ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 11, 2020

Online - 2455-3891
Print - 0974-2441

Review Article

EXPERIMENTAL ANIMAL MODELS OF PARKINSON'S DISEASE: AN OVERVIEW

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Received: 20 July 2020, Revised and Accepted: 04 September 2020

ABSTRACT

Parkinson's disease (PD) is the 2nd most common neurodegenerative disorder due to gradual loss of dopaminergic nerves in the substantia nigra in the midbrain which leads to motor symptoms: For instance, gait dysfunction, involuntary tremor, rigidity, and progressive postural instability. PD has no cure and available current treatment is only symptomatic. At present, the main treatment of PD relies on Levodopa that slowing down the disease development to some level but can lead to several side effects. The literature confirms the available models of Parkinsonism that is chemical-induced, that is, by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 6-hydroxydopamine-induced Parkinsonism furthermore transgenic models linked to monogenic alterations in SNCA, LRRK2, UCH-L1, PRKN, and PINK1 genes. In this review article, we conclude that the presently available neurotoxic models of PD that offer a platform for neuroprotective drug discovery.

Keywords: Parkinson's disease, Neurodegenerative, Experimental models, Neurotoxin.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i11.39131

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of the population over 55 years of age. The pathologic hallmark of the disease is the loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) and the presence of intracytoplasmic inclusions named Lewy bodies, formed mainly by synuclein and ubiquitin. Dopamine (DA) replacement is the main therapy, but as PD progresses, drug-related side effects emerge as well as patient's not responses to the treatment with increased risk of side effects. Since patients with PD have a normal lifespan, they must endure crippling symptoms for many years and also a severe impact on their quality of life. Hence, therapy aims to stop the continual loss of dopaminergic neurons along with preceding disease progression. This can be achieved only by neuroprotective strategies. The neuroprotection requires early intervention in the course of the disease. Later interventions that attempt to sustain or reconstruct the nigrostriatal system should be regarded as regenerative or restorative strategies instead of neuroprotective. Some of the signs are including all following characteristics: (1) Gradual loss of DA neurons in adulthood; (2) easily detectable motor deficits; (3) development of Lewy bodies; (4) based on a single mutation; and (5) short time course [1].

Parkinson characterized by the slow and gradual degeneration of dopaminergic neurons in the SN compacta and leads to a reduce level of DA in the striatum, tailed nuclei, and the putamen [2]. The progressive loss of dopaminergic neurons in the basal complexes is the most important pathological finding in the patient's brain. Destruction of these neurons results in the DA level in this area. After 50–60% of dopaminergic neurons are degraded and DA levels in the striatum decrease by around 80–85%, the symptoms of the disease appear as shown in Fig. 1. The exact molecular mechanism of the degradation of dopaminergic neurons and the incidence of PD is unclear; however, studies have shown that oxidative stress and mitochondrial dysfunction probably play a key role in the pathogenesis of PD; the loss of nigrostriatal dopaminergic neurons and the presence of intracellular cytoplasmic proteins, that is, Lewy bodies, are also involved. The cells are located in the nigrostriatal neurons in the SNpc are sent to putamen. The absence of these neurons, which typically contain small amounts of melanin, leads to depigmentation of SNpc [2]. The diagnosis of PD was based on two or more cardinal signs of PD, including resting tremor, cogwheel rigidity, bradykinesia, and postural reflex instability, and responsiveness to levodopa therapy. Risk factor of PD includes age, heredity, and exposure to neurotoxins.

