Neuroscience and Neurosurgery

Biology of Parkinsonism to enhance lifespan of mitochondria in Parkinson's patients

Al-Baraa Akram El-Sayed

Abstract
Lewy bodies and a noticeable loss of dopaminergic neurons in the substantia nigra are the two main symptoms of Parkinson's disease, which is a diverse, multifaceted, and frequently complex illness. Parkinson's disease also causes motor disability.
Although the precise PD pathophysiology is still being researched, one of the key pathogenic pathways thought to underlie PD development is mitochondrial dysfunction. Based on the premise that representations of HtrA2 (the mitochondrial precursor serine protease gene) might help to increase mitochondrial stress and transcriptional activation of the CHOP gene in response to nuclear stress. The goal of this article was to examine, through laboratory-based research, the function of HtrA2 and CHOP in stress signaling transmission and the ensuing activation of mitochondrial quality control in Parkinson's disease.
Key words:*Parkinson's disease, HtrA2, mitochondrial stress*

Introduction

Disease has been known as a "shaking palsy" since ancient times (India and Europe AD175). In 1817, Dr. James Parkinson published "An Essay on the Shaking Palsy," which included six cases of PD. After sixty years, French neurologist Jean Marie Charcot recognized the significance of the study and gave the illness his name.
Chemical variations in the brains of PD patients were discovered in the 1960s. Arvid Carlsson, Oleh Hornykiewicz, George Cotzias, and Melvin Yahr won the Nobel Prize in 2000 for their investigations that were started with reserpine-induced Parkinsonism in animal models and are currently considered the "gold standard" treatment for Parkinson's disease (1).
In the past 20 years, genetic research has helped us understand the pathophysiology of various diseases.

Definition:

Parkinsonism is a progressive neurological condition that affects how muscles move and is identified by any one or more of the following symptoms: tremor, stiffness, bradykinesia, and progressive postural instability. There may be obvious cognitive impairment.

Neuropathology of Parkinson's Disease

severe loss of black pigmentation (neuromelanin), which accounts for more than 80% of the loss of dopaminergic neurons in the pars compacta of the substantia nigra. The degree of motor impairment was associated with the decrease of black pigmentation. Clinical signs of

Parkinson's disease include other brain regions being impacted, such as the locus ceruleus and raphe cortex, when dopaminergic neurons are lost by more than 60% to 80%. Additionally, we observe the emergence of aberrant cytoplasmic structures, or Lewy bodies, in brain tissue.

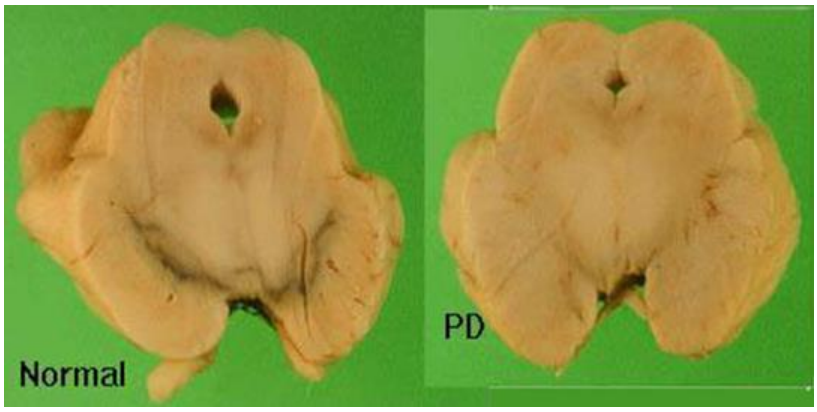

Figure 1. Left side represents a normal brain segment, whereas the right side represents a brain wounded by Parkinsonism. Post-mortem brain section demonstrating loss of black pigmentation in the substantia nigra in Parkinson's disease.

Causes:

For the majority of individuals, the disease's origin is unknown. The illness, especially the idiopathic variety, could result from:

1- Dopamine action in the corpus striatum of the basal ganglia responsible for motor control is decreased as a result of the death of dopaminergic neurons in the substantia nigra, a component of the extrapyramidal system.
2- Acetylcholine overproduction and/or overactivity results in aberrant signaling, which impairs the ability to govern muscular action.

Dopaminergic Neuron Anatomy

The corpus striatum contains the highest concentration of dopamine, which is also found in the frontal cortex, limbic system, hypothalamus, mesolimbic pathway, which originates in the Ventral Tegmental Area and projects to the limbic system (amigdala and Nucleus

accumbens), mesocortical pathway from the frontal cortex, and the tuberohypophyseal pathway, which regulates the pituitary gland.

Parkinson's Disease Pathophysiology

It is well recognized that the basal ganglia are crucial in regulating movement. In contrast to

acetyl choline, which is an excitatory neurotransmitter in the basal ganglia, dopamine is an inhibitory transmitter in the basal ganglia.
Without dopamine, inhibitory effects are lost and excitatory mechanisms are left unchecked, which causes excessive neuronal activity in the basal ganglia and results in rigidity and tremors from high muscular tone.

Parkinson's disease clinical presentation

1- Tremors

For several years, tremors while at rest may be the main symptom. These tremors decrease with exercise, although in some people, tremors may worsen while walking, feeling anxious, hot, or burning calories.

