Chapter 3

Melatonin in Parkinson's Disease

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Additional information is available at the end of the chapter

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1. Introduction

Parkinson's disease (PD) is characterized by the progressive depletion of pigmented neurons containing dopamine (DA) in the region known as substantia nigra pars compacta (SNpc) and by the presence of intraneuronal aggregates called Lewy bodies, which are enriched in filamentous α-synuclein and other proteins, that are often ubiquitinated before being destroyed [1]. The locus coeruleus, the dorsal motor nucleus, the autonomic nervous system and the cerebral cortex are additional neuronal fields and neurotransmitter systems involved in PD with consequent loss of noradrenergic, serotonergic and cholinergic neurons. These neuronal changes led to progressive non-motor symptoms like sleep abnormalities, depression and cognitive decline in the later stages of PD [2].

Currently, levodopa is widely prescribed for the treatment of PD. Although it is highly effective as a symptomatic treatment, levodopa is incapable of providing the long-term protection that is needed to impair the onset or progress of the disease [3]. In fact, in addition to a few specific mutations, oxidative stress and generation of free radicals from both mitochondrial impairment and DA metabolism play critical and important roles in PD etiology. Deficits in mitochondrial functions, oxidative and nitrosative stress, accumulation of aberrant and misfolded proteins, and ubiquitin-proteasome system dysfunction can represent the main molecular pathways that trigger the pathogenesis of sporadic and familiar forms of PD [4].

It is known that about 15% of PD patients has a family background of the disease and few specific mutations have been identified to be responsible for rare familial forms of the pathology: α-synuclein, parkin, UCH-L1, DJ-1, and PINK1 are genes found to be related to PD [5]. These genetic defects seem to affect a common molecular pathway related to the ubiquitin-proteasome system with exception of PINK1, which is related to mitochondrial metabolism [6].

Some, if not all, of these mutations are partially related to free-radical generation. High levels of free-radical, reactive oxygen species (ROS) and reactive nitrogen species (RNS) damage not only phospholipids and polyunsaturated fatty acids of mitochondrial bilayers but also mitochondrial DNA (mtDNA) and mitochondrial proteins [7]. Uncontrolled increase in these metabolites lead to free radical-mediated chain reactions which indiscriminately target proteins, lipids and DNA resulting in cell death [8], producing neurodegeneration, at least in part, through the mitochondrial apoptotic pathway [9]. Several experimentally PD models are used to study the pathogenesis of the disease. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin able to produce experimentally Parkinson's disease in humans and monkeys (Figure 1). When administered to animals, MPTP readily crosses the blood-brain barrier (BBB), where it selectively destroys DA neurons in the substantia nigra (SN). Once MPTP crosses the BBB, it enters astrocytes, where it is converted into the active metabolite 1-methyl-4-phenylpyridinium (MPP^+) by the action of the enzyme monoamine oxidase B (MAO B) [10]. MPP^+ leaves the astrocytes and via the DA transporter enters the dopaminergic neurons. First of all, MPP^+ accumulates into mitochondrial matrix, where it inhibits the Krebs cycle enzyme α-ketoglutarate dehydrogenase [11]. In addition, this metabolite inhibits complex I of the electron transport chain (ETC), causing increased generation of ROS, decreased adenosine triphosphate (ATP) production and nigral cell death [12,13]. MPP^+, by inducing nitric oxide synthase (NOS) expression in SNpc, has been shown to produce large amounts of nitric oxide (NO) that, reacting with O_2^- generates the highly toxic peroxynitrite ($ONOO^-$), a molecule that impairs mitochondrial functions causing irreversible inhibition of all ETC complexes [14] and neuronal cell death [15].

Together with MPTP, other toxin-based models frequently used to induce dopaminergic neurodegeneration include the neurotoxin 6-hydroxydopamine (6-OHDA), the herbicides paraquat (*N,N'*-dimethyl-4,4'-bipyridinium dichloride) and rotenone, and the fungicide maneb (Figure 1). They are capable of inducing the pathological hallmark of PD, the neuronal cell loss in the SN. The main contributing factor to this cell loss is mitochondrial dysfunction by inhibiting complex I, resulting in oxidative stress and eventually cell death [16]. In particular, neurotoxin 6-OHDA induces reduction of the antioxidant glutathione (GSH) and antioxidant enzyme superoxide dismutase (SOD) [17], increase of iron levels in SN [18] and inhibition of complexes I and IV in mitochondria [19] which lead to further oxidative stress. The herbicide paraquat having a structural similarity to MPP^+ directly inhibits complex I [20] and produces oxidative stress through redox cycling. The herbicide rotenone, extracted from tropical plants, easily crosses the BBB and accumulates inside the mitochondrial dopaminergic neuron, where it inhibits complex I. Maneb, on the other hand, induces the nigrostriatal dopaminergic neurodegeneration by inhibiting complex III [16].

Actually, considering that the existence of mitochondrial damage, due to oxidative stress, is the base of the disease which may lead to a decrease in the activities of mitochondrial complexes and ATP production, and as a consequence, a further increase in free radical generation, with the final consequence being cell death by necrosis or apoptosis, the use of antioxidants as an important co-treatment with traditional therapies has been suggested.

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There are several agents that are currently under investigation for their potential neuroprotective effects based on their capacity to modify mitochondrial dysfunction. These include creatine, melatonin (MLT), nicotine, nicotinamide, lipoic acid, acetyl-L-carnitine, resveratrol etc. (Table 1) [21]. Among these compounds, melatonin has shown to be effective in preventing neuronal cell death and ameliorating PD symptoms in several *in vivo* and *in vitro* PD models.

