Aluminum(III)-loaded phthalocyaniene induces the oxidation of amino acids that are important in the self-assembly of αS. The mechanism behind this effect relies on the generation of singlet oxygen. The aggregation kinetics studies indicate that the fully oxidized monomeric αS has no aggregation propensity, as these species neither self-assemble themselves, nor participate in the aggregation of non-oxidized αS. Future studies combined with cell-based toxicity assays will be needed to explore the possibility of practical applications of the conclusions of this work. Nevertheless, the results provide a detailed molecular understanding of the effects of photosensitizers on the aggregation behavior of αS, and might facilitate the potential development of light-mediated therapeutic agents for Parkinson disease.

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Yerba mate and chlorogenic acid activate AMPK, promote autophagy and decrease alpha-synuclein aggregation in cell culture

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Neuroprotection is a major goal in the study of neurodegenerative diseases such as Parkinson's disease (PD), where the progressive loss of dopaminergic neurons leads to severe motor and cognitive symptoms. Epidemiological evidence suggests that consumption of Yerba Mate (Ilex paraguariensis, YM), as well as coffee and green tea, is associated with a reduced risk of PD. These beverages contain bioactive compounds, notably polyphenols such as chlorogenic acid (CGA), which may contribute to neuroprotection. Previously we have demonstrated that YM showed protective effects on dopaminergic neurons in mesencephalic primary cultures but the underlying molecular mechanisms remain unclear, particularly those involving cellular pathways relevant to neuronal survival and protein homeostasis. This study aimed to explore whether YM and CGA modulate signaling pathways associated with neuronal protection, focusing on AMPK activation, autophagy induction, and α-synuclein (α-Syn) aggregation. We used the SH-SY5Y neuroblastoma cell line to evaluate AMPK phosphorylation following treatment with YM extract or CGA. Autophagy activation was assessed by immunocytochemistry (ICC) using LC3 as an autophagosome marker. To examine α-Syn aggregation, we employed the H4 neuroglial cell model co-transfected with SynT and Synphilin-1, which induces visible intracellular aggregates. Both YM and CGA increased AMPK phosphorylation in SH-SY5Y cells, suggesting activation of metabolic pathways linked to cell survival. ICC analysis revealed a higher number of LC3-positive vesicles, indicating a potential stimulation of autophagy. In H4 cells, treatment with YM or CGA reduced the number and size of α-Syn aggregates.

These results altogether support the idea that YM and CGA may promote neuronal metabolism by modulating AMPK signaling and autophagy, and by potentially attenuating α-Syn aggregation. This work contributes to our understanding of how dietary polyphenols may impact cellular pathways relevant to neurodegeneration, and highlights YM as a promising candidate for further investigation in the context of PD.

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Fluorescent labeled alpha-synuclein conformers for intracellular studies

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Alpha-synuclein (αS) is a cytoplasmic protein highly expressed in various brain regions. Albeit is an intrinsically disordered protein, under certain circumstances *in vitro*, it can form fibrilar aggregated species similar to amyloid fibrills. The presence of intracellular αS aggregated species is a hallmark of several neurodegenerative diseases identified as synucleinopathies, such as Parkinson's Disease. These aggregates are found in various cellular types such as dopaminergic neurons, as non-dopaminergic neurons and glial cells.
αS cellular internalization implies different unsolved mechanisms, thus is important to generate fluorescent αS species as an approach to study αS internalization and its interactions *in cellula*.
In this study, fluorescent αS species were generated by labeling monomer αS with Alexa-488 fluorophore. The yield of the reaction was calculated by spectrophotometry and obtained a ratio [Alexa-488]/[aS] = 0.2. Albeit a low yield, it is desirable to not alter the aggregation properties of the labeled monomer, so this labeling condition was preferred. Labeled αS fibrilar species were generated from labeled monomers. The kinetics of the formation reaction was similar to the non labeled control condition (t1/2: 23.36 h vs 22.53 h, control vs labeled) as well as the length of the fibrils (102.0 ± 46.0 nm vs 107.5 ± 37.9 nm, control vs labeled). In contrast, oligomeric species were labeled from unlabeled oligomers previously formed. Spectral properties were obtained from the labeled species and showed symmetrical spectra and similar emission properties.
Cellular studies were performed in the BV2 murine microglia cell line. Confocal microscopy and flow cytometry confirmed the internalization of aggregated labeled oligomers and fibrils, but not monomers after 3h incubation. Labeled fibril internalization was reduced in competition experiments with non-labeled fibrils, as well as after pretreatment of cells with a protease that digest membrane receptors.
The use of fluorescent labeled αS will allow further studies on the mechanism that modulates the cellular uptake for the different aggregated species.