Starting in the fingers, particularly the thumb, resting tremors with a frequency of 4 to 6 Hz can also affect the legs, lips, and tongue. It is distinguished by rough, intricate movements as well as the bending and extension of the fingers. Adduction and abduction motions of the thumb are distinctive characteristics of its tremors.
Supination and pronation motions characterize the directions of forearm tremors. Also susceptible to damage are the mouth, arms, legs, and feet. It is sporadic, present when at rest or distracted, but lessened when in motion.
Postural tremors that occur between 8 and 10 Hz are faster, more subtle, and have finer amplitude. It is present in posture or activity and carries over during movement.
Several anomalies can be found during a neurological examination, including:

1- Plantar responses are flexor, muscle strength and reflexes are normal
2- Hypomimia, a lack of facial expression, and glabellar tap sign, an excessive or unaccustomed blink reflex, are both signs of hypomimia.
3- As long as the natural age-related limitation of upward gaze is taken into account, eye movements during conventional clinical testing are normal.
4- Sensation is normal, and initially, cerebral capacities are unaffected
5- About one-third of individuals experience cognitive impairment as the condition worsens
6- As Parkinson's disease advances, other non-dopaminergic structures are involved, which can lead to various symptoms as loss of smell, sadness, dementia, autonomic dysfunction, and sleep disturbance.

In brief, stress and worry, dementia, minor thinking and memory issues, excessive sleep, and urine issues are the top priorities for both motor and non-motor symptoms.

Diagnosis of Parkinson's disease

Although there is no established diagnostic procedure for Parkinson's disease, the following are the main causes of Parkinsonism:

- Toxins, such as carbon monoxide and manganese toxicity, structural CNS lesions, metabolic conditions, and other neurological conditions
- The majority of instances are infrequent and are indicated by abnormal features, a history, or an examination

Frequently required to take into account 2 other diagnoses are:

- Parkinsonism brought on by drugs

- Parkinsonism plus syndromes, or the combination of neurological symptoms that are not typical of Parkinson's disease with parkinsonian traits

The most accurate test to identify dopaminergic depletion is imaging, however it can only be performed in specialized facilities and is highly expensive.

Induced Parkinsonism by Drugs

The fact that it is reversible makes it a crucial aspect, even though it could take weeks or months to do so after stopping the drug. Examples of medications that cause Parkinson's disease include haloperidol, atypical neuroleptics, lithium, amiodarone, valproic acid, and calcium channel blockers like flunarizine and cinnarizine. Dopamine antagonists also aggravate the condition and should be avoided whenever possible when treating Parkinson's disease patients.

Parkinson's condition Prognosis

It varies and is somewhat based on the age at which symptoms first appear; if symptoms appear in middle age, the condition is typically slowly progressive and likely to limit lifespan due to problems from immobility and susceptibility to fall. It is doubtful that onset after 70 will decrease life or get worse.

Cause of Parkinson's disease

Aging is the biggest risk factor for Parkinsonism, even though the most frequent cause is idiopathic (no known reason). Parkinson's disease has been linked to environmental variables like pesticides and occasionally poisons like MPTP. As a component of "ecstasy" (methylenedioxymethamphetamine), MPTP (methyl-phenyl-tetrahydropiridine) produced severe Parkinsonism in adolescent drug users, raising the possibility that environmental toxins may play a role in idiopathic Parkinson's disease. The same outcome may also result from physical harm, as with boxer Muhamad Ali.

The first Parkinson's disease-causing gene, SNCA, was discovered in 1997. It was found to be involved in about 2% of Parkinsonism, but 15% to 25% of patients had at least one family member who has the condition.

Currently, scientists have discovered that Parkinson's disease and around 26 genes. Genetic variants associated with early illness onset in people less than 50 years of age are extremely uncommon.

Ageing, genetic predisposition, and environmental factors are all known to play a role in the etiopathogenesis of Parkinson's disease. Protein misfolding and aggregation with mitochondrial dysfunction seem to be the key mechanisms involved in the process. Both routes cause the death of neuronal cells and may interact with one another, for instance, by

increasing radical oxygen species (ROS) and decreasing ATP.

The study of models containing these mutations will aid in understanding the mechanisms underlying neurological deterioration and the discovery of novel therapeutic approaches. Genes implicated in these pathways have been shown to be mutated in Parkinson's disease.

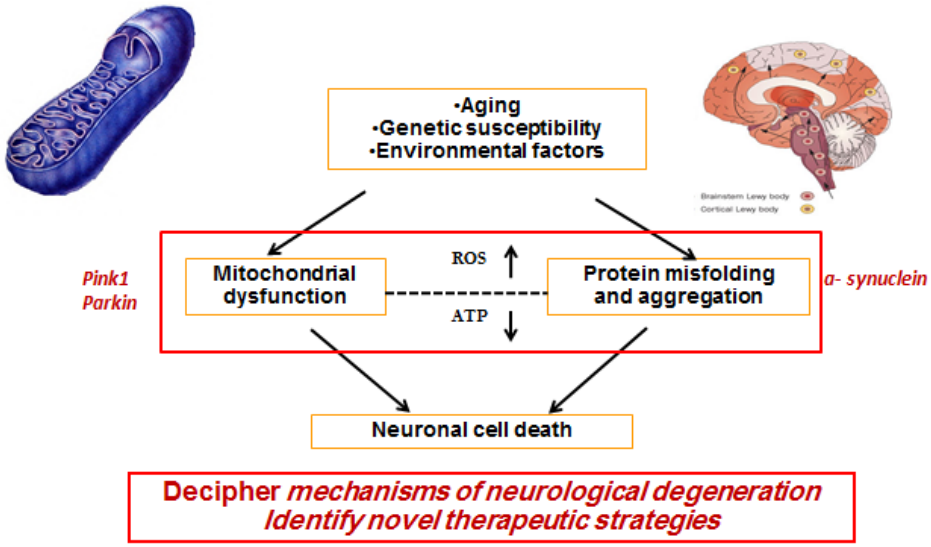

Figure 2. Diagram illustrating the etiopathogenesis of Parkinson's disease, including the main pathways that seem to be involved, such as protein misfolding and aggregation with

mitochondrial dysfunction, as well as some recognized contributing factors like genetic susceptibility and aging. Both routes result in the death of neurons, and they may interact with one another, for instance, by increasing reactive oxygen species (ROS) and decreasing ATP.