MLT is a natural hormone secreted by the pineal gland that easily crosses BBB. This hormone regulates and modulates a wide variety of physiological functions. Besides the well-known chronobiotic and sleep inducing properties [22], many other physiological effects have been ascribed to MLT, such as the modulation of cardiovascular [23] and immune [24] systems and the influence on hormone secretion and metabolism [25]. Other effects of MLT described in the literature include antitumor [26,27], anti-inflammatory [28], pain modulator [29], neuroprotective [30,31], and antioxidant [32] activities.

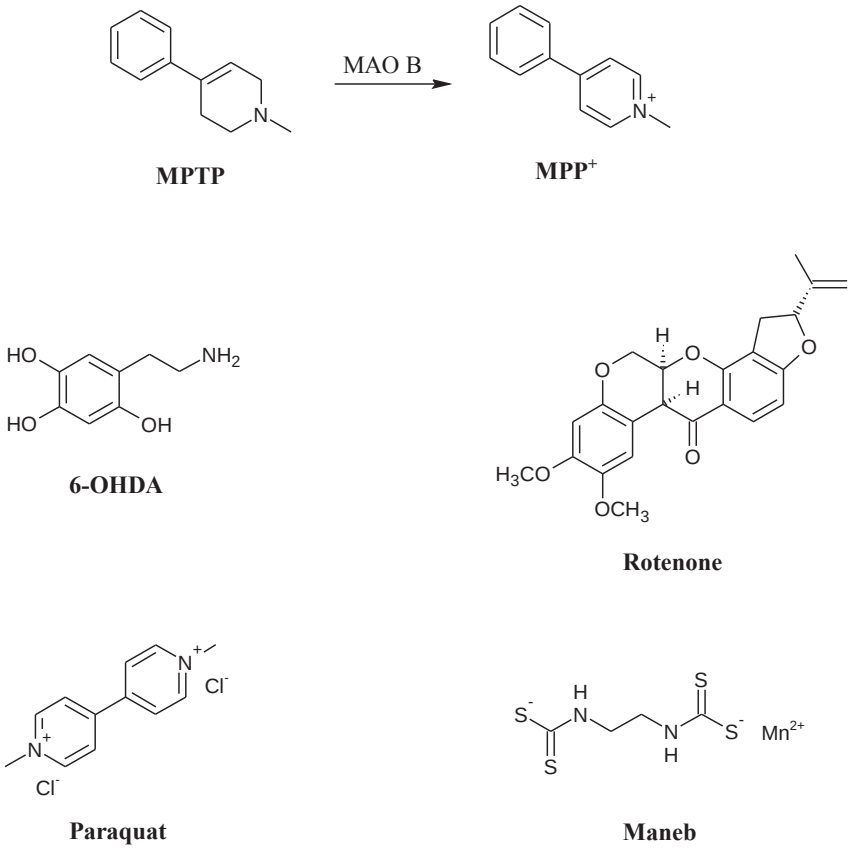
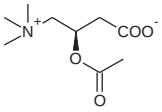
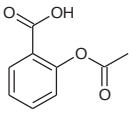
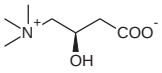
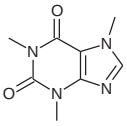
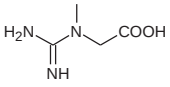
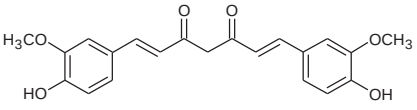


Figure 1. Toxins in experimental PD models.

Many *in vitro* and *in vivo* experimental models have contributed to demonstrate the role of MLT as an efficient radical scavenger against several reactive oxygen species (ROS), for example, the hydroxyl radical, the peroxynitrite anion, the superoxide anion, and singlet oxygen [33]. MLT has also been shown to enhance the production and the activity of several antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GRd), catalase, and glucose-6-phosphate dehydrogenase [34,35]. Furthermore, *in vivo* observations on the protective role of MLT in ischemic brain injury [36] or in animal models of PD [37] emphasize the therapeutic potential of this compound as a neuroprotective agent [38]. Moreover, MLT increases the efficiency of the electron transport chain thereby limiting electron integrity of the mitochondria and helps to maintain cell functions and survival [39]. Treatment with MLT counteracts the effects of MPTP in brain nuclei, increasing complex I activity, and the effects of MPTP on lipid peroxidation and nitrite levels in the cytosol and in the mitochondria of mice brain [40]. There is growing evidence that MLT antiapoptotic effects play an important role in neurodegeneration as well [41].

Agent	Structure
Acetyl-L-carnitine	
Aspirin (acetylsalicylic acid)	
Carnitine	
Caffeine	
Creatine	
Curcumin	

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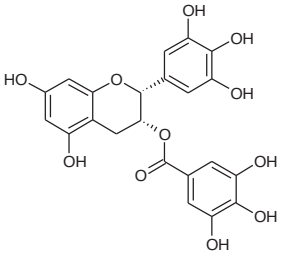
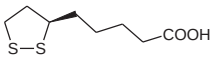
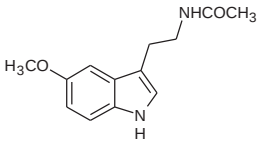
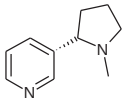
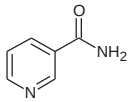
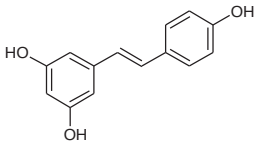
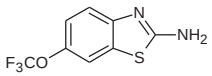
Agent	Structure
(–)-Epigallocatechin gallate (EGCG)	
(R)-Lipoic acid	
Melatonin (MLT)	
(–)-Nicotine	
Nicotinamide	
Resveratrol	
Riluzole	

Table 1. Neuroprotective agents in PD models.

