STUDY OF THE NEUROPHARMACOLOGICAL EFFECTS OF NARINGENIN, HARMINE AND ADENOSINE ON PARKINSONISM INDUCED IN RATS

Thesis

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Mevidette Adel El Madani

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LIST OF ABBREVIATIONS

Abbrev.	Meaning
6-OHDA	6-hydroxydopamine
AIF	Apoptosis-inducing factor
ATP	Adenosine Triphosphate
CAT	Catalase
CNS	Central nervous system
DA	Dopamine
DAergic	Dopaminergic
DMSO	Dimethyl sulfoxide
DOPAC	3, 4 dihydroxyphenyl acetic acid
GPX	Glutathione peroxidase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HVA	Homovanilic acid
$_i$NOS	Inducible nitric oxide synthase
MAO	Monoamine oxidase enzyme
MAO-A	Monoamine oxidase A enzyme
MAO-B	Monoamine oxidase B enzyme
MDA	Malondialdehyde
MPDP+	1-Methyl-4-phenyl-2, 3-dihydropyridine
MPTP	1-Methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
NEDD	N-(1-Naphthyl) ethylenediamine dihydrochloride
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
$NO^{\cdot}$	Nitric oxide radicle
NOS	Nitric oxide synthase
$O_2^{\cdot}$	Superoxide free radical

LIST OF ABBREVIATIONS (Cont...)

Abbrev.	Meaning
$ONOO^{\cdot}$	Peroxynitrite
ONOOH	Peroxynitreous acid
PARK1	Alpha synclein
PARK2	Parkin
PD	Parkinson's disease
ROS	Reactive oxygen species
RS	Reactive species
SN_C	Substantia nigra pars compacta
SOD	Superoxide dismutase
SULF	Sulfanilamide
UCHL-L_1	Ubiquitin carboxy-terminal hydrolase L_1

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PARKINSON'S DISEASE

Parkinson's disease (PD) is the most common neurodegenerative movement disorder with a prevalence of 1.8% in individuals of 65 years and older. It is characterized by the progressive depletion of dopamine in the caudate/putamen (striatum) resulting from the progressive loss of neurons in the substantia nigra pars compacta (SNc) **(Calne, 1992)**.

Resting tremors, postural instability, bradykinesia—all symptoms which result from the death of dopaminergic neurons in SNc. Neuropathological examination of PD shows several affected brain regions, but the loss of dopaminergic (DAergic) neurons in SNc is believed to be the most crucial **(Olanow and Tatton, 1999; de Rijk *et al*., 2000; Braak *et al*., 2004).**

At the time of clinical presentation approximately 50–70% of DAergic neurons in the nigrostriatal system have been lost. Surviving neurons may contain Lewy bodies, intracytoplasmic protein aggregates mainly composed of α-synuclein and believed to be a second neuropathological feature of PD **(Schapira *et al*, 1989; Spillantini *et al*., 1997).**

In about 95% of PD cases, there is no apparent genetic linkage (referred to as "sporadic" PD) but in the remaining cases, the disease is inherited. Some studies have indicated that both genetic and environmental factors might be involved in the etiology and pathogenesis of PD. Mutations of α-synclein (PARK1), parkin (PARK2) and ubiquitin carboxy-terminal hydrolase L-1(UCH-L1 or PARK5) genes have been found in rare familial PD **(Leroy *et al*., 1998)**. Moreover, there are increasing lines of evidence to suggest that genetic factors may also determine susceptibility for a majority of patients with idiopathic PD

(Piccini *et al*., 1999).On the other hand, numerous studies have shown that environmental factors such as pesticide exposure, are associated with an increased risk for PD **(Gorell *et al*., 1998; Menegon *et al*., 1998; Thiruchevlvam *et al*., 2000).**

A variety of in vitro and in vivo studies demonstrate that dopamine is a toxic molecule that may contribute to neurodegenerative disorders such as PD and ischemia induced striatal damage. The cytotoxic effect of dopamine has been assumed to originate either from the metabolism of dopamine [via the production of different reactive oxygen species (ROS)] or as a direct effect of the neurotransmitter itself in the neurodegenerative process. Indeed, the oxidation of the dopamine molecule produces a reactive quinone moiety that is capable of covalently modifying and damaging cellular macromolecules. This quinone formation occurs spontaneously but can be accelerated by metal ions (manganese or iron) and also arises from selected enzyme-catalyzed reactions. Macromolecular damage, combined with increased oxidant stress, may trigger cellular responses that eventually lead to cell death **(Stokes *et al*., 1999).**

Etiology and pathogenesis of PD

The etiology of Parkinson's disease and the mechanism of neuronal degeneration of the disease remain unknown. Several mutated genes have been identified in some familial cases of PD, suggesting that genetic factors may play a key role in the cause of the disease. Those familial forms of the disease are relatively rare and do not account for most cases (>90%) which are considered to be sporadic in nature **(Polymeropoulous *et al*., 1997; Kitada *et al*., 1998).**