Dopamine deficit in Parkinson's disease is supported by the following data

Reserpine causes some Parkinsonian symptoms. More than 80% of the dopamine in Parkinson's disease-related basal ganglia has been depleted post-mortem. Additionally, patients being treated aggressively with neuroleptics for schizophrenia experience Parkinsonian symptoms.
Parkinson's disease is brought on by MPTP, a neurotoxin that kills dopamine-producing cells. Parkinson's disease patients who are still alive have decreased dopamine uptake in their basal ganglia.

Parkinsonism drug treatment

The foundation of developing medication therapy for the condition is the central imbalance between the dopaminergic system (lower dopamine activity) and cholinergic system (increased acetylcholine activity).
Important criteria for the effects of DOPA therapy in Parkinson's disease include strong blood-brain barrier penetration and good absorption from the stomach via active amino acid transporter systems. Additionally, as DOPA has a half-life of between one and three hours, it should have a short half-life to prevent toxicity.

After the dosage, the peak plasma concentration occurs 30 to 2 hours later. In noradrenergic neurons, DOPA is quickly converted into dopamine (DA) by DOPA decarboxylase (DDC), making these substances pro-drugs. Next, it is converted into noradrenaline by monoamine oxidase (MAO) and catechol O-methyl transferase (COMT), which results in homovanillic acid (HVA).

Clinical response rates at the beginning of treatment are as follows: 40% symptom-free, 40% good response, 20% poor or no response and need for alternative treatment alternatives.

1-Levodopa/Carbidopa

Process of action

It is a dopamine precursor that enters the central nervous system (CNS) actively and is transformed there into dopamine. The blood-brain barrier cannot be crossed by dopamine alone. Levodopa should be given in a high dose when used alone to counteract the peripheral tissues' dopamine decarboxylase enzyme's ability to convert it to dopamine.

Levodopa is therefore effective when combined with carbidopa, another component. A peripheral decarboxylase inhibitor is carbidopa. Then, it prevents levodopa from peripheral decarboxylation and permits enough of it to penetrate the blood-brain barrier to be converted into dopamine in the brain.

Levodopa and carbidopa are used to reduce the amount of levodopa needed, which reduces the negative effects of peripherally raised dopamine levels, such as nausea, vomiting, cardiac arrhythmia, and hypotension.

Levodopa/Carbidopa side effects include

1- Vomiting, nausea, and heart arrhythmia (which are all lessened by the addition of carbidopa).
2- Motor fluctuation (wear-off and on-off phenomena). Levodopa/carbidopa formulations with sustained release can result in more consistent plasma levodopa levels, which is beneficial in some circumstances. Adding the total daily dose of levodopa and carbidopa and administering it in equal doses every two hours rather than every four to six hours is another way to address the on-off phenomena.
3- Confusion and hallucinations, particularly in the elderly. These psychotic adverse effects may be managed with the aid of atypical antipsychotics like clozapine.
4- Neuroleptic malignant syndrome may result with the abrupt cessation of levodopa.
5- Unusual uncontrollable motions (dyskinesia)
6- Disease progression is unaffected and may even be accelerated.

Dihydroxy phenyl alanine (DOPA) typically causes nausea, vomiting, postural hypotension, delusions, and hallucinations among its immediate adverse effects. Additionally, undesirable effects, particularly motor irregularities, are gradually emerging. Most patients see this effect after 3 to 5 years of taking L-DOPA, especially if a young patient is taking a high dose.

It comprises limb & axial dystonia, orofacial dyskinesia, involuntary movements, and even depression. It can happen as peak-dose phenomena or as a biphasic phenomenon, especially

during the build-up and wearing off phases, and this involuntary dyskinesia can be violent. Although management is challenging, it once again entails adjusting the dosage of levodopa to maintain stable levels in the brain and using substitutes, particularly dopamine agonists. Believed to be connected to L-DOPA's short pharmacological half-life, which results in striatal stimulation that pulses and eventually disrupted basal ganglia output.

Dopamine dysregulation syndrome (DDS), also known as a reward system disorder, is characterized by issues with self-control such addiction to drugs, gambling, or hypersexuality. Now, in order to prevent the growth of motor fluctuation, we want to extend the pharmaceutical

dopaminergic stimulation of the striatum. Neuroprotective and L-DOPA-sparing treatments are preferred in younger individuals.
L-DOPA should only be used to aid people with severe disabilities. Although all patients eventually require L-DOPA, we try to prevent it as much as possible in younger patients under 65 years old and instead prefer to utilize long-acting dopamine agonists initially with a half-life of at least eight hours. Therefore, we combine L-DOPA with peripheral COMT inhibitors such as entacapone, MAOB inhibitors such as selegiline, which may slow the progression of disease, or D1 and D2 agonists.

2- Dopamine receptor agonists*:*
Drugs that are easier to administer include pergolide, a D1 and D2 receptor agonist, and bromocriptine, a D2 receptor agonist and weak D1 receptor antagonist. It has a stronger effect and lasts longer. In treating the symptoms of Parkinsonism, these medications are less effective than levodopa, but they are considerably less likely to produce dosage variations or dyskinesia, though they will undoubtedly increase the latter once it has already started.
They are now most effectively administered either before the introduction of levodopa/carbidopa combination therapy (sinemet) or with it from the start, such as apomorphine, because they act directly on dopamine D2 receptors in the central nervous system. Their use in Parkinsonism is associated with a lower incidence of motor fluctuations and dyskinesias that occur with long-term levodopa therapy.
They are given orally, and the maximum daily dose of bromocriptine is 30 mg. The initial

dose is 1 mg, and it is raised to 2.5 mg every eight hours.