2. Neuroprotective agents for Parkinson's disease

Relevant preclinical studies have identified several compounds such as MLT, estrogen, nicotine, caffeine, riluzole, curcumin, aspirin, epigallocatechin-3-gallate (EGCG) and resveratrol, as neuroprotective agents in PD [42] (Table 1). Various prospective studies have suggested a strong association between tobacco smoking and a decreased risk of PD. Nicotine is one of the main constituents of tobacco and is known for its pharmacological effects, exerted by interaction with cholinergic nicotinic receptors in both central and peripheral nervous systems [43]. A recent clinical trial among six male PD patients demonstrated that chronic high doses of nicotine improved motor scores, reduced dopaminergic treatment and had a potential beneficial effect on striatal dopamine transporter density [44]. Chronic nicotine treatment partly protects against the MPTP-induced degeneration of nigrostriatal dopamine neurons in the black mouse, counteracts the disappearance of tyrosine-hydroxylase-immunoreactive nerve cell bodies, dendrites and terminals in the mesostriatal dopamine system and prevent striatal dopamine loss provoked by 6-OHDA administration in the substantia nigra [45-47].

17β-estradiol (E2) is a predominant sex hormone that acts on the whole body. Since several epidemiological studies have shown a greater incidence of PD in men than women, extensive research have investigated the possible neuroprotective effects of E2 in MPTP mice models and in 6-OHDA-injury model [48,49]. Estrogens alters MPTP-induced neurotoxicity in female mice with effects on striatal DA concentrations and release [50]. E2 prevents loss of dopamine transporter (DAT) and vesicular monoamine transporter (VMAT2) in substantia nigra, induces regulation of striatal preproenkephalin mPRNA levels in MPTP-lesioned mice, protects the SNpc of female rats from lesion induced by 6-OHDA and interacts with the insulin-like growth factor-1 (IGF-1) system to protect nigrostriatal dopamine and maintain motoric behavior after 6-OHDA lesions [51-53].

Caffeine is the most widely used psychoactive substance in the world due to its presence in coffee and other beverages. Several epidemiological studies have linked coffee intake with a lower incidence of PD, suggesting neuroprotective properties for caffeine and demonstrating its strong neuroprotective role in rodents for various injury models [54,55]. In particular, Chen and co-authors found that caffeine (10 mg/kg) was neuroprotective when administered 10 min prior to four injections of MPTP [56], attenuating the depletions in striatal DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and DAT-binding sites. The same effects were also established in a 6-OHDA model [57]. Several epidemiological studies suggested an interaction between estrogen and caffeine. It has been reported that caffeine attenuated the toxic effects of MPTP in male mice in a dose-dependent manner. In contrast, this results was not found in female mice and estrogen treatment also prevented this effect in young male mice [58].

Riluzole is a selective Na^+-channel blocker and some researchers have demonstrated its neuroprotective effects in rodents and in a primate model. Boireau and co-authors reported that riluzole neuroprotection in combination with MPTP was due to interference with MPP^+ production by MAO-B inhibition. The protective effect was confirmed in MPTP- treated mice, partially due to astrocyte activation [59].

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Many evidences reported that an important risk factor for the disease is aging [60]. It contributes to PD progression because of accumulative oxidative damage and decrease of antioxidant capacity. Genetic studies have also revealed that aging can be controlled by changes in intracellular NAD/NADH ratio regulating sirtuins, a group of proteins linked to aging, metabolism and stress tolerance in several organisms. Consistently, the neuroprotective roles of dietary antioxidants including for example, acetyl-L-carnitine, curcumin, epigallocatechin-3-gallate (EGCG), carnosine, resveratrol, etc. have been demonstrated through the activation of these redox-sensitive intracellular pathways.

In particular, acetyl-L-carnitine has been proposed to have beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders [21]. In fact, acetyl-L-carnitine treatment has been shown to prevent age-related changes in mitochondrial respiration and decrease oxidative stress biomarkers through the up-regulation of HO-1 (heme oxygenase-1), Hsp70 (heat shock protein 70) and superoxide dismutase-2 in senescent rats [61]. Acetyl-L-carnitine has shown to be neuroprotective through a variety of other effects such as the increase in protein kinase C (PKC) activity [62]. Moreover acetyl-L-carnitine has also been reported to attenuate the occurrence of parkinsonian symptoms associated with MPTP *in vivo*, and protects *in vitro* against the toxicity of neurotoxic MPP^+ [63].

Curcumin is an active polyphenolic compound of Turmeric (*Curcuma longa*), which is extensively used as dietary spice in Indian food. Curcumin is used as a food additive because of its yellow colouring properties and presents anti-inflammatory and antioxidant properties. Recent studies demonstrated the neuroprotective effects of pretreatment with curcumin in the 6-OHDA model in rats. Both motor deficits and neuronal damage were prevented by curcumin and by one of its main metabolites, tetrahydrocurcumin, which also had beneficial effects on the antioxidant status, with increasing GSH levels and activity of antioxidant enzymes. Curcumin inhibited, in fact, MAO-B activity which prevents the conversion of MPTP to its toxic metabolite MPP+ [64].

EGCG is a catechin ubiquitously found in plants and is an important substance in green tea. Interestingly, there are several epidemiological studies that investigated an association between tea and PD. Among tea drinkers, the risk of developing PD was lower than in non-tea drinkers [65]. This effect was thought to be especially influenced by EGCG, to which has been ascribed a wide range of therapeutic properties, including neuroprotection. In fact, green tea and EGCG prevented MPTP-induced neuron loss and inhibited the upregulation of striatal SOD and catalase enzymes [66].

Inflammation is believed to be one of the important factors in the pathogenesis of PD. Moreover, it had been demonstrated that the enzyme cyclooxygenase (COX) and other inflammatory proteins are elevated in PD. Therefore, there is a significant interest in non-steroidal anti-inflammatory drugs (NSAIDs), especially aspirin [42]. The aspirin has an additional free radical scavenging property in addition to COX2 inhibition. In a study reported by Marahaj and co-authors, aspirin (100 mg/kg) and paracetamol (100 mg/kg) prevented KCN-induced superoxide generation and lipid peroxidation. While paracetamol was a more effective antioxidant, aspirin completely blocked the debilitating effects of MPP^+ on striatal DA in rats, whereas paracetamol was only able to partially block this effect [67].

