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Melatonin as a Neuroprotective Agent in the Rodent Models of Parkinson's Disease: Is it All Set to Irrefutable Clinical Translation?

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Abstract Parkinson's disease (PD), a neurodegenerative disorder, is characterized by the selective degeneration of the nigrostriatal dopaminergic neurons, continuing or permanent deficiency of dopamine, accretion of an abnormal form of alpha synuclein in the adjacent neurons, and dysregulation of ubiquitin proteasomal system, mitochondrial metabolism, permeability and integrity, and cellular apoptosis resulting in rigidity, bradykinesia, resting tremor, and postural instability. Melatonin, an indoleamine produced almost in all the organisms, has anti-inflammatory, anti-apoptotic, and anti-oxidant nature. Experimental studies employing 1-methyl 4-phenyl 1, 2, 3, 6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), methamphetamine, rotenone, and maneb and paraquat models have shown an enormous potential of melatonin in amelioration of the symptomatic features of PD. Although a few reviews published previously have described the multifaceted efficacy of melatonin against MPTP and 6-OHDA rodent models, due to development and validation of the newer models as well as the extensive studies on the usage of melatonin in entrenched PD models, it is worthwhile to bring up to date note on the usage of melatonin as a neuroprotective agent in PD. This article presents an update on the usage and applications of melatonin in PD models along with incongruous observations. The impending implications in the clinics, success, limitations, and future prospective have also been discussed in this article.

Keywords Parkinson's disease · Melatonin · Rodent models · Neuroprotection

Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disease after Alzheimer's disease. It is characterized by the selective and progressive degeneration of the dopaminergic neurons of the nigrostriatal pathway leading to reduced level of dopamine in the striatum resulting in the onset of various clinical symptoms [1, 2]. While PD is contributed mainly by increasing age, environmental exposure to pesticides and heavy metals on the genetically susceptible individuals largely at the later phase of life is one of the most widely accepted suppositions of its etiology [2]. Rural living, well-water drinking, and farming have been found to be associated with the increased incidences of PD [1]. Environmental chemicals [manganese ethylene bisthiocarbamate (maneb), *N*,*N*′-dimethyl-4,4′-bipyridinium dichloride (paraquat), cypermethrin, rotenone, zinc, lead, iron, manganese, etc.] that preferentially and selectively degenerate the dopaminergic neurons are used to reproduce some of the features of the sporadic PD in the experimental animals. In addition to the environmental chemicals, many drugs, such as reserpine, methamphetamine, 6-hydroxydopamine (6-OHDA), and a contaminant of the synthetic heroin, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), when administered systemically in the experimental animals,

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produce many symptomatic, neurochemical, and anatomical features mimicking sporadic PD [1–3]. Genome and proteome approaches have identified several genes and proteins, which have offered clues to the genetic origin of PD in some individuals and also paved the way to develop the genetic models to understand its pathogenesis and to assess the efficacy of restorative agents [2].

Melatonin (*N*-acetyl-5-methoxytryptamine), an indoleamine, is a highly conserved anti-oxidant molecule secreted from the pineal gland, gastrointestinal tract, ovaries, testes, bone marrow, and eye lenses [4]. Melatonin and its metabolites, in general, offer anti-inflammatory, anti-apoptotic, anti-oxidative, and free-radical scavenging properties and protect against mitochondrial dysfunction [4–8], thereby regulating multiple biological processes in the body of an organism (Fig. 1). It is known to control the transcription, translation, and catalytic activities of the preventive antioxidants, including glutathione peroxidase, superoxide dismutase (SOD), and catalase under physiological and stress conditions [9]. Melatonin reduces the expression of adhesion molecules and pro-inflammatory cytokines, and regulates the expression of xenobiotic metabolizing enzymes and serum inflammatory indices [4, 8]. Furthermore, melatonin increases the activity of the mitochondrial complex I and complex IV, preserves homeostasis, improves respiration, enhances glutathione level, increases ATP synthesis, and decreases the harmful reduction in the mitochondrial membrane potential, which triggers mitochondrial transition pore opening and the apoptotic cascade [10, 11].

PD is the consequence of divergent contributors, including age and genetic and environmental factors, converging at a mysterious point leading to oxidative stress, inflammation, and selective neuronal death. Despite strong debate and conflicting evidences over various issues on PD, it is widely accepted that the disease is an outcome of an increased oxidative stress leading to irreversible dopaminergic neuronal death and is shared mainly by the impaired mitochondrial integrity and function, energy metabolism, and inefficient ubiquitin proteasomal system [2].

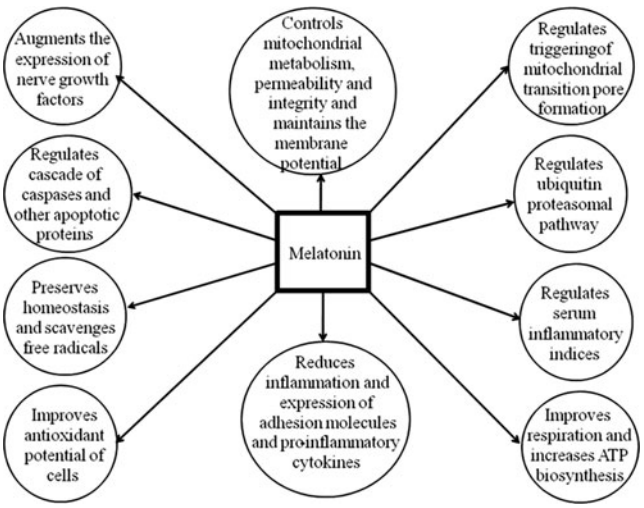

Fig. 1 Major biochemical, molecular, and histological events involved in PD, which could be significantly modulated by melatonin

Melatonin offers neuroprotection in a number of neurodegenerative diseases, including PD [7, 8]. Since melatonin is a powerful free-radical scavenger, naturally occurring anti-oxidant defense stimulator, potent anti-apoptotic agent, and a modulator of xenobiotic metabolizing enzymes, therefore, it may be considered to ameliorate the symptomatic features of PD [5, 6, 8, 9]. Melatonin is an ideal neuroprotective agent as it can easily cross the blood–brain barrier and enter the subcellular compartments, lacks toxicity as compared with many other neuroprotective agents, and possesses effective combating efficacy against free-radical-induced neuronal injury [12]. The presence of a considerable amount of melatonin in the mitochondria and growing evidences for mitochondrial dysfunction and oxidative stress in the dopaminergic neurons raise the likelihood of functional implication of melatonin in the mitochondrial and non-mitochondrial functions associated with PD [8, 13]. This is further supported by the fact that melatonin effortlessly enters the brain and cerebrospinal fluid owing to its smaller size and amphiphilic nature [12]. Melatonin is widely accepted as an alternative approach to ameliorate the symptomatic features of PD in experimental animals [8, 14–17].

