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Role of melatonin supplementation in neurodegenerative disorders

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1. ABSTRACT

Neurodegenerative diseases are chronic and progressive disorders characterized by selective destruction of neurons in motor, sensory and cognitive systems. Despite their different origin, free radicals accumulation and consequent tissue damage are importantly concerned for the majority of them. In recent years, research on melatonin revealed a potent activity of this hormone against oxidative and nitrosative stress-induced damage within the nervous system. Indeed, melatonin turned out to be more effective than other naturally occurring antioxidants, suggesting its beneficial effects in a number of diseases where oxygen radical-mediated tissue damage is involved. With specific reference to the brain, the considerable amount of evidence accumulated from studies on various neurodegeneration models and recent clinical reports support the use of melatonin for the preventive treatment of major neurodegenerative disorders. This review summarizes the literature on the protective effects of melatonin on Alzheimer disease, Parkinson disease, Huntington's disease and Amyotrophic Lateral Sclerosis. Additional studies are required to test the clinical efficacy of melatonin supplementation in such disorders, and to identify the specific therapeutic concentrations needed.

2. INTRODUCTION

The oxidative stress is a shift towards the pro-oxidant in the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increase in energy metabolism by aerobic pathways augments the intracellular concentration of free oxygen radicals, which in turn enhance the rate of the autocatalytic process of lipid peroxidation, inducing damage to structures, inhibition of cellular respiration, DNA alteration (i.e. base-pair mutations, deletions, insertions, sequence amplification) and proteins modification. The high content of lipids of nervous tissue, coupled with its high aerobic metabolic activity, makes it particularly susceptible to oxidative damage.

The great production of mitochondrial-derived superoxide anions is normally balanced by an efficient antioxidant system composed of free radical scavengers, metal chelators, metabolic enzymes and mitochondrial respiration itself that neutralize free radicals and their negative effects (1, 2). However, there are several para-physiological and pathological conditions, including aging (3), cancer or acute and chronic inflammation (4-6), where the oxidant/antioxidant homeostasis is impaired because of an excess of oxidants and/or a depletion of antioxidants,

leading to cytotoxic effects which play a role in their pathophysiology. Depending on its extent, oxidative stress may be either cause of minimal cellular damage, or provoke a serious injury such as apoptosis and necrosis (7).

Defense against all of these processes is dependent upon the capability of various antioxidants that are derived either directly or indirectly from the diet (8). However, since reactive oxygen species (ROS) also have useful role in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level. Unlike other antioxidants, melatonin does not undergo redox cycling. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (9).

Melatonin is mainly produced in the mammalian pineal gland from the neurotransmitter serotonin during the dark phase. Melatonin secretion from the pineal gland has been classically associated with circadian and circanual rhythm regulation, and with adjustments of physiology of animals to seasonal environmental changes (10). Melatonin production, however, is not confined exclusively to the pineal gland, but other organs and tissues including retina, Harderian glands, gut, ovary, testes, bone marrow and lens also produce it (11). Melatonin's activities transcend those of a hormonal modulator, since it influences the functions of tissues and cells not generally considered in the endocrine category (12). Various studies suggest a role for melatonin and its metabolites in the antioxidative defense in all organisms (13-16). Melatonin up regulates antioxidative defensive systems, including the activities of superoxide dismutase and glutathione peroxidase as well as the levels of glutathione (17).

Exogenous administration of melatonin can also entrain the circadian clock by a direct action on the central nervous system (CNS) and, thus, it represents a potential treatment for disoriented circadian clock in cases such as jet-lag, and in individuals with delayed or advanced sleep phase syndromes and sleep inefficiency (18).

The ability of melatonin to maintain cell integrity and its remarkably low toxicity has prompted to investigate its potential application in future therapeutic strategies for the treatment of ROS-derived diseases. This review provides a comprehensive discussion of the neuroprotective effects of melatonin and its potential clinical implications in those neurodegenerative diseases where free radicals-mediated insult is involved.

3. MELATONIN

Melatonin is ubiquitously found in the body: because of the amphiphilicity of its chemical structure, once synthesized (or after exogenous administration), it readily passes across all morphophysiological barriers (such as the blood-brain barrier) and diffuses to all cells compartment or body fluid. The majority of endogenous melatonin is directly released from the pineal gland to the cerebrospinal fluid (CSF) of the brain's third ventricle; from this location, melatonin readily diffuses into the surrounding neural tissue (19). A smaller fraction (up to 20 folds lower) is released into the capillary blood where it is distributed to all tissues (20). Besides the pineal gland, many other organs and tissues are responsible for its production, including retina, gastrointestinal tract, gonads, bone marrow and lens, thus suggesting its involvement in a number of yet undefined activities at a cellular and tissue levels which go beyond its classic functions as an hormone (12, 21).