Also resveratrol, a polyphenol compound, found in grapes and in red wine, has shown anti-inflammatory, anti-oxidant, and neuroprotective properties. The effects of resveratrol on the 6-OHDA injury in rats were studied by Khan and colleagues [68]. They have demonstrated that resveratrol was not only capable to protect neurons, but also to increase the activity of antioxidant enzymes and decrease the levels of thiobarbituric acid reactive substances (TBARS), protein carbonyl (PC), and phospholipase A2 (PA2), providing evidence for a possible antioxidant property. Then, pretreatment with resveratrol (50 and 100 mg/kg) prevented neuronal cell loss in the SN and striatal DA depletion, in 6-OHDA-injury model in rats it was neuroprotective and it has been shown to decrease mRNA and protein levels of TNF-α in COX2, suggesting that an anti-inflammatory mechanism underlies the protective effects of this polyphenol [69,70].

3. Melatonin

Melatonin (*N*-acetyl-5-methoxy triptamine, MLT), a triptophan derivative, is a highly conservative naturally occurring molecule present in a wide spectrum of organisms, including bacteria, fungi, plants, protozoa, invertebrates [71] and vertebrates. In vertebrates, MLT is primarily produced by the pineal gland with a marked circadian rhythm that is governed by the central circadian pacemaker in the suprachiasmatic nuclei (SCN) of the hypothalamus, the highest levels occurring during the period of darkness [72]. Extrapineal sites of MLT production include retina, Harderian gland, gut, bone marrow [74], platelets, and skin [75]. However, with the exception of retina, the physiological significance of these extrapineal sites is still a matter of debate. MLT was first isolated and identified in the bovine pineal gland by Lerner and coworkers in 1958 [76].

MLT acts as time-giver (*Zeitgeber*) in the regulation of circadian rhythms [77,78] and in synchronizing the reproductive cycle with the appropriate season of the year in photoperiodic species [8]. In non-photoperiodic species such as humans, MLT actions consist in consolidation of sleep and regulation of the circadian rhythm [9]. MLT actions, however, are not restricted to its role in the neuroendocrine physiology. Many other physiological effects have been ascribed to MLT, such as the modulation of cardiovascular [23] and immune [24] systems and the influence on hormone secretion and metabolism [25]. Other effects of MLT described in the literature include antitumor [26, 27], anti-inflammatory [28], pain modulator [29], neuroprotective [30, 31] and antioxidant [32] properties. MLT have also been associated with the cellular antioxidant defence since it is a powerful free radical scavenger, and it is able to induce the expression and/or the activity of the main antioxidant enzymes [79].

MLT exerts its actions by multiple mechanisms. Many of its physiological actions are mediated through activation of distinct MLT receptors expressed in a wide variety of tissues. Cloning studies have revealed at least three MLT receptor subtypes, two of which (MT_1 and MT_2) have been found in mammals and are localized in different areas of the central nervous system (CNS) as well as in peripheral tissues [80]. Moreover, a non-mammalian MLT binding site with a lower affinity profile (MT_3) has been found in hamster brain and characterized as a MLT-

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sensitive form of the human enzyme quinine reductase 2 [81]. MLT is also a ligand for retinoid orphan nuclear hormone receptors referred to as RZRα and RZRβ at concentrations in the low nanomolar range. Both receptors are present in the central and peripheral nervous system and have been associated with cell differentiation and immune response regulation [82,83]. The melatonin MT_1 receptor is coupled to different G proteins that mediate the inhibition of adenylyl cyclase and the activation of phospholipase C [84], while the MT_2 receptor is coupled to a number of signal transduction mechanisms, among them phosphoinositide production, inhibition of adenylyl cyclase and guanylyl cyclase [80].

Tryptophan serves as the precursor for the biosynthesis of MLT (Figure 2). It is converted into serotonin via 5-hydroxytryptophan. Serotonin is then acetylated to form *N*-acetylserotonin by arylalkylamine *N*-acetyltransferase (AANAT or NAT), one of the key enzyme in MLT synthesis. *N*-acetylserotonin is then converted to MLT by hydroxyindole-*O*-methyltransferase (HIOMT) which has been identified as the rate-limiting enzyme in the biosynthesis of pineal MLT [85]. In all mammals pineal MLT biosynthesis is synchronized to light/dark cycle by the SCN, which receives its input from the retinohypothalamic tract. Special photoreceptive retinal ganglion cells containing melanopsin as a photopigment are involved in the projection from retina [86]. Fibers from the SCN pass through a circuitous route involving the paraventricular nucleus of the hypothalamus and then proceed to innervate pineal gland as postganglionic sympathetic fibers. Norepinephrine released from these fibers binds to postsynaptic adrenoceptors whose activation induces an increase in cyclic adenosine-3′,5′-monophosphate (cyclic AMP) accumulation and a subsequent activation of NAT [87].

MLT has two important functional groups which determine its specificity and amphiphilicity: the 5-methoxy group and the *N*-acetyl side chain. Due to its lipophilic nature and pK_a, MLT readily crosses the BBB. Once formed within the pineal gland, the majority of MLT diffuses directly towards the cerebrospinal fluid of the brain's third ventricle, while another fraction is released into the blood stream where it is distributed to all tissues. The brain has much higher concentrations of MLT than any other tissue in the body [88].

Circulating MLT is partially bound to albumin and can also binds to hemoglobin [89,90]. MLT is mainly metabolized in the liver via hydroxylation reaction by cytochrome P450 monooxygenases. This reaction is followed by conjugation with sulfuric or glucuronic acid, to produce the principal urinary metabolite, 6-sulfatoxymelatonin. Conjugated MLT and minute quantities of unmetabolized MLT are eliminated through the kidney. In addition to hepatic metabolism, oxidative pyrrole-ring cleavage appears to be the major metabolic pathway in other tissues, including CNS [91].