The genetic basis for PD had long been controversial. In the last few years several gene mutations have been discovered to cause PD in a small number of families. Ten monogenic forms of PD, labeled PARK 1-10, have been identified. They present either as autosomal dominants or autosomal recessives. The autosomal recessives particularly PARK2 are much more common; though still infrequent **(Tanner *et al*., 1999).**

Unlike familial cases of PD that often are characterized by an early age of onset, sporadic PD is an age related disorder usually starting in the sixth or seventh decade of life and progressing over a period of 10 to 20 years**.** Current hypotheses for sporadic PD include combinations of the aging process, genetic propensity and environmental exposures leading to oxidative stress, mitochondrial dysfunction, microglial activation and excitotoxicity **(Gandhi and Wood, 2005; Savitt *et al*., 2006).** Additionally, dysfunction of the protein degradation system, also known as ubiquitin proteasome system may play a key role in PD pathogenesis associated with genetic mutations in parkin, UCHL1 and α-synuclein **(McNaught *et al*., 2003)**. All these events constitute a vicious cycle, and any one of them could initiate neuronal cell death rapidly recruiting the others **(Halliwell, 2006).**

It was found that mice lacking UCHL1 gene show widespread neurodegeneration, formation of protein aggregates and increased oxidative damage **(Castegna *et al*., 2004).** Damage to mitochondria [e.g. by neurotoxins such as 1-methyl-4-phenylpyridinium ion (MPP+) or rotenone] generates more ROS from the electron transport chain and causes oxidative damage that modifies proteins and other biomolecules **(Moore *et al*., 2005).**

Oxidized and nitrated proteins are usually removed by the proteasome; its inhibition allows abnormal proteins to accumulate and

produces oxidative stress. Potential mechanisms include, increased mitochondrial ROS production **(Sullivan *et al.*, 2004)** and increases in neuronal nitric oxide synthase (nNOS) activity, producing more nitric oxide free radicle (NO•) **(Lee *et al.*, 2001).** Formation of abnormal proteins resulting from gene mutations or of excessive amounts of normal proteins (e.g. α-synuclein, Cu-Zn-SOD) due to gene duplications or triplications could overload the proteasome. Indeed, injecting the proteasome inhibitor lactacystin into mouse or rat substantia nigra produced neurodegeneration, movement disorders and protein aggregation **(Zhang *et al*., 2005).**

Protein aggregates may stimulate reactive species (RS) formation from neurons and activate microglia. Finally, RS-producing agents could initiate neurodegeneration and may inhibit proteasome function directly (e.g. by oxidative or nitrative inactivation of proteasome subunits) or indirectly (e.g. by interfering with ubiquitination) **(Halliwell, 2006).**

Dopamine (3, 4 dihydroxyphenethylamine) not only acts as a central nervous system neurotransmitter for neurons involved in regulating movement (nigrostriatal pathway) and motivated behavior (mesolimbic pathway), but it is also a central component of neuroendocrine axes (hypothalamus). Dopamine serves as an intermediate in the synthesis of both norepinephrine and epinephrine in the peripheral and central nervous systems. A number of disease states such as schizophrenia, PD and also in addiction are thought to involve aberrations in dopamine neurotransmission **(Stokes *et al*., 1999).**

Dopaminergic receptors are classified into two sub-families; D_1 sub-family, which consists of D_1 and D_5 receptors and D_2 sub-family with D_2, D_3 and D_4 receptors. The dopaminergic receptors are built with about

400 amino acids and contain seven α-helices participating in ligand binding. Signal from the activated receptor is transmitted to the effector proteins via G proteins. The most important is the pathway linking substantia nigra and striatum (nigrostriatal) which produces 75% of the brain dopamine and participates in the regulation of motor activity. Other pathways are the mesolimbic pathway, which participates in the regulation of emotional and cognitive functions, as well as mesocortical and tubero-infundibular pathways. For fulfilling the functions of dopamine, the simultaneous stimulation of D_1 and D_2 receptors is necessary, a process called obligatory synergism **(Missale *et al.*, 1998).**

Dopamine oxidation products which accumulate in PD **(Spencer *et al.*, 1998)** can damage mitochondria and inactivate the proteasome **(Keller *et al.*, 2000)**. The environmental hypothesis posits that PD-related neurotoxin exposure can enhance neurodegeneration results from exposure to a dopaminergic neurotoxin. Theoretically, the progressive neuro-degeneration of PD lead to death of neurons due to chronic exposure to neurotoxin (chronic death neurotoxin exposure) or by limited exposure initiating a neurodegeneration cascade of deleterious events **(Langston *et al.*, 1983).**