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Structural studies of lipidic alpha-synuclein aggregates derived from multiple system atrophy patients

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Accumulation of α-synuclein (αSyn) aggregates in the human brain is a hallmark of synucleinopathies such as Parkinson's disease (PD), Multiple System Atrophy (MSA), and Dementia with Lewy bodies (DLB). Understanding the structure of αSyn aggregates is essential for the development of therapeutic drugs and diagnostic tracers targeting these diseases. Although various αSyn structures have been reported, most were formed in the absence of lipids or extracted from patient brains using detergents.

In this study, we extracted αSyn aggregates from the cerebellum of MSA patients using a detergentfree sucrose gradient method and propagated the aggregates by seeding in the presence of lipids. Using NMR spectroscopy and cryo-electron microscopy, we found that aggregates of different densities showed distinct structural features. Compared with the lipidic αSyn aggregates of type 2 (L2) structure, they appeared more asymmetric and heterogeneous.

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Alpha-synuclein accumulation correlates with lipid metabolism dysregulation in mouse neurotoxicity-based models

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The accumulation and pathological aggregation of α-synuclein is a hallmark in the pathogenesis of a group of neurodegenerative disorders known as synucleinopathies, including Parkinson's disease. Our goal was to characterize α-synuclein expression and associated metabolic changes in mouse models of neurotoxicity. To this end, we employed two neurotoxicants linked to parkinsonism: iron overload and the dithiocarbamate pesticide maneb (MB).

The iron overload model was induced by repeated intraperitoneal administration of iron saccharate (333 mg/kg; 4 doses) every five days. The pesticide-induced model was established through sub-chronic exposure to MB (40 mg/kg; 12 doses), administered intraperitoneally twice a week.

In both experimental models, we observed increased α-synuclein expression in mice midbrain, accompanied by a significant loss of dopaminergic neurons. This neurodegenerative process was associated with pronounced glial activation, as evidenced by elevated immunoreactivity of glial fibrillary acidic protein (GFAP) in astrocytes and ionized calcium-binding adapter molecule 1 (Iba1) in microglia. Notably, these neuropathological events were accompanied by a shift in lipid metabolism, characterized by increased levels of free cholesterol, reduced triglyceride content, and upregulation of the lipogenic transcription factors Sterol Regulatory Element Binding Protein (SREBP) 1 and 2. These lipid alterations coincided with a pronounced oxidative stress characterized by lipid peroxidation along with the activation of ferroptotic pathways, as indicated by decreased levels of glutathione peroxidase 4 (GPX4), reduced glutathione content, and downregulation of the cystine/glutamate antiporter SLC7A11. In line with these molecular changes, both the iron- and MB-treated groups exhibited significant motor deficits in rotarod and open field behavioral tests, highlighting functional impairment of the dopaminergic system in these models of neurodegeneration.

Taken together, our results contribute to establish a link between lipid metabolism alterations and ferroptosis with α-synuclein accumulation in mice midbrain. These findings support the hypothesis that synucleinopathies should be considered not only as proteinopathies but also as lipidopathies.

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Effect of pioglitazone on oxidative stress and mitochondrial function in a murine model of alpha-synucleinopathy

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Parkinson's disease (PD) is a progressive neurodegenerative disorder and the most prevalent clinical phenotype of α-synucleinopathy. It is characterized by pathological aggregation of α-synuclein, mitochondrial dysfunction, and oxidative stress, particularly in the nigrostriatal pathway. Pioglitazone (PGL), a PPARγ agonist, has shown neuroprotective and antioxidant effects in preclinical studies.