A crucial role for melatonin and its metabolites as potent free radical scavengers and antioxidants has been confirmed not only *in vitro* but also in several *in vivo* studies over the years (22). Animals treated with different toxics known to promote free radicals production (including paraquat, LPS or safrole) showed a significant reduction in the oxidative damage when concomitantly treated with melatonin (23-25).

The presence in melatonin structure of an electron-rich aromatic indole ring which functions as an electron-donor may explain its ability to reduce and neutralize electrophilic radicals, thus protecting intracellular proteins, DNA and lipids from the oxidative damage (26). Indeed, experimental studies have shown that, compared to classical antioxidants, melatonin is significantly more efficient; in fact, because of its amphiphilic feature, differently from other free radical scavengers that are either hydrophilic or lipophilic, melatonin can limit oxidative damage in both the lipid and aqueous phases of cells (27). Nonetheless, its antioxidant activity is not limited to free radicals scavenging, but also involves other indirect mechanisms, including the stimulation of several antioxidative defensive enzymes (e.g. superoxide dismutase, glutathione reductase and glutathione peroxidase) (17, 28-30) and the down-regulation of pro-oxidant enzymes, in particular 5- and 12-lipo-oxygenase and nitric oxide (NO) synthases (31, 32). Additionally, recent studies showed that melatonin effectively binds and inactivate endogenous iron, thus suppressing the Fenton reaction and the consequent overproduction of ROS (33).

Melatonin is metabolized by cytochrome P-450 mono-oxygenases liver isoenzymes through its hydroxylation at the 6-carbon position to yield 6-hydroxymelatonin (34). This reaction is followed by conjugation with sulphate, to produce the principal urinary metabolite, 6- sulphatoxymelatonin or, to a lesser extent, with glucuronic acid. In the last step, conjugated melatonin and little quantities of unmetabolized melatonin are excreted through the kidney. In addition to hepatic metabolism, oxidative pyrrole-ring cleavage appears to be the major metabolic pathway in other tissues, contributing to about one-third of the total catabolism, but the percentage may be even higher in certain tissues, including CNS (16). The primary cleavage product is the kynuric derivative N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), which is then converted to N^1-acetyl-5-methoxykynuramine (AMK) (35). Both AFMK and AMK