ETIOLOGY

- Degeneration of neurons in the substantia nigra pars compacta.
- Degeneration of nigrostriatal (dopaminergic) tract results in a deficiency of DA in striatum >80% (Fig. 2).
- Inequity between the excitatory amino acids acetylcholine and inhibitory neurotransmitter DA described in Fig. 3.
- Oxidation of DA in the presence of iron
  - Generally quenched by glutathione.
  - Aging related alterations – DNA and lipid membranes.
- Genetic
  - α-synuclein (synaptic protein).
  - Parkin (a ubiquitin protein ligase).
  - UCHL1.
  - DJ-1 protein.
- Environmental causes
  - Infectious mediators – Encephalitis lethargica.
  - Environmental toxins –1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP).
  - Acquired brain injury.
- Excitotoxicity
  - ↑Glutamate, the normal excitatory transmitter in neurons.
  - Activated NMDA receptor.
  - Ca^{++} load ↑.
- Energy metabolism and aging
  - ↓ Role of (mitochondrial-electron transport system and MPTP) complex.
  - ↑Oxidative stress by free radicals.
- Other common factors
  - Cerebral atherosclerosis.
  - Viral encephalitis.
  - Due to the SIDE effects of some antipsychotic drugs (i.e., phenothiazines, butyrophenones, and reserpine).
  - As a result of pesticides, herbicides, and industrial chemicals.

ANIMAL MODELS OF PD

Animal models are a vital aid to find out the preliminary pathogenic tool and therapeutic strategies for clinical disorders. For model use