MLT seems to function *via* a number of means to reduce oxidative stress. It can develop its action at two levels: as a direct antioxidant, due its ability to act as a free radical scavenger, and as an indirect antioxidant, since it is able to induce the expression and/or the activity of the main antioxidant enzymes.

MLT is a powerful free radical scavenger since it is able to remove H_2O_2, $^{\bullet}OH$, peroxinitrite anion ($ONOO^-$), singlet oxygen ($^1O_2$), $O_2^{\bullet-}$ and peroxyl radical ($LOO^{\bullet}$). MLT, as an electron-rich molecule, is able to interact with free radicals through consecutive reactions giving rise to

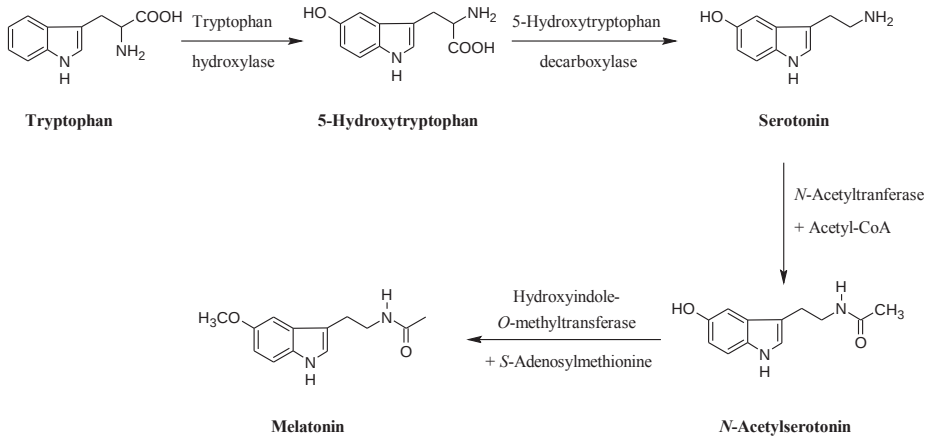

Figure 2. Biosynthetic pathway of melatonin.

many stable compounds that can be excreted by urine. In fact, the MLT antioxidant mechanism implied a free radical scavengers cascade, since secondary, and even tertiary metabolites are also efficient free radicals scavengers, like *N*-acetyl-*N*-formyl-5-methoxykynuramine (AFMK) and *N*-acetyl-methoxykynuramine (AMK) (Figure 3) [92,93]. The formation of such metabolites from MLT implies that, unlike classic antioxidants, melatonin does not produce prooxidant reactions and, even more, AMK and AFMK, in all the mitochondrial studies where comparisons were made, were more potent than MLT itself [94].

The large subcellular distribution of MLT allows its interaction with almost any kind of molecule, diminishing oxidative damage in both lipid and aqueous environments. This is supported experimentally by numerous data that show that MLT is able to protect lipids in the cellular membranes, proteins in the cytosol and DNA in the nucleus from free radical damage [95]. MLT gets free access to all cell components especially in the nucleus [96] and mitochondria [97], where it seems to accumulate in high concentration. In addition, MLT interacts with lipid bilayers of mitochondria, stabilizing its inner membrane [98], an effect that improves ETC activity [99].

Apart from its direct scavenging activity, MLT confers indirect protection against oxygen species through its capability to increase the gene expression and/or activities of antioxidant enzymes. This regulatory role is also mediated by the metabolites of MLT [34,35]. The expression of enzymes, such as GPx, GRd and SOD, related to the endogenous antioxidant system of the cells and the mitochondria, are under genomic regulation of MLT [100,101]. Some antioxidant properties of MLT are attributable to a genomic effect in the regulation of the activities of other antioxidant enzymes such as inducible (iNOS) and mitochondrial (mtNOS) isoforms of nitric oxide synthase [102]. MLT also inhibits neuronal nitric oxide synthase (nNOS) activity because of its binding to the calcium-calmodulin complex [103].

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The pineal production of MLT exhibits an unambiguous circadian rhythm with its peak near the middle of scotophase and basal levels during the photophase. The amount of MLT produced by the pineal gland of mammals changes as animals age. The tendency is that pineal MLT production wanes with advanced age. In humans, MLT production not only decreases in the aged but also is significantly lower in many age-related diseases as Alzheimer's, Parkinson's and Huntington's disease [104,105] and cardiovascular disease [106,107].

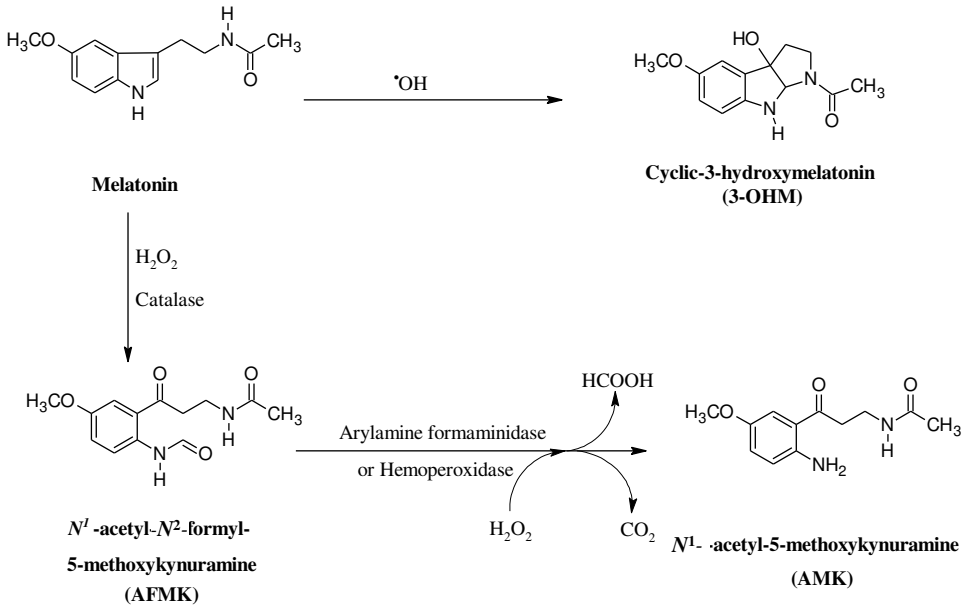

Figure 3. Melatonin oxidation.