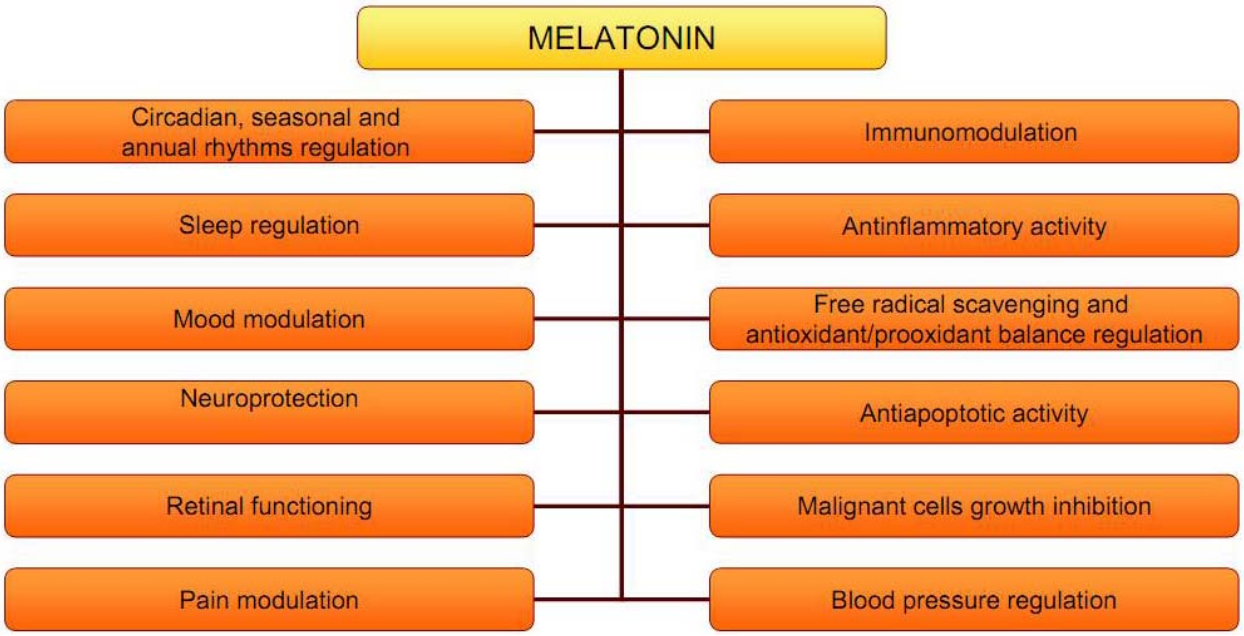

Figure 1. Principal effects of melatonin

are likewise free radical scavengers (36), which easily cross the blood-brain barrier and form metabolites by interactions with reactive oxygen and nitrogen species (37). It was recently reported that AMK is a better antioxidant than its precursor AFMK (38), and a more efficient NO scavenger than melatonin (39). AMK also exhibited anti-inflammatory properties, due to its capacity to inhibit and down regulate cyclooxygenase 2 (COX-2), thus limiting PGE2 production (40).

Melatonin has also shown to reverse chronic and acute inflammatory processes, probably due to a direct interaction with specific binding sites located in lymphocytes and macrophages (41-46). Experimental and clinical data have shown that melatonin reduces adhesion molecules and pro-inflammatory cytokines including IL-6, IL-8, and tumor necrosis factor (TNF)-alpha and modifies serum inflammatory parameters. As a consequence, melatonin may improve the clinical course of illnesses which have an inflammatory etiology (46).

4. MECHANISMS OF ACTION OF MELATONIN

Besides its antioxidant activity, melatonin exhibited many other properties, some of them potentially applicable for therapeutic purposes, including sedative, anxiolytic, antidepressant, anticonvulsant, and analgesic effects (Figure 1) (47). All these activities involve both receptor-mediated and receptor-independent processes: the formers normally occurs at physiological concentrations, whereas the latters usually require higher supraphysiological/pharmacological melatonin concentrations (48). Receptor-independent effects of melatonin are associated to its direct binding to calmodulin, with consequent inhibition of Ca2+/calmodulin-dependent kinase II (49) and Ca2+-dependent membrane translocation of protein kinase C (50).

Melatonin receptors include both G-protein coupled membrane and nuclear receptors (RZR/ROR). The two G-protein-coupled metabotropic melatonin receptors have been cloned and classified based upon their kinetic properties and pharmacological profiles into MT1 and MT2 subtypes (51). They belong to the seven transmembrane receptor family, and the binding to these receptors results in the inhibition of adenyl cyclase. MT1 and MT2, which are the primary mediators of the physiological actions of melatonin, are expressed in various tissues, including central and peripheral nervous system, thus further confirming melatonin capacity to pass the blood-brain barrier (52).

Melatonin is also a ligand for retinoid orphan nuclear hormone receptors, referred to as RZR-alpha and RZR-beta, although showing lower sensitivity (53). Both receptors are present in the central and peripheral nervous system and have been associated with cell differentiation and immune response regulation (54). Furthermore, Steinhilber *et al.* reported that melatonin-induced RZR-alpha receptors activation can down-regulate the expression of 5-lipooxygenase, an important inflammatory mediator, in B lymphocytes, thus modulating the inflammatory response (55).

A third putative mammalian melatonin receptor (MT3), the cytosolic quinine oxydoreductase 2 (QR2) enzyme, has been recently proposed (56). Although the physiological role of this enzyme is not known yet, its inhibition induced by melatonin seems to be related to melatonin indirect anti-oxidant properties, and over-

expression of this enzyme may have deleterious effects (57).

5. ROLE OF MELATONIN IN NEURODEGENERATIVE DISEASES

Despite the etiology of brain degenerative diseases is multifactorial and still partially undefined, oxidative stress is thought to play a crucial role in eliciting most of neurological and age-related disorders. In recent decades, many research groups have focused their attention on the potential role of melatonin in neuroprotection (58-60), basing on the observation that several central as well as peripheral nervous system neurodegenerative diseases (including Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis) share the reduced capacity to maintain the balance between free radical formation and antioxidative mechanisms as a common critical factor (30).