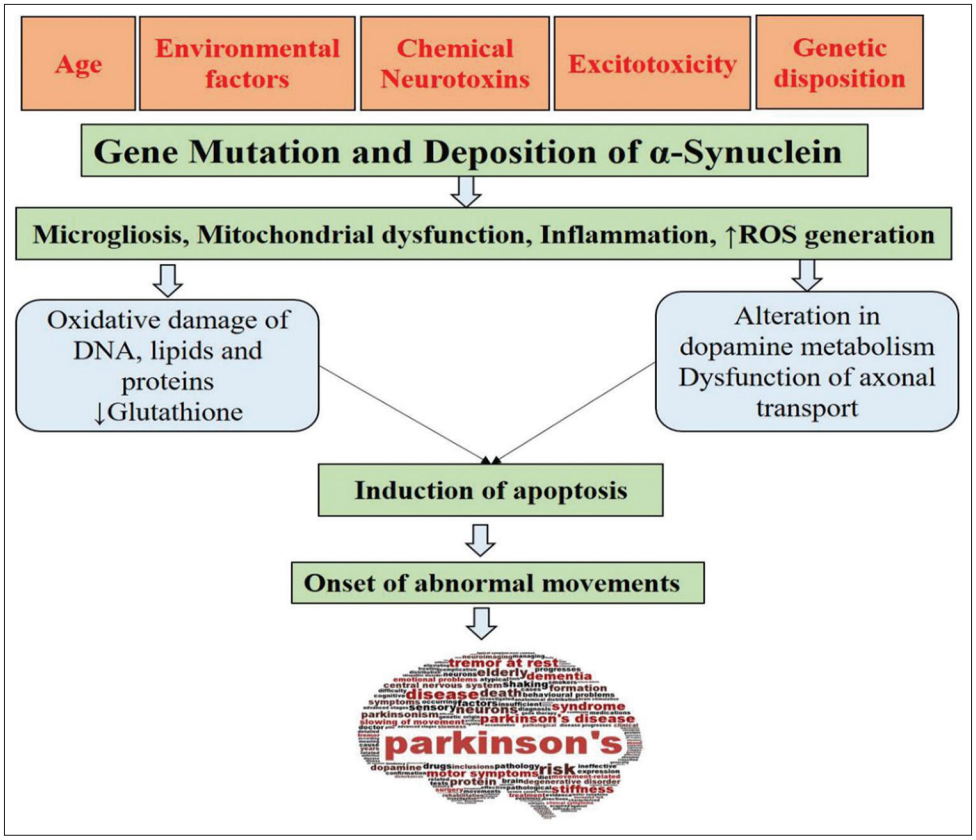

Fig. 1: General steps involved in pathophysiology of Parkinson's disease

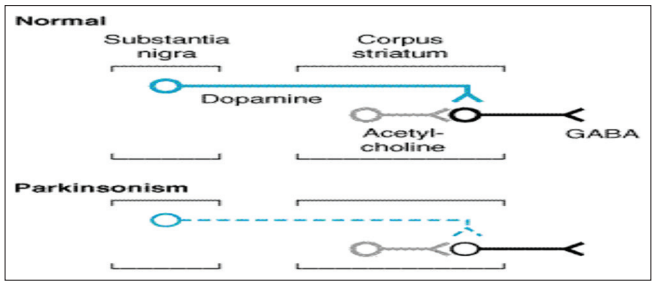

Fig. 2: Deficiency of dopamine in striatum

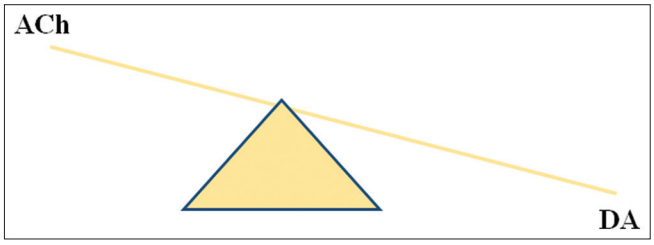

Fig. 3: Imbalance between acetylcholine and dopamine

of an animal model confirms the striatal DA deficiency associated Parkinsonian symptoms. The Levodopa (L-DOPA) was first use to compensate for striatal DA loss. L-DOPA still remains the standard treatment of PD [3]. There are multiple animal models of PD, but the vast majority of electrophysiological data used the monoaminergic neuron-selective toxins 6-hydroxydopamine (6-OHDA) and MPTP. 6-OHDA moves into neurons through monoamine transporters, where it undergoes oxidation to toxic free radicals [4]. It is infused directly into the brain; however, it cannot cross the blood-brain barrier. If targeted to the medial forebrain region, it causes complete DA denervation at the ipsilateral hemisphere. It can also be injected into monoaminergic terminal, cause death of all neurons projecting into those areas [5]. This may confine DA loss to specific nuclei, though dopaminergic cell bodies often send collaterals to multiple regions. This is an important consideration of neuronal death, which may progress over 2 weeks or longer [6]. The MPTP model emerged after i.v. drug users inadvertently produced MPTP from meperidine and developed acute Parkinsonism [7]. MPTP produces DOPA-responsive Parkinsonism in primates and causes severe monoaminergic depletion, specifically in mice but not in rats [5]. MPTP can be administered systemically or through intracarotid infusion to primates and can be used to create Hemiparkinsonism. MPTP-treated animals demonstrate regional differences in striatal dopaminergic denervation, dopaminergic cell death, and noradrenergic cell death, similar to PD. Some primate species (notably African Green monkeys) develop a classic rest tremor, while others (notably macaques) develop an intermittent higher frequency postural/action tremor [8] The detail about the chemical induced model was mentioned in Table 1.

MPTP model

MPTP is the tool of choice for investigations of the mechanisms involved in the death of DA neurons in PD. MPTP has been shown to be toxic in a large range of species. The most popular species, besides primates, is the mouse and rats were found to be resistant to this toxin [9]. A quantity of intoxication regimes or direction methods had been used in mouse [10,11] and in primates [12-14] for years; MPTP primarily causes damage to the nigrostriatal DA pathway with a profound loss of DA in the striatum and SNc [7].