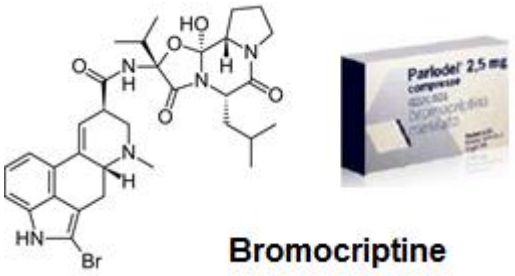

Figure 3. Chemical make-up of bromocriptine, which is sold as Parlodel 2.5 mg in tablet form.

The pergolide dosage is 50 mg initially, followed by 250 mg every eight hours, and up to 3000 mg per day if necessary.
Anorexia, nausea, vomiting, constipation, hypotension, confusion, hallucinations, digital vasospasm, cardiac arrhythmia, nasal congestion, and mental problems are just a few of the negative effects they might cause.

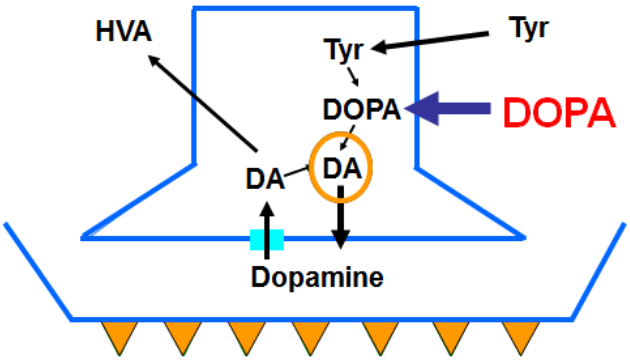

Figure 4. Therapy with DOPA supplements for Parkinson's disease HVA stands for homovanillic acid and DA stands for dopamine.

Monoamine oxidase (MAO) inhibitors that are selective

MAO is an enzyme that has two isozymes, MAO-A and MAO-B. Both are tangentially present. Monoamine oxidase inhibitors (MAOIs) are a class of medications that impede the oxidative metabolism of dopamine in the brain. Examples of these medications are selegiline and rasagiline. They reduce how quickly dopamine in the brain breaks down. They themselves have a rather weak anti-parkinsonian effect.

There have been some concerns about its safety; however these are debatable issues that are the focus of continuing research. Their morning dose is typically 5 to 10 mg.

They are utilized as levodopa/carbidopa adjuncts in patients who have motor tremor or a deteriorating response to levodopa. They are selective MAO-B inhibitors that can prevent dopamine breakdown centrally.

Selegiline produces a by-product that metabolizes like an amphetamine, which implies that it

may give some people hallucinations and insomnia. Dietary tyramine, which can be found in cheeses, smoked meats, fish, and red wine, is broken down by MAO-A in the periphery. In order to minimize the severe hypertensive effects of either of these medications, it is advisable to avoid tyramine-rich foods when taking either one. If you also block MAO-A, you will get tachycardia, hypertension, vomiting, and headache.

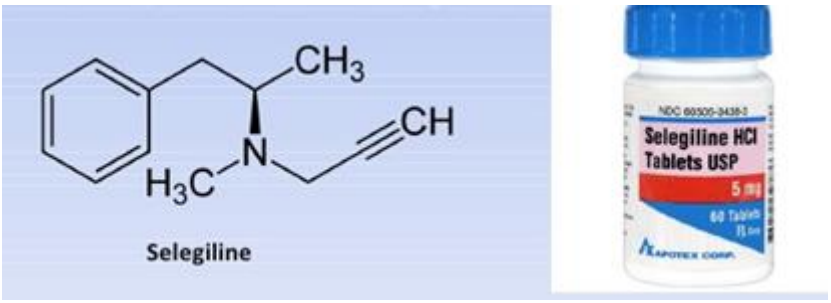

Figure 5. Selegiline's chemical makeup and its commercially available tablet form, selegiline HCl 5 mg

4- COMT blockers

Levodopa's peripheral methylation into 3-O-methyldopa is prevented by these drugs, such as Tolcapone and Entacapone, resulting in longer-lasting plasma levels of the drug and more consistent dopaminergic activation of the brain.

Tolcapone has a longer duration of action and exerts both central and peripheral effects. Entacapone has a quick onset of effect. In individuals with inadequate or fluctuating responses to levodopa, the two medications are used as an adjuvant, shortening the time that the patient experiences an effect.

Entacapone blocks COMT from metabolizing L-DOPA in the peripheral nervous system into 3-methoxy-4-hydroxy-L-phenylalanine (3-OMD), which is difficult to pass the blood brain barrier (BBB). Entacapone and carbidopa or benserazide are somewhat comparable drugs pharmacologically.

When used with levodopa, entacapone (200 mg) prolongs the effects of each dose and lessens motor swings. As a result, the levodopa dosage can be lowered and administered less frequently.