Because of its ability to inhibit the ubiquitination-dependent proteasomal activity and to regulate numerous neuronal functions by receptor-dependent and receptor-independent mechanisms, melatonin is widely tested for its therapeutic efficacy against established rodent models of PD [7, 8, 16, 18, 19]. However, its wide relevance in clinics for the same purpose is not yet clearly established, and the knowledge generated from epidemiological studies is limited and inconclusive [20, 21]. It is worthwhile to present an update of the information as melatonin is found to be effective even against the recently developed and validated pesticides-induced animal models [1, 8, 22]. This article describes an update on the various rodent models and the interpretation of the usage of melatonin therein, followed by the possibility of its real applications in humans, the challenges associated with its applications, and the possible approaches to overcome these limitations.

Melatonin and 6-OHDA

6-OHDA is possibly the first chemical reported to degenerate the cell bodies of the dopaminergic neurons in the substantia nigra and fibers in the striatum and also induces microglial activation [23, 24]. It is known to produce several

symptomatic features in the experimental animals mimicking sporadic PD owing to its ability to induce free-radical generation, inhibit mitochondrial electron transport chain complexes I and IV, and induce apoptosis [23, 25–27]. Despite the fact that 6-OHDA cannot readily cross the blood–brain barrier due to its hydrophilic nature (Fig. 2) and is not an environmental chemical to which humans are exposed in their day-to-day life, it is one of the most widely used neurotoxins for understanding PD pathogenesis and assessing the treatment outcomes.

Melatonin effectively prevents apoptosis and protects against cell death caused by both low and high doses of 6-OHDA [28]. It is known to restore 6-OHDA-induced loss of tyrosine hydroxylase (TH)-positive cells, i.e., the dopaminergic neurons in the substantia nigra, absence of terminals in the dorsolateral striatum ipsilaterally, and behavioral deficits [29]. Melatonin prevents lipid peroxidation and significantly recovers the striatal dopaminergic function by restoring TH activity and dopamine content in 6-OHDA-induced PD in rats [30]. Melatonin is found to encounter apomorphine-induced unilateral rotation in 6-OHDA-treated rats [31]. The inhibitory effect of melatonin on apomorphine-induced rotational behavior is found to be dose dependent [32]. Melatonin partially restores the striatal dopamine and its metabolites and elevates the expression of dopamine-1 receptor in 6-OHDA-induced PD in rats. Melatonin also offers protection against apoptosis in naive and neuronal PC12 cells by preventing the 6-OHDA-induced reduction in the mRNA of many anti-oxidant enzymes as estimated by the cell viability assays, number of apoptotic cells, and quantification of DNA fragmentation [33]. Although behavioral anomalies and complex I enzyme activity