In 1982, the dopaminergic neurotoxin MPTP, an analog of the narcotic meperidine (Demerol), was accidentally discovered [15]. Young drug addicts developed an idiopathic Parkinsonian syndrome after intravenous administration of (MPPP: 1-methyl-4-phenyl-propion-oxypiperidine) known as a "synthetic heroin" [15,16]. MPTP was the neurotoxic contaminant liable for the effect. Most of the biochemical,

neuropathological, and clinical characteristics observed in these addicted groups matched exactly to the cardinal symptoms of PD as in human with the exception of formed Lewy bodies [15]. A more recent study of these patients provided evidence for a stable and irreversible PD induced by MPTP [17]. Today, MPTP represents the most important and frequently used Parkinsonian toxin used in preclinical models [18,19] and has a competitive advantage over all other toxic PD models because: (i) It cause directed a specific intoxication of dopaminergic structures and (ii) induces similar virtual symptoms as found in human PD [19]. MPTP is extremely lipophilic and after systemic administration speedily crosses the blood-brain barrier. Then after, the 1-methyl-4-phenyl-2,3-dihydropyridium is formed by protoxin MPTP in non-dopaminergic cells (particularly in astrocytes and serotonergic neurons) by monoamine oxidase B (MAO-B) and then extemporaneously oxidizes to 1-s (MPP^+) [19,20]. Thereafter, MPP^+ is released into the extracellular space by an unidentified mechanism. The polar molecule MPP^+ is not able to enter dopaminergic cells freely; thus, its uptake depends on active plasma membrane carrier systems.

MPP^+

The uptake of MPP^+, the active metabolite of neurotoxin MPTP, was studied in various mammalian cell lines transfected with the cloned human and rat DA transporters and compared with rat striatal synaptosome preparations. Only in neuronally derived cell lines such as NGlOS-15, NSPOY, and SK-N-MC cells, MPP^+ for the cloned transporters comparable to that of DA as seen in rat striatal synaptosomes. In non-neuronally derivative cells such as COS-7, CHO, and Ltk- cells rapidly or forever stating the transporters, the K, of MPP^+ was 10-fold higher. The everlasting expression of either the duplicated human or rat DA transporters conferred to SK-N-MC cells exposure to the cytotoxic effects of MPP^+ in low concentrations. The extent of this action was depend on the apparent level of the DA transporters and could be exactly antagonized by the catecholamine acceptance inhibitor mazindol. There were no substantial alterations in the susceptibility to MPP^+ of cells stating similar levels of either the human or rat DA transporter. The demo for the 1st time of a quantifiable connection between the cellular appearance of the plasma membrane transporter and the level of the cytotoxic actions of MPP^+ advises that known alterations in the susceptibility of various brain areas to MPP^+ cytotoxicity might be associated to their actual content of DA uptake regions.

In addition, the intrinsic differences in the DA transporter proteins of humans and rats are not probably liable for the marked amplified susceptibility of primates to the neurotoxic effects of MPTP, as compared to rats [21,22].

6-OHDA

6-OHDA is a hydroxylated referent of the natural neurotransmitter DA [23]. It was originally isolated by Senoh [24]. Its biological effects were reported in works [25] and confirmed that 6-OHDA induces efficient and long-lasting noradrenaline depletion in sympathetic neurons [25,26]. 6-OHDA is one of the most common neurotoxins used in the assessment of central catecholaminergic estimates in the nigrostriatal system through *in vivo* and *in vitro* run down models [23,27-29]. 6-OHDA induced toxicity ensuing a preferential uptake of 6-OHDA by DA and noradrenergic transporter molecules [30]. Inside neurons, 6-OHDA accumulates in the cytosol and induces cell death without apoptotic characteristics [31]. Electron-microscopic studies have provided evidence for the ability of 6-OHDA to destroy adrenergic nerve terminals after systemic injection [32,33]. Furthermore, 6-OHDA was shown to cause ultrastructural changes in lizards and rats [34]. Non-neuronalcells like adrenocortical cells in case of inlizards and rats. However, the uptake of 6-OHDA into synaptic vesicles of adrenergic terminals is not necessary for its degenerating effect because pretreatment with reserpine prevents both the reduction of tyrosine hydroxylase (TH) in sympathetically innervated organs and ultrastructural changes of adrenergic nerve endings [35]. 6-OHDA has to be injected stereotactically into the brain for better results. Other preferred injection sites are the substantia nigra, medial forebrain bundle, and striatum [36,37]. Fig. 4 represented the induction of Parkinsonism.