4. Mitochondria and melatonin

Mitochondria are organelles found almost ubiquitously in eukaryotes, that play a central role in the cell physiology; in fact, besides their classic function of energy metabolism, these organelles perform many other functions including the distribution of energy through the cells, energy/heat modulation, ROS regulation, calcium homeostasis, and apoptosis control. In mitochondria important metabolic pathways take place including fatty acids β-oxidation, pyruvate oxidation, Krebs cycle, lipids and cholesterol biosynthesis. Many of these processes are functions required for the wellbeing of the cells and of the human beings. The inner mitochondrial membrane is rich in proteins, half of which are involved in oxidation-reduction reactions with transport of electrons and in oxidative phosphorylation (OXPHOS). The oxidative phosphorylation, coupled to electron transport chain (ETC), allows the synthesis of

adenosine triphosphate (ATP), a molecule rich in energy, via the enzyme complex ATP synthase.

Human mitochondria contain their own genome (mitochondrial DNA, mtDNA), a circular double stranded-molecule. The human mitochondrial chromosome contains 37 genes (16,569 base pairs), including 13 that encode subunits of respiratory chain/oxidative phosphorylation proteins; the remaining genes code for rRNA and tRNA molecules necessary to the protein-synthesizing complex of mitochondria. About 99% of the mitochondrial proteins are encoded by nuclear DNA (nDNA); so these proteins have to be imported into mitochondria. Mitochondrial proteins synthesized in the cytosol possess mitochondrial targeting signals that direct them to the appropriate compartment (outer or inner membranes, intermembranes space and matrix) within the organelle. Transport across outer and inner membranes needs a complex machinery including the presence of ATP, docking proteins, chaperonins and proteases, and it involves unfolding and refolding of the proteins to be translocated.

NADH produced in the cytosol by glycolysis and in the mitochondria by oxidation of pyruvate, fatty acids β-oxidation, and Krebs cycle, are oxidized by respiratory chain transferring electrons to O_2, that is converted to water. The primary function of mitochondria is to generate ATP (from ADP and phosphate by adenin nucleotide and phosphate translocators and FoF1 ATP synthase) through the ETC resulting in OXPHOS. The ETC, located in the inner mitochondrial membrane, comprises a series of electron carriers grouped into four enzyme complexes: complex I or NADH ubiquinone reductase, complex II or succinate ubiquinone reductase, complex III or ubiquinol cytochrome c reductase, and complex IV or cytochrome c oxidase. The end product of the respiratory chain is water generated after reduction of O_2 by mitochondrial complex IV; this process needs the addition of four electrons to each oxygen molecule. However, about 5-10% of the oxygen is involved in production of hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{\bullet-}$), and the extremely reactive hydroxyl radical ($^{\bullet}OH$) [108]. These three molecules are ROS and represent endogenous oxidotoxins. The mitochondria for action of the enzyme nitric oxide synthase (mtNOS) can also produce nitric oxide ($NO^{\bullet}$) from L-arginine [109], which can be converted into various reactive nitrogen species (RNS), such as nitrosonium cation (NO^+), nitroxyl anion (NO^-) and peroxynitrite ($ONOO^-$) [110]. These free radicals are detoxified or their peroxidation products are decomposed by the natural antioxidant defense system as SOD, glutathione redox cycle, catalase and coenzyme Q. Mitochondria not only generate ROS/RNS, but are also the main target of their actions [111]. Small fluctuations in the steady-state concentration of ROS/RNS may play a role in intracellular signaling [112]. Several mechanisms take part in the control of ROS/RNS production. Among these the enzyme SOD, localized in the inner side of the inner mitochondrial membrane, remove $O_2^{\bullet-}$ [113]. When formed, $O_2^{\bullet-}$ is immediately dismutated to H_2O_2 by cytosolic or mitochondrial superoxide dismutase. As H_2O_2 is the precursor of the highly damaging $^{\bullet}OH$, it is imperative that H_2O_2 is removed very quickly.

The enzyme GPx metabolizes H_2O_2 to water and O_2; GPx in this reaction also converts reduced GSH to its oxidized form (GSSG). In turn, GSSG is reduced to GSH by the action of the enzyme glutathione reductase (GRd) in the presence of NADPH [114,115]. These enzymes form part of the endogenous antioxidant defense system suppressing ROS/RNS levels both in the cells

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and in the mitochondria. Under normal conditions, MLT reduces mitochondrial hydroperoxide levels and stimulates the activity of GPx and GRd, enzymes involved in the GSH-GSSG balance [116]. The indoleamine MLT is also able to neutralize the oxidative stress induced by high doses of *t*-butyl hydroperoxide, restoring GSH levels and GPx and GRd activities. However, vitamins C and E have no such effect under the same conditions [116].

Other antioxidants such as ascorbate, ubiquinone and α-tocopherol can participate in the mitochondrial antioxidative defense system, but without to be able to convert $O_2^{\bullet -}$ to O_2. However, uncontrolled increase in these metabolites leads to a series of reactions which target proteins, lipids and DNA resulting in cell death by necrosis or apoptosis. In recent years, several findings support the antioxidant effect of MLT in mitochondrial homeostasis [99,117,118].