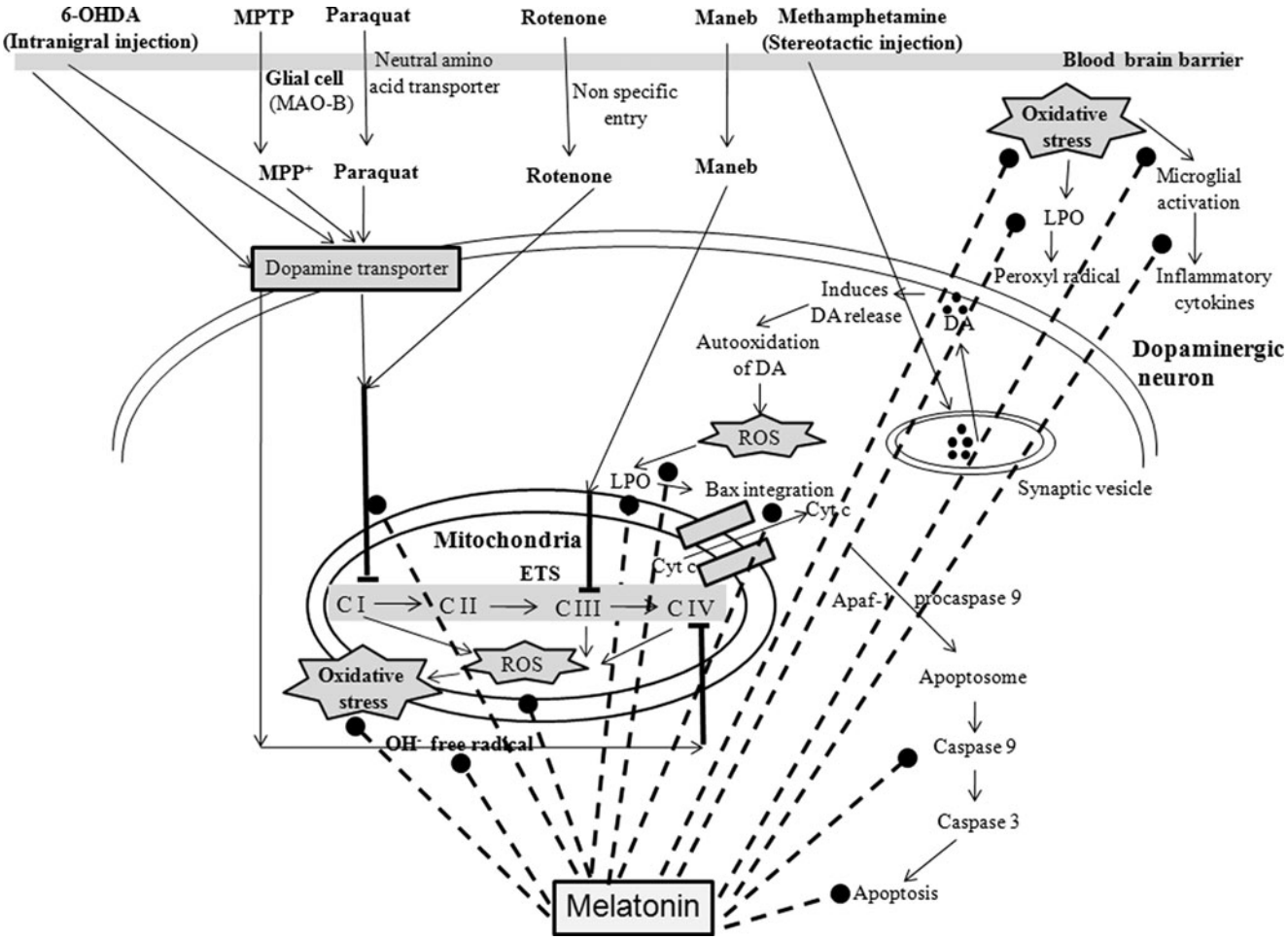

Fig. 2 Diagrammatic representation of the 6-OHDA, MPTP, paraquat, rotenone, maneb, and methamphetamine-induced neurodegeneration. The routes of various neurotoxins across the blood–brain barrier and their subsequent passage through the dopaminergic neurons are shown in the figure along with the subsequent major steps responsible for neurodegeneration (*solid arrows*). From the bottom of the image, *dotted lines with circular end* are projected to show how melatonin acts on the various steps involved in toxins-induced degeneration (*CI*, *CII*, *CIII* and *CIV*—mitochondrial complexes I, II, III, and IV; *ROS*—reactive oxygen species; *LPO*—lipid peroxidation; *DA*—dopamine; *Cyt*—cytochrome; *ETS*—electron transport system; *MAO-B*—monoamine oxidase-B enzyme)

works independent to melatonin, 6-OHDA-induced mitochondrial complex I deficiency, which increases reactive oxygen species production, reduces ATP synthesis, and leads to energy failure, may be taken care of by melatonin [31] (Fig. 2).

Most of the studies have shown that the anti-oxidant, anti-apoptotic, and anti-inflammatory efficacy of melatonin play critical roles in ameliorating symptomatic features of 6-OHDA-induced PD. However, melatonin exacerbates 6-OHDA-mediated motor impairments and cannot work as a universal remedy [34]. Despite rare conflicting reports suggesting the negative effects of melatonin, most of the studies decisively favor that melatonin offers neuroprotection against 6-OHDA [35].

Melatonin and MPTP

MPTP has been widely used to develop PD symptoms in the experimental animals to understand the pathophysiology and to assess the efficacy of anti-PD drugs. As MPTP is a lipophilic molecule, therefore, it readily crosses the blood–brain barrier [7]. The development of this model system is prompted by the PD phenotype seen during 1980s in the synthetic heroin addicts in North California [36]. It was Langston and his co-workers who identified MPTP, the real culprit, present in the synthetic heroin [37]. MPTP effectively elicits most of the critical features of PD in humans and primates, and a few of them can also be seen in the experimental rodents [7, 38]. Although MPTP does not induce the formation of non-fibrillar Lewy body in the nigrostriatal tissues and many phenotypic symptoms in the rodents, such as resting tremor, it is known to degenerate the dopaminergic neurons by inducing oxidative stress. MPTP induces the free-radical generation owing to its ability to inhibit mitochondrial complex I. MPTP alters nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system in addition to the activation of microglial cells and neuroinflammation [39]. MPTP is known to induce apoptosis via the activation of caspases and augmentation of DNA fragmentation [26, 40] (Fig. 2).

Melatonin, on the other hand, ameliorates the progressive impairment of the mitochondrial function, prophylactically reduces the oxidative damage and lipid peroxidation in MPTP-induced PD [41–43], and protects TH-positive nerve terminals [44, 45]. Since melatonin easily enters the brain, therefore, it inhibits the damage elicited by the chronic administration of MPTP, as measured by the nigral cell count, TH protein level, and other ultra-structural features related to PD pathology [46]. Melatonin is well known to reduce the MPTP-induced increase in the striatal 6-OHDA and protect against dopamine depletion [14]. MPTP-mediated neuroinflammation is a complex event initiated by the phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK) and translocation of nuclear factor kappa-B (NF-kB) (Fig. 3). Melatonin resists MPTP-induced degeneration of the dopaminergic neurons, nitrosative and oxidative stress, levels of intracellular calcium ion and phosphorylated p38 MAPK, translocation of NF-kB, and release of pro-inflammatory cytokines [47, 48] (Fig. 3). According to a study, the neuroprotective effect of melatonin against MPTP-induced dopaminergic neurotoxicity partially owes to its hydroxyl radical scavenging property [49]. MPTP down-regulates the expression of growth factors; however, melatonin increases the expression of growth factors, such as fibroblast growth factor 9 by up-regulating heme oxygenase-1 and gamma-glutamylcysteine synthetase expressions, thereby resisting the MPTP-mediated toxicity [50]. Melatonin ameliorates MPTP-induced inhibition of the mitochondrial electron transport chain and increase in oxidative damage as well as dysfunction of the glutathione system and blocks caspase-3 activation and cellular apoptosis [51, 52]. Melatonin potentially attenuates MPTP-induced nigrostriatal dopaminergic injury by impeding the increase of oxidized/reduced glutathione ratio owing to its anti-oxidant property [52, 53]. Melatonin counteracts MPTP-induced apoptosis as well as MPTP-dependent DNA fragmentation in vivo [54]. Melatonin also encounters MPTP-induced c-Jun-N-terminal kinase and caspase-dependent signaling leading to the dopaminergic neurodegeneration [55].