ROTENONE

Complex I is the first enzyme of the respiratory chain. Rotenone having a high affinity to inhibit complex [38]. It is commonly used as an organic pesticide and in lakes and reservoirs to kill nuisance fish. Since it is extremely lipophilic, it crosses biological membranes easily and not requires specific transporters (unlike MPPþ) and enters the brain very rapidly [39]. The ability to inhibit complex I without significantly affecting respiration is due to the "threshold effect" seen in brain [40,41]. Scientist estimated the highest brain concentration of rotenone to be about 30 nM, close to that off KI for complex I. The

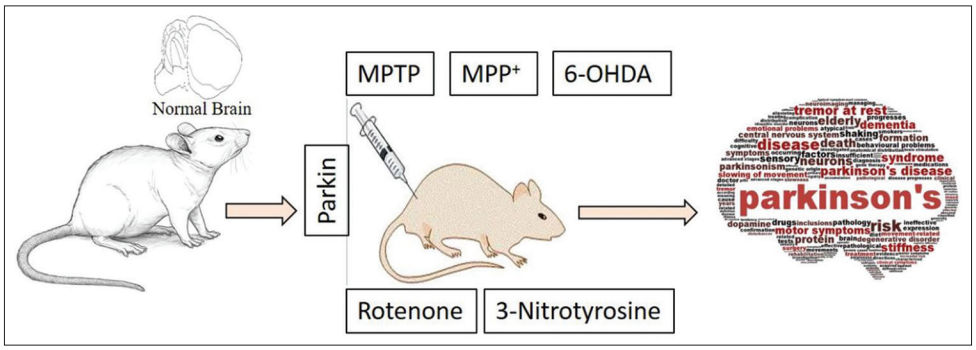

Fig. 4: Animal models showing chemical-induced Parkinsonism

Table 1: Drug tempted animal models of Parkinson's disease

Sr. no.	Name of model	*In vivo/In vitro*	Dose	Animal used	References
1	MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine)	*In vivo*	15–20 mg/kg	Mice, rats, cat	[63,64].
2	MPP^+ (1-methyl-4-phenylpyridinium)	*In vivo*	0.086–0.430 mg/kg	Mice	[21,22,65-67]
2	6-OHDA (6-Hydroxydopamine)	*In vivo*	0.05–0.032 mg/kg	Rats	[68,69].
3	Rotenone	*In vivo*	2–3 mg/kg	Rats	[70,71]
4	Transgenic α-Synuclein	*In vivo*	0.075 mg/kg	Mice	[72]
5	3-nitrotyrosine	*In vivo*	0.4 mg/kg	Mice	[73]
6	Reserpine	*In vivo*	0.1 mg/kg	Rats, Mouse	[74]
7	Parkin	*In vivo*	0.4 mg/kg	Mice	[75]
8	Amphetamine	*In vivo*	2.5 mg/kg	Rats/Mice	[76,77]

concentration of rotenone and the degree of inhibition was uniform across brain regions and was similar in other organs such as heart, liver, and kidney.

Chronic enteric contact to rotenone in rats initiates symptoms similar to PD, with DA degeneration at nigrostriatal [1]. The rotenone-administered animal model also produces all of the behavioral features as that of human PD. Importantly, many of the degenerating neurons have intracellular inclusions that resemble Lewy bodies morphologically. These inclusions show immunoreactivity for α-syn and ubiquitin as did the original Lewy bodies [42]. Rotenone can be administered by intraperitoneal injection, intravenous and subcutaneous injection [43]. Recently, rotenone has also tested in mice through direct infusion to the brain [44].