Apoptosis and necrosis are two types of cell death occurring in neurodegeneration. Apoptosis (programmed cell death) occurs naturally under normal physiological conditions; on the contrary, necrosis is caused by external factors such as toxins, infections and trauma. Apoptosis is characterized by cell shrinkage, cytoplasm contraction, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation, plasma membrane bleb formation and apoptotic body formation [119]. Many of these changes are activated by a family of caspases, i.e. proteases that in their active site possess a cysteine and cleaves the substrates after aspartate residues. Apoptotic cells are rapidly sequestered by phagocytosis before they can lyse and cause an inflammatory process [120]. Necrosis does not involve any DNA or protein degradation and is accompanied by swelling of the cytoplasm and of the mitochondria with membrane ruptures. Both apoptosis and necrosis involve a change in mitochondrial membrane permeabilization (MMP) [121].

MMP causes the opening of a nonspecific pore in the mitochondrial membranes, known as the mitochondrial transition pore (MTP), that allows the passage of any molecules of >1500 Da across this membrane. This pore can be rapidly closed by chelation of calcium ion. Because MTP allows also rapid passage of protons (H^+), its opening causes depolarization of mitochondria and uncoupling of oxidative phosphorylation without synthesis of ATP. If the MTP remains open, ATP levels can be totally depleted; on the contrary, transient opening of the MTP can be involved in the mitochondrial-mediated apoptosis through the proteins released from mitochondria. Among these apoptogenic proteins we know cytochrome c [122], the serine protease HtrA2/Omi [123], and endonuclease G [124].

Permeabilization events, which occur at points where outer and inner mitochondrial membranes are in contact, involve association of several proteins from different districts of the cell and the mitochondria [125]: cytosol (hexokinase), outer mitochondrial membrane (peripheral benzodiazepine receptor and voltage dependent anion channel or VDAC), mitochondrial inner membrane space (creatine kinase), inner mitochondrial membrane (adenine nucleotide translocator or ANT) and mitochondrial matrix (cyclophilin D).

Two main considerations suggest a role for MLT in mitochondrial homeostasis. As it is known, mitochondria produce high amounts of ROS and RNS. Besides, mitochondria depend on the GSH uptake from the cytosol, even if they have GPx and GRd to maintain redox cycling. Thus,

the anti-oxidant effect of melatonin and its ability to increase the levels of GSH may be of great importance for mitochondrial physiology [126]. The fact that the inhibition of CN^- on complex IV of the mitochondrial ETC is removed by MLT, also supports its intramitochondrial role [127]. A protective effect of MLT against MPP^+-induced inhibition of complex I of ETC has been also shown [128].

The effects of MLT on mitochondrial ETC have been also studied on submitochondrial particles from rat liver and brain mitochondria [129]. MLT at 1 nM concentration significantly increased the activity of the complexes I and IV of ETC in rat liver submitochondrial particles, whereas 10-100 nM MLT stimulated the activity of the same complexes but in brain submitochondrial particles. The indoleamine counteracted CN^--induced inhibition of complex IV, restoring the levels of Cyt aa3. This effect was of physiological significance, since the MLT increased the ETC and OXPHOS activities with a consequent increase of ATP synthesis [129]. In addition, due the high redox potential of MLT (-0.98 V), this molecule can donate directly electrons to complex I of the ETC [130].

The effect of MLT (10 mg/kg) on ETC complexes from rat liver and brain mitochondria has been also studied in vivo. Martin et al. [116] have found that MLT increases the activity of the respiratory chain complexes I and IV and ATP synthesis in a time-dependent manner after mitochondrial damage induced by ruthenium red [116].

Recently, the role of MLT on cardiolipin and mitochondrial biogenesis was studied [131]. Cardiolipin, a phospholipid located in inner mitochondrial membrane, is required for several mitochondrial bioenergetic processes as well as for the activity of transport proteins. Alterations in cardiolipin structure and acyl chain composition have been associated with mitochondrial dysfunction under a variety of pathological dysfunctions. The authors [131] reported that MLT protects the mitochondrial membranes from oxidation-reduction damage by preventing cardiolipin oxidation.

5. Melatonin and Parkinson's

In the last decade, many research findings provide scientific evidence for the protective role of MLT in a number of oxidative stress related diseases, especially Alzheimer's [132] and Parkinson's diseases [133], being the protective actions of the indoleamine attributable to its direct and indirect antioxidative properties. The first evidence of a significant relationship between Parkinson's disease and MLT derived from the evidence of a reduction in the concentration of circulating MLT in PD patients as a consequence of a decreased activity of the pineal gland [134]. After its antioxidant properties were uncovered, melatonin has been successfully tested in several *in vivo* and *in vitro* PD models.

MLT was found to inhibit *in vitro* the prooxidant effects of dopamine and L-dopa [135] and to be more effective than the vitamin E analog, trolox, in preventing dopamine autooxidation [136]. Melatonin was also reported to prevent in the MPTP model the rise in lipid peroxidation products in the substantia nigra (SN) of MPP^+-treated rats and, additionally, to preserve tyrosine hydroxylase (TH) activity, which is normally decreased after toxin treatment [69].

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When the 6-OHDA model was used instead of MPTP ones to induce dopaminergic degeneration, MLT administration restored the motor deficits elicited by apomorphine co-treatment with 6-OHDA [137] and also completely prevented the rise in neural lipid peroxidation products and partially rescued striatal dopaminergic levels after lesioning with 6-OHDA [138]. The protective action of MLT against dopaminergic neuronal degeneration was also expressed by reduction of the DNA fragmentation induced by MTPT [139] and of mitochondrial complex I deficiency observed after 6-OHDA administration [140]. MLT also counters MPTP-induced c-Jun-N-terminal kinase and caspase-dependent signaling leading to the dopaminergic neurodegeneration [141]. It has been reported that MLT partially preserves the GSH concentrations in SN of MPTP-treated rats [142,143]. The antioxidant activity of MLT was supposed to be the major mechanism underlying MLT's protection in these PD models. The protective function of MLT also include its antiapoptotic effects. MLT has been reported to rescue dopamine neurons from spontaneous cell death in low-density seeding culture [144].