Melatonin is reported to produce complete protection against MPTP-induced dopaminergic neurodegeneration even in in vitro system [56]. The major factors responsible for melatonin-mediated neuroprotection against MPTP-induced neurodegeneration are its anti-oxidative, anti-apoptotic, and anti-inflammatory properties and its ability to regulate many associated sequential events. According to a report, melatonin is not a universal remedy and may pose considerable problems in neurological diseases characterized by dopamine depletion [34]; however, its huge potential as a neuroprotective agent and experimental evidences have strongly contradicted the hypothesis. Additionally, some studies claim that melatonin does not inhibit monoamine oxidase-B and therefore could be unable to protect against the MPTP-induced chronic reduction in the striatal dopamine content [57]. Although contradictions have been reported in a few studies, the reasons for such contradictions are difficult to interpret. However, variations may arise because of the time points considered for the isolation and fractionation of the nigrostriatal tissues and mitochondrial preparations under various experimental paradigms. Variations in the results across studies could be maximally minimized when the time of the isolation of tissues and mitochondrial fraction may be taken into account [58].

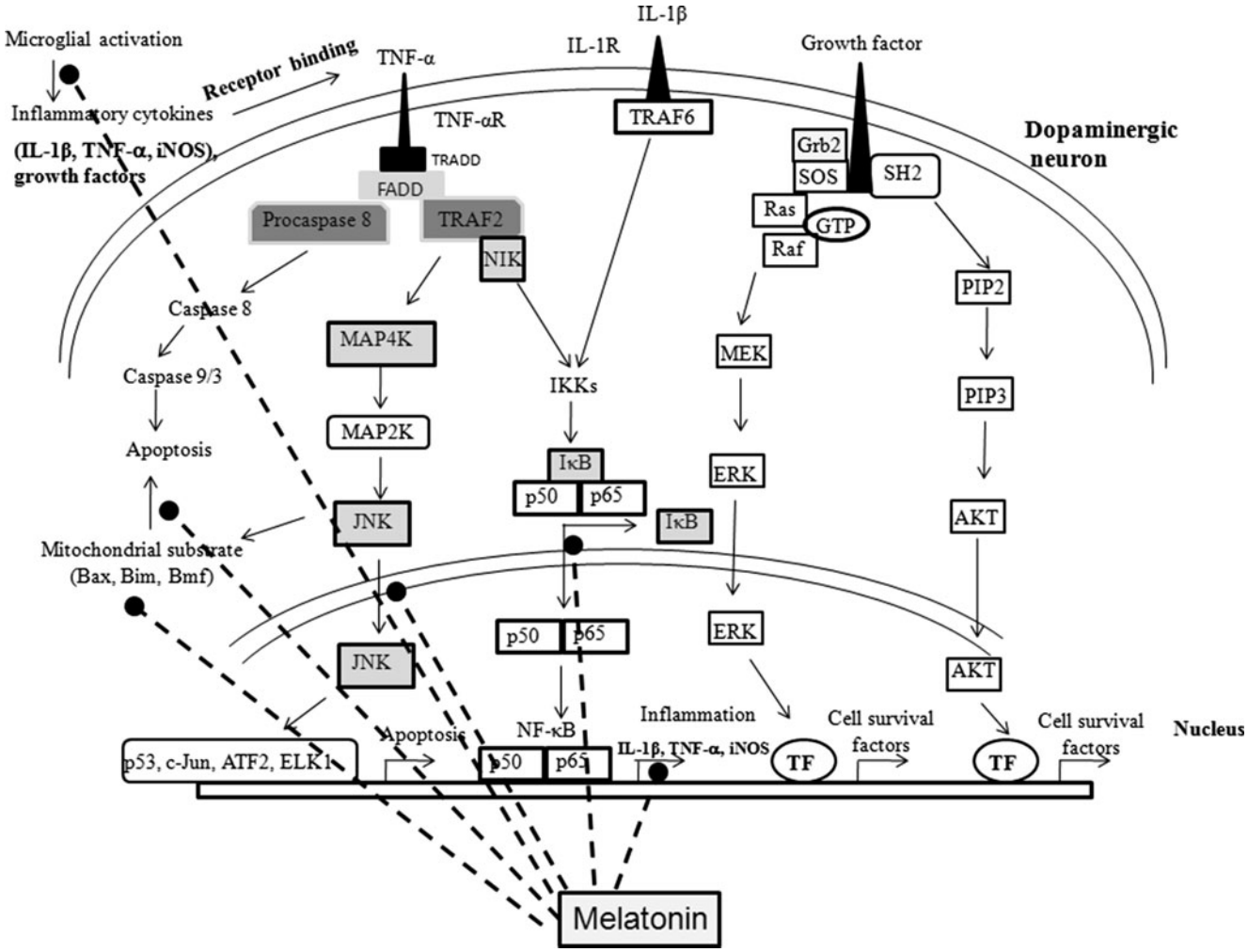

Fig. 3 The figure shows toxins-mediated microglial activation, which subsequently release the inflammatory cytokines. These cytokines further bind to respective receptors at the cell surface of the dopaminergic neurons leading to enhanced production of the pro-apoptotic and inflammatory proteins leading to neuronal cell death (*solid arrow*). The complete signal transduction pathway is mentioned in the image along with the involvement of phosphorylation of various signaling molecules. The effects of melatonin are shown via the *dotted lines circled at the end* against various events responsible for neurodegeneration (*TNF-α* tumor necrosis factor-α, *TNF-αR* tumor necrosis factor-α receptor, *IL-1β* interleukin-1β, *IL-1R* interleukin-1β receptor, *iNOS* inducible nitric oxide synthase, *SH2* Src homology 2 domain, *FADD* fas-activated death domain, *TRAF 2* tumor necrosis factor-α receptor associated factor 2, *TRAF 6* tumor necrosis factor-α receptor associated factor 6, *MAP2K* mitogen-activated protein 2 kinase, *MAP4K* mitogen-activated protein 4 kinase, *JNK* c-Jun N-terminal kinase, *IkB* inhibitor of kappa B, *NF-kB* nuclear factor kappa B, *TF* transcription factor, *Bax* B-cell lymphoma 2 associated protein X, *Bim* B-cell lymphoma 2 interacting mediator of cell death, *Bmf* B-cell lymphoma 2 modifying factor, *IKKs* inhibitor of kB kinase, *NIK* nuclear factor-kB inhibitory kinase, *ERK* extracellular signal-related kinase, *Grb2* growth factor receptor-bound protein 2, *SOS* son of sevenless (ras-specific nucleotide-exchange factor), *MEK* MAP kinase kinase, *ATF2* activating transcription factor 2, *ELK 1* E twenty-six like transcription factor 1, *Apaf 1* apoptotic protease activating factor 1, *AKT* Ak transforming serine/threonine protein kinase, *PIP2* phosphatidylinositol diphosphate, *PIP3* phosphatidylinositol triphosphate, *TRADD* tumor necrosis factor receptor type 1-associated death domain)