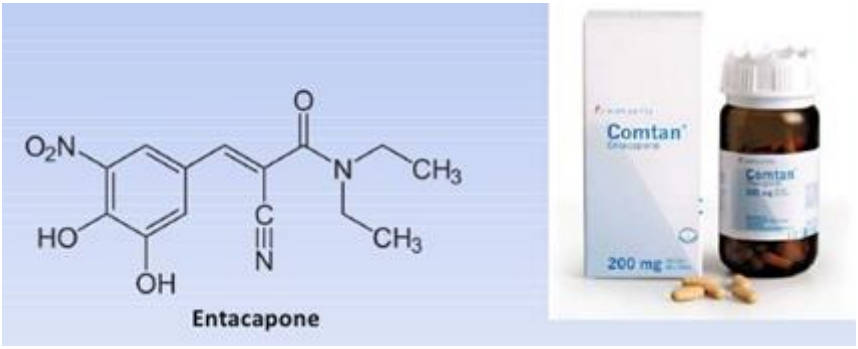

Figure 6. Entacapone's chemical make-up and its tablet form, comtan 200 mg, on the market

5- Amantadine

It functions as an influenza antiviral agent and an ongoing antiparkinsonian medication.

Although its exact method of action is unknown, it could:-

1- Boost dopamine production
2- Cholinergic system inhibition
3- Blocking NMDA receptors that are excitatory.
In mild occurrences of the illness, it may be utilized. It is more effective than anticholinergic medications but less effective than levodopa. After receiving medication for 6 to 8 months, tolerance to its effects develops.
Anorexia, nausea, constipation, postural hypotension, restlessness, and confusion are a few of its adverse effects.

6- Anticholinergic medications

They restore the balance between dopaminergic and cholinergic neurons because they are muscarinic receptor antagonists. This equilibrium seems to make up for the overall dopamine function deficiency in some way. Acetyl choline release is severely blocked by dopamine, particularly D2 receptors, when the region of the brain with the highest concentration of acetyl choline, the striatum, reaches the beak. Hypokinesia, stiffness, and tremor are mostly caused by cholinergic hyperactivity resulting from dopamine deficiency, which over stimulates the indirect pathway. Benzatropine is an instance of an anticholinergic medication.

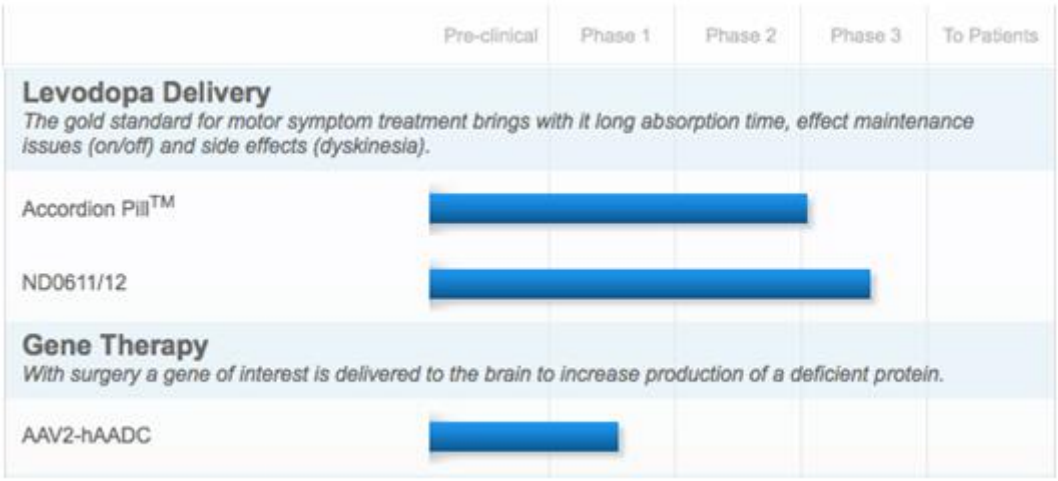

Figure 7. Levodopa delivery, a new approach for treating Parkinson's disease that is regarded as the gold standard for treating motor symptoms, comes with long absorption times, effect maintenance problems, a "on/off phenomenon," and side effects like dyskinesia, such as accordion pill and ND0611/12. Another intriguing treatment is gene therapy, which combines surgical editing of a target gene before delivery to the brain to boost the synthesis of a defective protein.
Alternative remedies include surgery and gene therapy. But some recently approved medications exist to improve motor problems. The first medication is called Rytary, and it was authorized in 2015 to treat Parkinson's motor symptoms. Levodopa and carbidopa are both available in immediate and prolonged release versions as Rytary, a capsule. Carbidopa is

a medication that facilitates the entry of levodopa into the brain, where it is transformed into

dopamine, the neurotransmitter that is absent in Parkinson's disease.
Rytary seeks to lessen Parkinson's disease's motor symptoms such tremors, stiffness, and

slowness. It can be used at any stage of Parkinson's disease, although it is more frequently given when a patient's symptoms are not adequately controlled or when there are long "off time" periods when their medicine is not functioning as intended and their symptoms come back.
The total daily dose needed with rytary is higher than with other levodopa formulations, including sinemet, however administration may be less frequent. In other words, fewer tablets may be taken more frequently, typically three to five times per day. Depending on the patient, the duration of Rytary's effects might range from 4 to 6 hours and begin in about 30 minutes.