In this work, we used a rat model of a-synucleinopathy induced by the intranigral dministration of β-sitosterol-β-D-glucoside (BSSG), which replicates key pathological features of a-synucleinopathies. Male Wistar rats were divided into three groups: BSSG, mock (DMSO), and BSSG + PGL (10 mg/kg/day/for 30 days). After the treatment period, the substantia nigra pars compacta (SNpc) and striatum were dissected and processed for biochemical analysis.

PGL treatment significantly reduced oxidative damage in the nigrostriatal pathway. Specifically, PGL decreased lipid peroxidation levels (TBARS assay) and attenuated the formation of reactive oxygen species (ROS) in both the SNpc and striatum compared to the BSSG group. Additionally, PGL increased the reduced glutathione (GSH) concentrations and enhanced the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase. Mitochondrial complex I activity, impaired in the BSSG model, was also significantly improved in PGL-treated animals.

These findings suggest that PGL mitigates oxidative stress and restores mitochondrial function in a model of a-synucleinopathy. By enhancing redox imbalance and mitochondrial dysfunction in the nigrostriatal system, PGL may offer therapeutic potential as a disease-modifying agent in PD.

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Characterization of neuronal exosomes purified from plasma of Parkinson's Disease patients

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Introduction: Parkinson's disease (PD) is the most common motor neurodegenerative disorder worldwide. One of the key proteins involved in its pathophysiology is α-synuclein (α-syn), which can form toxic oligomers in specific brain regions, contributing to the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Exosomes (small extracellular vesicles) have been proposed as a potential mechanism for the propagation of α-syn throughout the brain. Since PD is typically diagnosed at advanced stages and current treatments are only palliative, identifying reliable biomarkers is essential for early detection and personalized interventions.

Materials and Methods: We quantified neuronal exosomal α-syn levels in plasma samples from PD patients using Western blot and ELISA. We also analyzed the correlation between exosomal α-syn levels and clinical features of PD.

Results: Plasma-derived neuronal exosomes from PD patients showed significantly elevated levels of α-syn compared to healthy controls. Moreover, α-syn levels positively correlated with disease severity as measured by the Hoehn and Yahr scale.

Discussion: These findings support the use of neuronal exosomal α-syn as a potential biomarker for PD, offering promise for earlier diagnosis and personalized treatment strategies based on disease stage and symptom progression.

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Periphery to brain spreading of alpha-synuclein in novel Parkinson's Disease mouse models

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Objectives:

Parkinson's disease (PD) is a neurodegenerative disorder linked to the aggregation of α-synuclein (αSyn), which forms toxic Lewy bodies. While PD's motor symptoms are largely due to αSyn-related dopamine neuron dysfunction in the substantia nigra, its origins may trace to peripheral areas like the gastrointestinal (GI) tract or olfactory system. Using novel transgenic mouse models, we explored αSyn's spread from the gut and vomeronasal organ to the brain, mimicking PD's early-stage non-motor symptoms. Our novel transgenic mice demonstrate transsynaptic αSyn transmission via the vagus nerve and offer insight into αSyn's GI and olfactory propagation pathways, suggesting two potential origins for PD.

Methods:

Two new PD mouse models, Vitras and Vitras6J, have been used in this study. The former expresses human, 1-120 truncated a-syn in the gut and olfactory epithelium in an a-syn null background, while the latter is the same transgenic mouse expressing 1-120 human synuclein but with mouse endogenous a-syn in the background.

Results:

In Vitras mice, α-synuclein (αSyn) was observed to propagate from the vomeronasal organ to the brain and from the gastrointestinal tract first to the myenteric plexus and then to the brain with notable transmission through the vagus nerve. This transsynaptic spread resulted in αSyn accumulation in brain regions associated with the vagus, such as the dorsal nucleus of vagus nerve and later to the solitary nucleus. These findings support the hypothesis of dual origins for Parkinson's pathology through peripheral routes linked to the gut and olfactory systems.

Conclusions:

This study provides evidence that α-synuclein can propagate to the brain from both the gastrointestinal tract and olfactory system, supporting a dual-origin hypothesis for Parkinson's disease. These findings highlight potential pathways for early disease spread and may inform future strategies for targeting Parkinson's pathology at its peripheral origins.

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