Melatonin and Paraquat Either Alone or in Combination with Maneb

Epidemiological evidences have prompted investigators to develop pesticides-induced animal models to understand the molecular and biochemical events leading to PD and to develop therapies to encounter the disease [22, 59, 60]. Paraquat, an herbicide, possesses structural similarity with 1-methyl 4-phenylpyridinium ion (MPP^+), the primary metabolite of MPTP, and induces the nigrostriatal dopaminergic neurodegeneration almost in the same fashion as MPTP. Unlike MPTP, paraquat is a polar molecule and crosses the blood–brain barrier through neutral amino acid transporter. Maneb, a fungicide, induces the nigrostriatal dopaminergic neurodegeneration along with paraquat much more potentially than paraquat alone. Paraquat inhibits the mitochondrial complex I; however, maneb inhibits the complex III (Fig. 2). Both of them when administered together lead to free-radical generation, and inhibit proteasomal function and energy metabolism more potentially than

that of alone. These pesticides generate superoxide, hydroxyl, and fatty acyl radicals leading to DNA fragmentation and apoptosis [60–65]. Paraquat also induces microglial activation and inhibits NADPH oxidase activity as observed with MPTP [24, 36].

Melatonin prevents the dopaminergic neurodegeneration by inhibiting free-radical generation, neuroinflammation, and apoptosis induced by paraquat and maneb possibly in the same fashion as in the case of MPTP. Since maneb and paraquat combined model is a relatively newer animal model of PD, the studies on the effect of melatonin against this model system are limited [8]. Melatonin and its metabolite *N* (1)-acetyl-5-methoxykynuramine inhibit the expression and activity of the mitochondrial inducible nitric oxide synthase and prevent mitochondrial failure [17] in many PD models (Fig. 3). Likewise, melatonin reduces the inducible nitric oxide synthase, nitrite content, oxidative stress, and apoptosis in maneb- and paraquat-induced PD phenotype in mouse [8]. Melatonin combats paraquat-induced oxidative stress, regulates the recycling of reduced nicotinamide adenine dinucleotide (NADH), and defends against paraquat-induced NADH depletion [66, 67]. Melatonin checks paraquat-mediated DNA damage and genotoxicity by foraging the hydroxyl and other free radicals [68, 69]. Similarly, melatonin reduces paraquat-induced mortality in rats and increases the lethal dose 50 [70]. Melatonin is found to inhibit the maneb-induced alpha-synuclein aggregation and mitochondrial dysfunctions and neurodegeneration at a night-time physiological blood concentration [71]. Despite little knowledge about the applications of melatonin against maneb- and paraquat-induced toxicity, it can be inferred from the studies conducted so far that melatonin could be equally effective in this model system as in 6-OHDA and MPTP.

Melatonin and Rotenone

Rotenone, a lipophilic compound, readily crosses the blood–brain barrier, inhibits the mitochondrial electron complex I, causes free-radical generation, and reduces ATP biosynthesis (Fig. 2). Rotenone leads to the dopaminergic neurodegeneration and reduces the number of TH-positive neurons of the nigrostriatal pathway. Although degeneration caused by rotenone is non-specific in nature, it is the only neurotoxin which exhibits well-defined Lewy body formation and aggregation of alpha-synuclein along with depletion of glutathione, disruption of axonal transport, and onset of several critical histological, biochemical, and pathological hallmarks of sporadic PD [23, 27, 72]. Since rotenone does not rely on the dopamine transporter uptake to exert neurotoxicity, therefore, it could be considered as an ideal model to assess neuroprotection [73].

Keeping in view of its close resemblance with the sporadic PD, several studies have been performed to assess the neuroprotective potential of melatonin against this model system. Melatonin prevents the nigrostriatal neurodegeneration and alpha-synuclein aggregation [72] induced by rotenone. Melatonin scavenges rotenone-induced hydroxyl radicals and restores the decreased glutathione level and changes in the catalytic activities of superoxide and catalase in the substantia nigra [74]. Even in chronic rotenone-based *Drosophila* model system, melatonin alleviates both locomotor impairment and the dopaminergic neuronal loss [75]. Although melatonin does not directly alter free calcium ion concentration or rotenone-induced inhibition of mitochondrial complex I, it is known to inhibit calcium ion and rotenone combined-induced oxidative stress in isolated rat brain mitochondria [76] (Fig. 2). Contrary to the above findings, melatonin is reported to potentiate the striatal catecholamine depletion and terminal loss and degeneration of the cell bodies of the nigral dopaminergic neurons, thereby exacerbating many features of rotenone-induced PD in rats [73]. Additionally, melatonin alone elicits an alteration in the striatal catecholamine level showing that melatonin does not offer neuroprotection against rotenone-induced PD in rats [73]. Overall, melatonin offers neuroprotection against rotenone model by inhibiting the free-radical generation, resisting TH-positive neuronal loss, and restoration of the dopamine level in the striatum.

Melatonin and Methamphetamine/Amphetamine

Methamphetamine-induced striatal dopamine depletion is considered as one of the important models to mimic some of the PD pathology, and chronic or intermittent treatment induces temporary or permanent disturbance in the dopaminergic system [77, 78]. Moreover, methamphetamine increases protein-1 and cyclic adenosine monophosphate response element binding protein expressions by activating the respective transcription factors and also directly acts on the mesencephalic cell nuclei [79]. Amphetamine leads to an increase in the level of alpha-synuclein and decrease in the phosphorylated TH and mitochondrial complex I proteins [15].