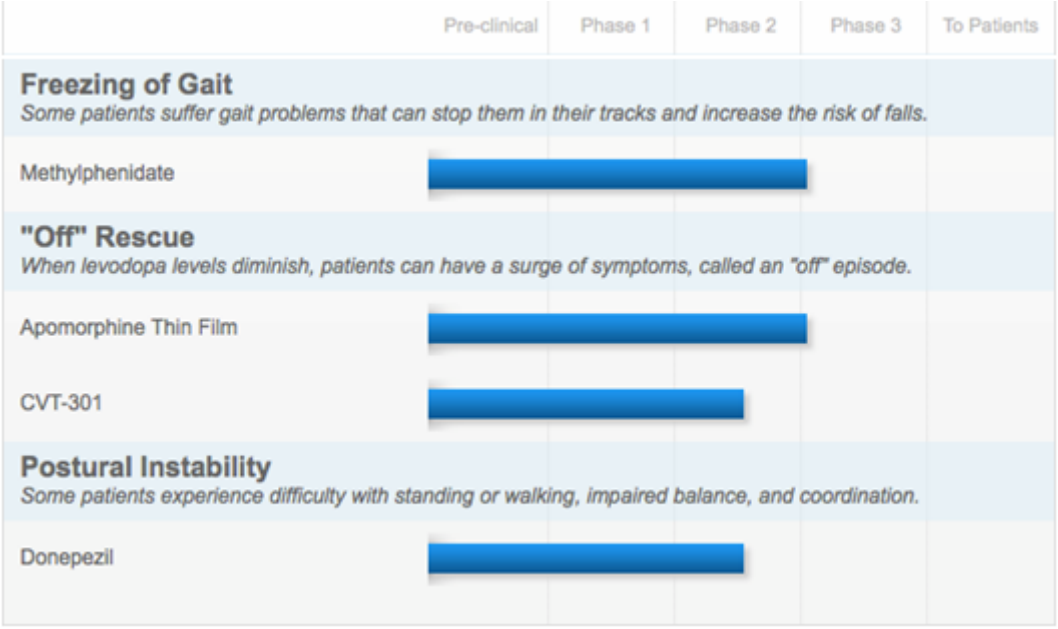

Figure 8. Chart illustrating methods used in experiments to cure Parkinsonism's motor symptoms. Since some patients have gait issues that can cause them to halt in their tracks and increase their risk of falling, methylphenidate can be administered to treat freezing of gait. As a last resort, "off" can be treated with apomorphine thin film and CVT-301. Patients in this situation could experience an increase in symptoms known as "off" episodes when levodopa levels drop. Clinical trials using donepezil are being conducted to address postural instability because some patients struggle to stand or walk and have poor balance and coordination.

Duopa, which was also approved in 2015 for the treatment of Parkinson's disease motor symptoms such tremors, slowness, and stiffness, is the second medication that has recently received approval. Carbidopa and instant release levodopa are combined in a gel formulation that is intended for continuous infusion into the small intestine, where levodopa is absorbed. Bypassing the stomach, which can empty slowly and erratically in Parkinson's disease, this

form of delivery prevents poor absorption and variable effectiveness of oral drugs. Duopa is pumped into the intestine through a surgically implanted tube by an external device that is worn

throughout daily activities. Levodopa levels are normally kept constant in the blood for 16 hours a day with the goal of reducing total daily "off time".

When a medicine is not performing as intended and symptoms reappear, it is most frequently utilized in patients who have motor difficulties, such as dyskinesia, and extensive "off time".

Figure 9. The chart outlines each stage of alternative and promising medicines that are being developed to treat disease exacerbations. This list includes anti-oxidants like glutathione and inosine because excessive oxidation can cause neuronal death, anti-inflammatory medications like pioglitazone because inflammation can cause neuronal death, and neurotrophic factors like GDNF, CERE-120, and PYM 50028 (cogane) because they help rebuild and protect neurons.

The structure and operation of mitochondria:

In eukaryotic cells, mitochondria play key roles in fundamental cellular and metabolic processes like energy production (ATP production) through oxidative phosphorylation (OXPHOS), calcium and metabolite supply, inflammation, intracellular signaling, cell proliferation, and apoptosis.

Dopaminergic neurons' survival and ability to operate heavily depend on proper mitochondrial homeostasis. One of the primary pathogenic mechanisms driving the emergence of PD is mitochondrial dysfunction, which has received widespread acceptance (2).

Over 2% of people worldwide over the age of 65 are affected by Parkinson's disease (PD), the second most common age-related neurodegenerative condition of the central nervous system (CNS).

Lewy bodies and significant dopaminergic neuron degeneration in the substantia nigra pars compacta (SNpc) are hallmarks of the neuropathology of Parkinson's disease (PD), which causes

severe deficiencies in striatal dopamine (DA) and impairs motor function. Some of the clinical motor characteristics of PD include bradykinesia, resting tremors, slowness of movement, muscular rigidity, and postural instability. Additionally, patients may experience non-clinical symptoms such late-stage dementia, autonomic dysfunction, cognitive changes, depression, and sleep difficulties.

Nuclear DNA and mitochondrial DNA (mtDNA) gene mutations or environmental stress are key causes of mitochondrial dysfunction, as are changes in mitochondrial structure and dynamics, dysregulation of transcription factors, and impairment and aggregation of protein components (3). Accordingly, mutations in a broad range of primary genes have been implicated in mitochondrial dysfunction and are known to play a role in autosomal dominant and recessive forms of PD, such as u-Synuclein (SNCA, PARK1), Parkin (PARK2), Phosphatase and tensin homolog (PTEN)-induced putative kinase I (PINK/, PARK6), Leucine-rich repeat kinase 2 (LRRK2) and DJ-/ (PARK7) (4).

Mitophagy, also known as organellar quality control, removes mitochondrial dysfunctions by lysosomal degradation. Numerous cellular processes, such as cellular stress and differentiation, can cause mitophagy to occur (5). Importantly, two of the genes that are mutated in PD, PINK/ and Parkin, are coordinators of the PINK I/Parkin-mediated mitophagy process (6). This process is carried out by complexes of molecules that recognize dysfunction at mitochondria and target them for degradation through the autophagy or/mitophagy process done by ATG complexes.

Due to an increase in mtDNA deletions, mice lacking HtrA2/0 mi protease activity displayed increased overexpression of CHOP, decreased mitochondrial function, and premature aging in brain cells (7).