PD epidemiological studies have suggested an association with the environmental toxin rotenone, a mitochondrial complex I inhibitor. In recent years, *Drosophila melanogaster* has been used as a model for several neurodegenerative diseases, including PD. Coulom and Birman studied for several days the neurodegenerative effects of a chronic exposure to rotenone in *Drosophila melanogaster*. After several days of treatment, flies presented characteristic locomotor impairments that increased with the dose of herbicide. Immunocytochemistry analysis demonstrated a dramatic and selective loss of dopaminergic neurons in the brain of all treated flies. The addition of L-dopa into the feeding medium rescued the behavioral deficits but not neuronal death, as is the case in human PD patients. On the contrary, the antioxidant MLT alleviated both symptomatic impairment and neuronal loss, supporting the idea that this agent may be beneficial in the treatment of parkinsonism [145].

MLT has been shown to protects PC12 cells from both apoptosis and necrosis induced by high doses of 6-OHDA [146,147]. Since 6-OHDA induced cellular toxicity is mediated by increased free-radical generation, the antioxidant properties of MLT presumably account for its ability to suppress both necrosis and apoptosis. Numerous data suggest a role for MLT in mitochondrial homeostasis [148]. It has been reported that MLT increases the activities of respiratory complexes I and IV in a time-dependent manner after *in vivo* administration to rats [129] and maintains GSH homeostasis in the mitochondrial matrix under increased oxidative stress; these actions are not shared by either vitamin C or vitamin E [117]. Mitochondria in the cell are the major source of ROS, owing to the leakage of electrons through the electron transport chain. Due the critical role of mitochondria in programmed cell death and PD, it is conceivable that actions at the mitochondrial level mediate at least some of MLT apoptotic effects. It has been reported that MLT induces ATP production, increasing the activity of the mitochondrial oxidative phosphorylation (OXPHOS) enzymes [129]. The indoleamine also protects mitochondrial DNA, which is particularly vulnerable to oxidative damage, thus indirectly helping to preserve mitochondrial metabolism.

Since mitochondria play a critical role in the pathogenesis of PD, it is conceivably that actions at mitochondria level mediate some of MLT antiapoptotic effects. The beneficial actions of MLT in PD has been widely investigated not only on the basis of its neuroprotective efficacy

assessment but also because of the down regulation of MLT receptors in the nigrostriatal region of PD brain [149]. There is growing evidence of sleep–wake boundary dysfunction in PD. REM sleep behavior disorder (RBD) which is characterized by loss of normal skeletal muscle tone with prominent motor activity and dreaming, has been associated with PD and/or other forms of dementia, with a tendency for RBD to precede the onset of parkinsonism. There is some clinical evidence that MLT can be a useful add-on therapy for RBD in PD [150].

6. Conclusions

PD is a highly debilitating condition that concerns thousands of family in the world and annually cost millions of euro for treatment. This disease has occasionally a genetic basis, but the signs of PD develop after free-radical damage to the substantia nigra pars compacta. Moreover, neuroinflammation and mitochondrial dysfunction participate in the ethiology of this neurodegenerative disorder and contribute to the increase of oxidative damage to the dopaminergic neurons.

The mitochondria in cells play a myriad of different and important functions, so any alteration in these organelles could have a considerable impact on the functionality of the cells and also the entire body. Mitochondria are also the site of generation of reactive oxygen and nitrogen species (ROS/RNS) and the subsequent widespread deleterious effects (oxidation and/or nitrosylation of mtDNA, oxidation of phospholipids and proteins) of these intermediates. These effects lead also to the opening of the mitochondrial transition pore, release of Cyt c and the activation of the events that culminate in apoptosis.

Abnormal mitochondrial functions (decreased respiratory complexes activities, increased electron leakage, opening of the mitochondrial transition pore) have all been shown to play a role in the pathophysiology of neurodegenerative disorders such as PD, AD and HD. Mitochondrial involvement in PD is revealed by deficiency of mitochondrial complexes I and IV, decreased ATP production with a parallel reduction in GSH levels.

Among the substances involved in maintaining mitochondrial biogenetics, a number of *in vivo* and *in vitro* studies indicate that MLT may emerge as a major therapeutic candidate to preserve bioenergetic function of mitochondria.

MLT is a molecule present in all creatures from prokaryotes to human beings. It is an antioxidant that protected organisms from oxidative stresses and apoptosis and mediates seasonal physiological functions, is a signal of dark/light promoting also sleep, modulates the immune system, and inhibits the growth of several cancer. Indoleamine is an antioxidant that directly scavenges ROS/RNS produced during the normal metabolism of mitochondria and it indirectly promotes the activity of the antioxidant enzymes including SOD, catalase, GPx and GRd.

It has also been documented that the ability of MLT to quell the oxidation-reduction processes, with the formation of free radicals, is due to its conversion to metabolites, such as cyclic 3-OHM, AFMK and AMK. Considering the cascade of reactions that include AFMK and AMK, a MLT can scavenge about ten ROS/RNS. MLT increases the activity of ETC and the ATP

synthesis, reducing at the same time the oxygen consumption; then, it avoids an excess of ROS/RNS, preventing PTP opening and apoptosis.

Considering that this hormone is an endogenous, nontoxic, antioxidant molecule without known side-effects, it should be considered as a useful agent in PD patients as a treatment with other conventional therapies. Although MLT is an important molecule and possibly has a great future in PD research, it should be extensively tested across multiple populations for efficacy and real effects along with the side effects at the efficacious doses. Future therapeutic strategies could be directed at identifying and developing MLT analogues as drugs with more powerful inhibitory effects on the mitochondrial cell death pathway, slowing the progression of neurodegenerative diseases.

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