The protective effects of melatonin on methamphetamine-induced changes in the nigrostriatal system have been widely studied [78, 80, 81]. Methamphetamine-induced increase in free-radical formation owing to incomplete oxidative phosphorylation and mitochondrial damage leading to a failure of cellular energy metabolism followed by a secondary excitotoxicity could be regulated by melatonin [78] (Fig. 2). It is found that methamphetamine-induced biosynthesis of the reactive nitrogen species, peroxynitrite, which leads to the striatal dopamine depletion, is significantly reduced by melatonin

[80]. Amphetamine-induced changes in alpha-synuclein, phosphorylated TH, and mitochondrial complex I proteins are significantly reversed by melatonin [15]. Melatonin is reported to rescue methamphetamine-induced depletion of the striatal dopamine and dopamine transporter binding sites [82]. Melatonin effectively inhibits JNK 1 activation and reduces apoptotic signaling cascade and autophagic phenotype in methamphetamine-induced autophagy in a dopaminergic neuronal cell line [81] and therefore is useful in treating PD as JNK inhibition can be used to treat PD [83] (Fig. 3). Melatonin counteracts amphetamine-induced loss of cell viability and oxidative stress, and causes a decrease in alpha-synuclein expression and an increase in ATP synthesis in a human dopaminergic neuroblastoma cell line [84]. Melatonin alleviates methamphetamine-induced neurotoxicity, oxidative and nitrosative stress, and also suppresses the expressions of interleukin1-beta, interleukin-6, and tumor necrosis factor-alpha [85]. Additionally, melatonin is reported to protect against methamphetamine-mediated calpain-dependent cell death and increase cell viability [86]. Similarly, melatonin reduces the levels of Bax, Bcl-2, and cleaved caspase 3 proteins and thereby protects against cell death in methamphetamine-treated dopaminergic neuronal cells [87] (Fig. 2). Melatonin activates the mammalian target of rapamycin (mTOR) signaling pathway, phosphorylates mTOR and its downstream target, the eukaryotic initiation factor 4E-binding protein, and inhibits methamphetamine-mediated autophagy in vitro [88]. Melatonin abolishes methamphetamine-induced degeneration of nerve terminals in the neonatal rat brain and partially restores the expressions of TH, synaptophysin, and growth-associated protein-43 [89]. Melatonin protects the dopaminergic and serotonergic neurons against methamphetamine-generated oxidative stress [90]. On the contrary, melatonin is reported to aggravate the methamphetamine-induced deficits in the serotonergic and dopaminergic systems, such as the suppression of the activities of TH and tryptophan hydroxylase and reduction in the contents of dopamine and 5-hydroxytryptamine [91]. Melatonin also reduces the amphetamine-mediated toxic insults in the dopaminergic neurons by preserving the levels of VMAT-2 and phosphorylated TH [92]. Melatonin suppresses the amphetamine-induced overexpresssion of inducible nitric oxide synthase mRNA and defends against nitrosative stress-induced brain damage [93] (Fig. 3).

Other Rodent Models and Melatonin

Unfortunately, melatonin is not yet tested against most of the recent PD models, which might have relevance to humans. We are exposed to many chemicals, such as heavy metals and pyrethroid pesticides in our daily life; therefore, these chemicals are being employed to develop the animal models. Melatonin is not yet checked against metals and cypermethrin-induced nigrostriatal dopaminergic neurodegeneration, as these are relatively newer animal models [94–97].

Melatonin and Possible Explanations of the Neuroprotective Potential

Free-radical generation, mitochondrial pore formation, and oxidative stress-induced neuronal cell death are critical in PD, and any substance which prevents opening of mitochondrial pores and preserves the mitochondrial function may act as a neuroprotective agent [98]. Melatonin reduces neuroinflammation, oxidative stress, and cell adhesion, and restores mitochondrial function [4,8] and also acts as an important component of the brain's anti-oxidant defense system against catecholamine auto-oxidation and protects against the consequent dopaminergic neurodegeneration [99]. This idea gained momentum from the fact that pineal gland meets the criteria of the neuroendocrine system, and dysfunction of the pineal gland may be associated with the pathophysiology and clinical manifestations of PD [100]. Although initially it was considered that melatonin offers neuroprotection by restoring its level or by improving the restoration of the expression of TH by reducing its oxidation [7], several theories have been documented till date.

Inhibition of Dopamine Release

An integrated relationship between dopamine and melatonin is essential for normal physiology, and any imbalance between the two may lead to PD [101]. But as the physiological levels of melatonin decrease with age, therefore, its importance in the total antioxidative defense capacity of an organism is being investigated [102]. Melatonin inhibits the release of dopamine in the striatum and limbic system, and decreases the blinking rate in PD patients, suggesting a functional link among blink rate, melatonin secretion, and the striatal dopaminergic functions [103]. PD may show sleep-related symptoms, and a significant improvement in the subjective sleep disturbance by melatonin suggests its relevance in PD [20, 104].

Antioxidant and Free-Radical Scavenger

As PD imprints long-lasting physiological and pathological permanent effects on the pathogenic proteins, proteasomal machinery, mitochondrial physiology, metabolism, permeability and viability and cellular integrity and apoptosis,

therefore, anti-oxidants may help in the amelioration of the symptomatic features of PD. Melatonin, reported to be a potent free-radical scavenger, forages a variety of reactive oxygen and nitrogen species, including hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, and peroxynitrite anion. Furthermore, it can improve the cell survival and functions by stimulating anti-oxidant enzymes, increasing the efficiency of the electron transport chain and promoting ATP synthesis [6]. Even the pharmacological levels of melatonin are used to combat oxygen and lipid peroxides-induced toxicities [7]. It is several times more potent than vitamin C and E in protecting tissues from oxidative injury when compared at an equivalent dosage [105]. Melatonin, alone or in combination with deprenyl, defends against the dopaminergic neurodegeneration, as they suppress the hydroxyl radical formation during dopamine autoxidation in vitro [106, 107]. Melatonin regulates circadian modulation of Na+/K+-ATPase and Na+/H+ exchanger owing to its anti-oxidative and membrane fluidity modulating properties [108]. Melatonin-mediated rhythmic modulation of malondialdehyde and intracellular glutathione contents during day and night emphasizes the role of melatonin as an antioxidant and its function against oxidative stress [109].