Materials and methods

1. ATP Assay

ATP levels were measured using Cell Titer-Glo (Promega), as directed by the manufacturer. The genotypes were briefly cultivated on a 96-well dish, then given medication. Using ATP standard curves and serial dilutions of a 50 L solution of ATP in culture media, ATP levels were measured for analysis. 30 minutes were spent incubating the cells. A SpectraMax (MS) spectrophotometer was used to measure the chemiluminescence at a wavelength of 570 nm. Additionally, the protein content, as determined by the Bradford assay, was used to normalize the ATP levels.

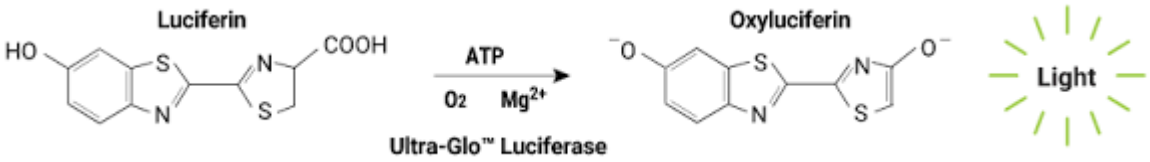

Figure 10. An ATP assay that displays luciferin's transformation into oxyluciferin serves as an example of a chemiluminescence process.

2. Bradford Assay

Using the Bradford assay, protein levels were calculated for normalization purposes. The cells were cultivated in 96-well plates, rinsed in phosphate buffered saline (PBS), and then treated with Bradford solution (Sigma, Germany) for 30 minutes. PBS was then used to wash the cells again. For each experiment, a standard curve was created using a BSA stand, a stock solution (in PBS), and several dilutions in the Bradford solution.

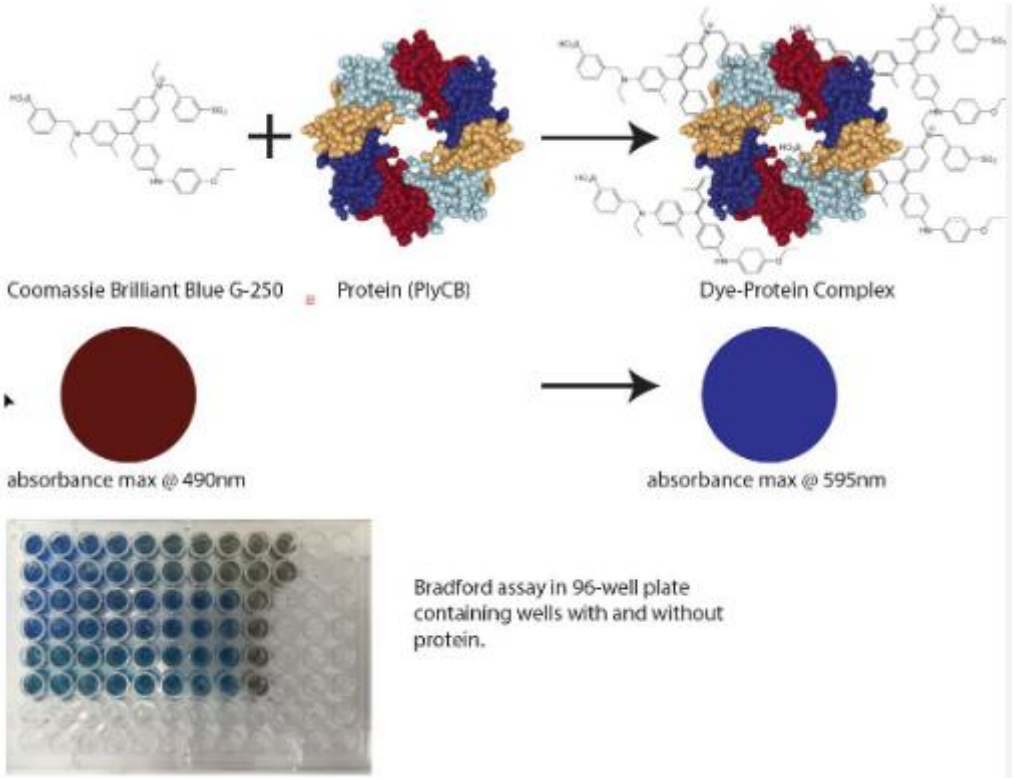

Figure 11. 96-well Bradford assay plates

Results

The several genotypes examined in this study were exposed to 0.5 mM of ADEP4 and ACP5, as well as 50 mM of the neurotoxic 6-OHDA. MTT Assay was then used to measure the vitality of the cells. Cell viability revealed that the levels of the various genotypes' viability decreased in a concentration-dependent manner. Cell viability in the HtrA2 KO/CHOP genotype was primarily decreased when compared to the vehicle control (DMSO). HtrA2 depletion markedly increased the susceptibility to 6-OHDA treatment (p 0.0001).

These results indicate a drug concentration-dependent influence on the cell viability of the genotypes, indicating that depletion of cellular content might be owing to high concentrations of drug treatment with 6-OHDA. Therefore, the concentration of the drug will be proportionate to the number of alive.