RESERPINE

Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2. The obstruction of DA vesicular acceptance results in the growth of neurotoxic DA oxidation byproducts [45]. DA along with molecular oxygen form DA-quinones, which can reduce the antioxidant levels mainly glutathione produces reactive oxygen species (ROS) throughout this process [46]. In addition, enzymatic breakdown of DA (through MAO) it increases the formation of ROS [47]. Oxidative damage occurs when the production of ROS exceeds the ability of the antioxidant system [48]. Furthermore, it showed that L-DOPA administration alleviated the reserpine-induced akinetic state, indicating that behavioral recovery is levodopa dependent. This led to the major hypothesis, later confirmed in humans [49], that the motor symptoms of PD result from striatal DA depletion [50]. The discovery that striatal DA deficiency resulted in PD like symptoms prompted the development of the "reserpine animal model." Systemic reserpine administration depletes the stores of DA at the nerve terminals and induces hypokinetic state in rodents. These moments deficits are due to the loss of DA storage capacity in intracellular vesicles [51].

PARKIN

Parkin is an E3 ubiquitin ligase that functions in the ubiquitin-proteasome system. Mutations in parkin are a cause of familial PD and are also seen in some young, sporadic PD cases [52,53]. Several parkin mice have been generated, typically produced by deletionatexon3, exon7, or exon 2 in the PRKN gene [54-59]. However, they show no substantial DA-related behavioral abnormalities. Some of these KO mice exhibited impaired DA release [55,60] and reduced norepinephrine levels in the olfactory bulb and spinal cord with an irregular nigrostriatal area but without damage of SNc neurons [54]. Only the parkin-Q311X-DAT-BAC mice exhibit multiple late onsets and progressive hypokinetic motor deficits, age-dependent DA neuron degeneration in the SNc and a significant reduction in striatal DA and dopaminergic terminals in the striatum [61]. Recently, overexpression of T240R-parkin and of human WT parkin induced progressive and dose-dependent DA cell death in rats [62].

DISCUSSION

Experimental Parkinsonism was incited in animals through various neurotoxins such as MPTP, 1-methyl-4-phenylpyridinium (MPP^+), 6-OHDA, Rotenone, 3-Nitrotyrosine, and Parkin. They induce selective dopaminergic neuron death or defects by producing increasing ROS. Further on continuation administration of neurotoxin causes aggregation of alpha synuclein. Further PD induced by genetic approaches, it includes transgenic models and viral vector-mediated models based on genes linked to monogenic PD. Environment affects the manifestation of symptoms and neurodegenerative scantling in animal models. These models can be combined to study the relationship between genetics and environment and untangle the heterogeneity and mechanisms underlying PD.

CONCLUSION

In this review article, we provide a comprehensive summary of the current experimental animal models of Parkinsonian disease. By retrieving and analyzing relevant literature, we found that a numerous neurotoxin drug, that is, MPTP, MPP+, 6-OHDA, rotenone, transgenic α-Synuclein, 3-nitrotyrosine, reserpine, parkin, amphetamine, etc., is available which provide a platform for anti-Parkinsonism drug discovery.

ACKNOWLEDGMENT

The authors offer their sincere thanks to Dean R&D Integral University for providing technical support and assigning Communication reference no IU/R&D/2020-MCN000928 for further communication; we also extend our sincere thanks to Head and Dean Faculty of Pharmacy, Integral University, Lucknow.

CONFLICTS OF INTEREST

The authors affirm no conflicts of interest, financial, or otherwise.

AUTHORS' CONTRIBUTIONS

All authors take considerably subsidized to the writing of this review article. Mohd Imran, who is the first author and Asif Iqbal, the last author, has collected all the relevant literature and sorted the significant articles for this review. Dr. Anuradha Mishra, the second author, recommended the theme and helps out in collection and screening of the information in the article and reviewed the article for final submission. Ms. Afreen Usmani, the third author, played a critical part in this article who edited the article for improved graphics and assisted in the concluding endorsement for publication version.

CONSENT FOR PUBLICATION

Not applicable.

AUTHORS' FUNDING

Nil.

DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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