Mitochondrial Modulator

As inhibition of mitochondrial complexes I to IV have been implicated in the pathogenesis of PD, therefore, a modulator of any of these, if not all, may be used to protect the dopaminergic neurons from damage. Melatonin preserves mitochondrial homeostasis, enhances mitochondrial glutathione level, and preserves proton potential and ATP synthesis by stimulating complex I and IV activities [11]. Melatonin increases the activity of the complex I and complex IV, and improves mitochondrial respiration, increases ATP synthesis under normal and stressful conditions, and also ameliorates the harmful reduction in the mitochondrial membrane potential that may trigger mitochondrial transition pore opening and the apoptotic cascade [10] (Fig. 2; Table 1). Melatonin protects against the deficits in the mitochondrial complexes leading to free-radical mechanisms, both directly via reactive oxygen species production and indirectly by decreased ATP synthesis and energy failure [31].

Anti-apoptotic Molecule

Apoptosis is one of the major critical events in the pathophysiology of neurodegenerative diseases, especially PD. Melatonin exhibits the apoptotic gene and protein expression patterns similar to many neuroprotective agents [110]. Melatonin also displays an extremely low index of mortality and even its high concentration does not significantly affect the expression of the mitochondrial Bcl-2 family members, bcl-2 and Bax [98]. Melatonin also inhibits oxidative stress-induced NF-kB activation, one of the main molecular hallmarks of the apoptotic events in PD [111] (Figs. 2 and 3, Tables 1 and 2).

Extracellular signal-regulated kinase (ERK) signaling is involved in the transcription of various genes responsible for cell survival. Melatonin increases the phosphorylation of ERK and activates the mitogen-activated protein kinase (MAPK) pathway in gonadotropin-releasing hormone-secreting neuronal cell line (GT1–7 cells) [112]. Similarly, Ak transforming serine/threonine kinase (Akt) is one of the most important mediators of growth factor-induced cell survival and regulates cell proliferation, apoptosis, and cell-cycle progression [113]. Melatonin is known to induce Akt phosphorylation via melatonin receptor- and phosphatidyl inositol-3-phosphate kinase-dependent pathways in primary astrocytes [114], and defends against neuronal death in the hippocampus [115] and cerebral ischemia [116] (Fig. 3).

Growth Factor Promoter

Melatonin attenuates the compensatory contralateral increase in the striatal glial cell-derived neurotrophic factor (GDNF) expression, supporting a physiological role for melatonin in correcting the expression of growth factors, which is normally defective in PD [117]. Melatonin induces GDNF and melatonin receptor expressions, which supports a functional role for the MT1 receptor, as they are co-localized and their interaction possibly offers neuroprotection against PD [118]. An induction of GDNF mRNA by melatonin also shows how it maintains the nigrostriatal dopaminergic integrity, as GDNF is essential to protect the neurons [119].

Advantages

Melatonin is an ideal neuroprotective agent in the amelioration of symptomatic features of PD owing to its lesser toxicity, ability to enter the brain, and combating efficacy against free-radical-induced neuronal injury ([12]; Table 2). As PD is mainly an aging-related disease characterized by an increased oxidative stress and reduced or altered expression of anti-oxidant, anti-inflammatory, toxicant metabolizing, apoptotic, and energy metabolism-related genes, and melatonin possesses the potent anti-oxidant, free-radical scavenging, anti-apoptotic, and anti-inflammatory properties, therefore it can effectively encounter the altered expression of several genes and proteins

Table 1 Summary of a few studies showing the major effects of melatonin against chemicals-induced cellular and animal PD models

Animal model	Salient features	Effects of melatonin	References
6-OHDA	Inhibits the mitochondrial complexes I and IV, causes non-selective degeneration of the dopaminergic neurons and produces Lewy body	Inhibits neuroinflammation and protects the dopaminergic neurons by restoring complex I activity, reduces microglial activation, apomorphine-induced rotations, oxidative stress and attenuates apoptosis	[7, 30–32]
MPTP	Inhibits the mitochondrial complex I and causes selective, however, mainly acute degeneration of dopaminergic neurons and does not form distinct Lewy body	Inhibits neuroinflammation and 6-OHDA production, protects the dopaminergic neurons by reducing TH-positive cell loss and hypolocomotion, restores mitochondrial complex I activity, decreases microglial activation, attenuates mitochondrial DNA damage, prevents dysfunction of glutathione system, blocks caspase-3 activation, protects the nigrostriatum from oxidative stress, and reduces oxidative stress and lipid peroxidation	[14, 41,44–49, 51–53]
Rotenone	Inhibits complex I of the electron transport chain, non-selectively degenerates the dopaminergic neurons, induces acute neurodegeneration and causes Lewy body formation	Prevents the nigrostriatal neurodegeneration and alpha-synuclein aggregation, reduces glutathione depletion and catalase activity, increases SOD activity, protects the mitochondria from oxidative stress, and increases cell viability	[72, 74, 76]
Maneb and paraquat	Inhibit complex I and III and degenerate the dopaminergic neurons	Protects the dopaminergic neurons and inhibits the expression of pro-apoptotic genes	[8]
Methamphetamine/ amphetamine	Degenerates the dopaminergic neurons and reduces dopamine packaging in synaptic vesicles	Attenuates VMAT-2 reduction and inhibits inducible nitric oxide synthase expression, increase in alpha-synuclein, decrease in phosphorylated TH and the mitochondrial complex I proteins	[15, 84, 92, 93]

Table 2 Tabular representation of the possible therapeutic applications of melatonin and the plausible limitations of therapy based on the knowledge gathered from various experimental models of PD

Animal model	Potential therapeutic applications	Limitations of therapeutic applications	References
6-OHDA	Protects against oxidative stress-induced apoptosis and restores the striatal dopaminergic function and behavioral deficits	Any modulation in the basal level of regular apoptosis must be checked Possibly exacerbate behavioral deficits and impairment of motor function	[28–30, 33, 34]
MPTP	Protects TH immunoreactivity of nerve terminals against reactive oxygen species and oxidative stress-induced apoptosis, and prevents the dopamine depletion in the striatum, PD progression, and the interaction of MPP^+ and other toxins with the mitochondrial complex I	Perturbation in the physiological balance between the body fluid concentrations of melatonin and dopamine owing to exogenous melatonin delivery may exacerbate neurodegeneration Stage-specific response needs elucidation Whether interaction is prohibited in vivo, irrespective of the route of exposure, needs to be sorted out Any modulation in the basal level of regular apoptosis must be checked	[14, 41, 44, 46, 47, 51, 101]
Maneb and paraquat	Resists oxidative stress-induced neurodegeneration and inhibits the aggregation of alpha-synuclein and mitochondrial dysfunction	Extrapolation of the similar result in human subjects needs validation	[8, 71]
Rotenone	Restores antioxidant enzymes in the substantia nigra and prevents the nigrostriatal neurodegeneration	Possibly potentiate the striatal catecholamine depletion and degeneration of the nigral dopaminergic neurons	[72–74]
Methamphetamine/ amphetamine	Encounters oxidative and nitrosative stress, subsequent autophagy and apoptosis and restores native conformation and structure of PD-related proteins, such as alpha-synuclein, TH, and mitochondrial complex I	Any modulation in the basal level of regular autophagy and apoptosis must be checked Melatonin may aggravate the disease	[15, 34, 78, 80, 81]