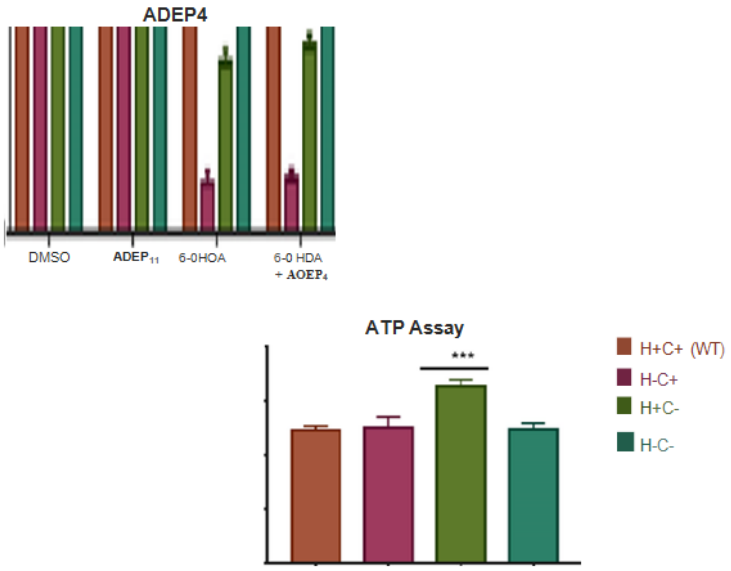

Figure 12. Shows that the HtrA2 KO/CHOP genotype produces less ATP. HtrA2 depletion markedly raised the responsiveness to pharmacological therapy Energy production loss was noticeably larger in HtrA2 KO/CHOP genotype treated just with 6-OHDA compared to vehicle control (DMSO). When combined with either ADEP4 (A) or ACP5 (B), the HtrA2/CHOP KO genotype's energy level was noticeably reduced. The HtrA2/CHOP KO genotype had considerably more ATP product ions (the data are provided as the mean SEM). One- or two-way ANOVA was used for the statistical analysis, and P 0.05 was required for Bonferroni's multiple comparisons against the vehicle control and between the indicated groups. •P <0.05, "*P<0.01, "*"P <0.001

HtrA2 KO/CHOP genotype produces less ATP:

Through an ATP assay nominalized by the Bradford method for measuring protein levels, ATP generation was ascertained. Similar to the cell viability experiment, the ATP assay showed a concentration-dependent decrease in energy production among the various genotypes and represented both the metabolic status of the cells and their viability. The findings demonstrated, as shown in figure (7), that HtrA2 deletion dramatically increased responsiveness to pharmacological treatment. The HtrA2 KO/CHOP genotype treated exclusively with 6-OHD A substantially lost more energy than the vehicle control (DMSO) (p 0.0001).

Discussion

The HtrA2 gene, which has 4152 DNA nucleotides and eight exons, is found on the forward strand of chromosome 2. A 49 kDa protein with 458 amino acid (aa) residues is produced by the gene. The trimeric pyramidal structure of the full-length translated protein, which is found in the mitochondrial intermembrane space (IMS) and undergoes a complex allosteric activation route to define HtrA2 proteolytic control, specificity, and activity. The serine protease domain (150–343 aa) with the catalytic triad residues (Ser306, His198, and Asp228), the transmembrane domain (105–125 aa), the one PDZ domain (364–445 aa) at the C-terminal end (8), and the mitochondrial localization sequence (1–40 aa) make up the protease.

HtrA2 proteases have the capacity to activate or synchronize many signaling pathways linked to PQC. The mature HtrA2 protein is released from the mitochondrial intermembrane space (IMS) into the cytosol in response to apoptotic stressors, which cause the N-terminus to break. The molecular architecture of human HtrA2 has been extensively studied structurally, but the precise mechanism by which the protein is activated is still unknown (9).

The bZIP domains of the CHOP protease are highly conserved in humans and several eukaryotic organism types, according to bioinformatics studies. The mouse CHOP and human CHOP (Homo sapiens) protein sequences (obtained from the Protein Data Bank) exhibit 88% identity and 91% similarity along their complete lengths, according to NCBI BLAST analysis. High sequence conservation was found in the bZIP domains of the investigated species according to a Clustal Omega multiple sequence alignment with CHOP protein sequences from various species (10).

The DNA-binding and dimerization motifs in the CHOP's bZIP domains are structurally comparable to those in other bZIP domains (11).

The actions of three interconnected processes—oxidative phosphorylation, the tricarboxylic acid cycle (TCA), and fatty acid-oxidation metabolism—help mitochondria regulate the generation of ATP. ATP is depleted as a result of improper mitochondrial biogenesis and an increase in oxidative stress, which triggers glycolysis (12). Environmental changes and genetic alterations may potentially have an impact on the enhancement of this glycolytic pathway (13).

According to earlier research, mice with HtrA2 deletion had lower mitochondrial mass and potential, which led to ATP depletion (14). As a result of the decreased ATP production, the UPR may become dysfunctional, which is linked to an increase in oxidative stress and is likely to cause further mitochondrial malfunction and pathogenic processes for neurodegeneration and apoptosis (15).

Conclusions

Ultimately, the evidence from the present study supports the involvement of HtrA2 and CHOP in the transmission of stress signals and the subsequent activation of mitochondrial quality control in Parkinson's disease. The transcriptional activation of mitochondrial quality control signals is hampered by the loss of CHOP activity. The molecular quality control marker Hsp60 and the organellar quality control marker Atg5 both significantly increase when HtrA2 is lost.

In the HtrA2 KO/CHOP genotype, 6-OHDA drug treatment resulted in decreased cell viability and ATP product ions, showing a drug concentration-dependent effect that reflects both the cell viability and the metabolic condition of the cells. Cell viability and ATP production were dramatically increased when 6-OHDA was combined with ADEP4 or ACP5, showing that these medicines may have a protective impact while counteracting the effects of 6-OHDA. The results offer fresh proof that decreased levels of HtrA2 may be linked to elevated levels of mitochondrial stress and transcriptional activation of the nuclear stress response gene CHOP, both of which are linked to Parkinson's disease.

This is a significant approach to enhance and improve the life span related to mitochondria and can help us in the earlier diagnosis of Parkinson's disease.

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