critical in PD pathogenesis [7, 8, 12, 35]. Since melatonin is naturally present in all organisms, could resist multiple anomalies associated with PD unlike most of the anti-PD molecules, and is a potent modulator of xenobiotic metabolizing enzymes, therefore, it has immense potential to be a remedy for PD. Melatonin may be considered as a "wonder" or "many in one" molecule if it succeeds substantially in epidemiological and clinical investigations globally.

The role of melatonin, a potent endogenous antioxidant, has been widely validated not only on the basis of its neuroprotective efficacy assessment but also because of the down-regulation of melatonin receptors in the nigrostriatal region of PD brain [120]. Although melatonin may cause a few side effects, which include nausea, headache, nightmares, and reduced blood flow after high doses and long-term exposures, according to most of the reports, melatonin is totally safe with short-term usage [121, 122]. Despite a report based on a case–control study, which has shown that melatonin improves the subjective quality of sleep but does not restore or improve the motor dysfunction, melatonin is an ideal remedy that can be tested in clinics. The negative result could be probably because of small sample sizes used in the study [21].

Regular melatonin intake by aged individuals may protect from the aging-related depredation, but the results of such a therapy would not be known possibly for decades [123]. The impact of application of melatonin in neurodegenerative disease may get certification only after clinical disease course outcome data become available [124]. Successful scientific evidences through comprehensive human population-based studies will provide real application of melatonin against PD in clinics.

Limitations

Despite neuroprotective experimental evidences of melatonin, clinical works with melatonin did not observe a remarkable therapeutic efficacy, and a few preclinical and clinical works have also shown that melatonin may even worsen the disease condition [34, 101]. Melatonin has been validated as a neuroprotective agent against most of the well-established animal models of PD (Table 1); studies have also shown that it offers an excellent neuroprotective efficacy against Alzheimer's disease but not PD [11]. Similarly, multiple effects of melatonin against several complicated disorders may create problems for its proper, complication-free, and widespread usage against PD. For example, a lower rate of cancer mortality or incidence in PD patients speculates the risk or preventative factors common to both diseases, including the lifestyle factors and genetic susceptibility. Lower melatonin concentration predicts a higher cancer and lower PD risk [125]. Application of high doses and long-term exposures of melatonin have been associated with a few minor complications (Table 2), although no major problems have been associated with its usage so far to the best of our knowledge.

The major causes of limitation of melatonin usage in clinics have been the elusive onset and pathogenesis of the disease and lack of suitable fingerprints for its early diagnosis. PD is mainly a sporadic or idiopathic disorder, and the pathogenesis is often slow and progressive. Since the disease is characterized by the irreversible degeneration of dopaminergic neurons, therefore, the permanent cure of PD is not yet possible with any known therapies. Similarly, none of the animal models developed so far completely or substantially mimics sporadic PD, which indicates that the conclusions drawn from animal experimentations may or may not be completely reproduced in clinical interventions. Melatonin plays a dual role in homeostasis and disease etiology, thereby requiring appropriate assessment of PD pathogenesis in a proper model system employing multiple biochemical, histological, molecular, and phenotypic parameters. Like all other drugs used in PD till date, melatonin does not regenerate the dopaminergic neurons in the nigrostriatal pathway; therefore, the permanent cure using melatonin as a gold standard is also not feasible. Melatonin is an important molecule and possibly has a great future in PD research, but it needs to be extensively tested across multiple populations for efficacy and real effects along with the side effects at the efficacious doses, and only if everything goes alright, it may be considered worthwhile in clinics.

Future Directions

Melatonin has been recommended for testing against the onset or progression of PD because of its multiple beneficial effects, lack of toxicity, and endogenous nature [7]. However, either its wide application as a therapeutic agent to encounter sporadic PD is not widely conducted or such information is not extensively reported in peer-reviewed, highly rated journals. It is essential to monitor the effects of melatonin in the chronic PD models mimicking sporadic PD more appropriately, employing behavioral, biochemical, histological, ultra-structural, genetic, molecular, genomic, and proteomic tools. Such testing is relevant and may be performed for better doable application of this endogenous molecule against a disease with an elusive pathophysiology and no permanent cure.

Complimentary and combination therapies, such as use of melatonin with dopaminergic therapy, offer more relevant and potent effects on motor and non-motor symptoms; however, clinicians need to ensure that complementary and combinational therapies should be used appropriately without reducing the benefits of each other [126]. Melatonin in combination with stem cell transplantation is found to be more effective as compared with any of them alone [127].

As stem cell therapy is projected to treat PD after successful animal experimentations, its combination with melatonin could be a viable approach to restore normal histopathology and biochemistry in PD patients [127].

Owing to multiple assets of melatonin that include anti-oxidant and free-radical-scavenging properties, anti-inflammatory potential, and its beneficial effects against mitochondrial dysfunction, its metabolites and synthetic analogs need to be tested for highest activity and lowest side effects [128–130]. If such strategies succeed, best melatonin analog could be tested either alone or in combination with other potential therapies to achieve the maximum neuroprotection and minimum toxicity in the sufferers of PD. Endogenous melatonin protects exogenous dopamine precursor from auto-oxidation, and their systemic co-administration increases its bioavailability and long-term application possibly without measurable toxicity. A cocktail therapy of multiple neuroprotective agents that possess lesser toxicities can possibly offer more neuroprotection and rescue the dopaminergic neurons more efficiently than the current treatment strategies with extremely high doses of a single anti-oxidant in the early stages of PD [131].

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