Indeed, the whole nervous system is particularly prone to oxidative and nitrosative stress damage. This susceptibility depends on the inherent biochemical and physiological characteristics of the brain: high metabolic activity utilizing a disproportionately large amount (20%) of the total oxygen inhaled (61), small quantity of endogenous scavengers, wide axonal and dendritic networks, high content of polyunsaturated fatty acids representing valid substrates for the formation of ROS. Also, the presence of high concentrations of metals like iron can contribute to free radical damage by catalyzing the formation of reactive hydroxyl radicals, inducing secondary initiation of lipid peroxidation and by promoting the oxidation of proteins (62, 63). Abnormally high levels of iron have been demonstrated in a number of neurodegenerative disorders including Alzheimer's disease and those characterized by nigral degeneration such as Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy (64).

Recent studies suggested that melatonin neuroprotective activity may be related to its ability to accumulate at the mitochondrial level due to its lipophilicity (65), and to protect brain mitochondrial membranes from free radical attack, thus maintaining mitochondrial homeostasis and inhibiting mitochondrial cell death pathways (66). In particular, melatonin acts not only by lowering electron leakage, but also inhibiting the opening of the mitochondrial permeability transition pore (mtPTP), thus maintaining the mitochondrial respiratory electron flux (67). This aspect is particularly important since the efficiency of mitochondrial electron transport system decreases with age; thus, a correlation between the decline in brain mitochondrial activity and the etiology of neurodegenerative disease has been suggested by several authors.

Astroglial cells dysfunction seems to be particularly involved in neurodegenerative damage; in physiological conditions, astrocytes participate to several brain functions (like neuronal development, synaptic activity and cellular repairing after brain injuries) and contribute to neuroprotection through inactivation of ROS (68). Nevertheless, "activated" astrocytes may induce the inducible nitric oxide synthases (iNOS), leading to an excessive formation of NO (and its toxic metabolites), which inhibits the mitochondrial neuronal respiration and causes cellular energy deficiency and, eventually, neuronal death (69). Glia activation seems to originate by a chronic inflammatory state causing an immune response, with cytokines production (interferons and interleukins) and macrophages, lymphocytes, and other immune system cells involvement. The consequent, sustained release of large amounts of NO has been related to several neurological diseases with inflammatory component, including HIV-1-associated dementia, Alzheimer's disease, multiple sclerosis and stroke (70, 71).

Besides its traditional role as an antioxidant and free radical scavenger, recent works show that melatonin can also modulate astrocyte reactivity or death through an upregulation of anti-oxidative astrocytic defenses: melatonin protection against NO-induced impairment is also associated with decreased upregulation of oxidative stress-responsive genes, such as Hsp70, whose expression is a valid indicator of astroglial stress (72).

Melatonin also prevents specifically the activation of the pro-inflammatory enzymes COX-2 and iNOS in glioma cells, thus indicating an anti-inflammatory action. Importantly, melatonin did not alter COX-1 protein level, one of the major disadvantages of non-specific non-steroidal ant-inflammatory drugs (NSAIDs). The mechanism through melatonin limits the nitrite/nitrate production and reduces iNOS expression in glioma cells involves NF-κB pathway (73). A large body of evidence confirms that melatonin and its metabolites show broad spectrum of antioxidant activities, together with the absence of any harmful side-effects even at high doses (74) and with the ability to be taken up rapidly by neural structures, supporting the clinical use of melatonin in defense of morphological and functional integrity of the CNS.

The efficacy of melatonin and its metabolites in either reducing the severity or delaying the onset of neurodegenerative disorders has been estimated in various conditions such as Alzheimer's or Parkinson's disease (73, 75-78), amyotrophic lateral sclerosis (48, 79), and neural trauma (80, 81).

Moreover, data from clinical tests showed that even at the higher concentrations (dosages up to 1g melatonin daily for 30 days), melatonin is safe and well-tolerated by humans (82, 83), which could encourage its long-term administration for therapeutic uses.

5.1. Melatonin in Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by a progressive loss of neurons, especially cholinergic neurons of the basal forebrain. Clinical manifestations of the disease consist in an irreversible memory impairment, cognitive dysfunction, and behavioral changes. AD can occur in any decade of

adulthood, but it is the most common cause of dementia in people older than 70 (84). As a consequence, given the increasing longevity of population in developed countries (and in absence of effective treatments), the incidence of AD is expected to dramatically grow in the future.

Although its etiology is largely unknown, there is increasing evidence suggesting that neuroinflammation, immune activation, nitrosative and oxidative stress play a critical role in the pathogenesis of AD (85). The postmortem histopathological analysis confirmed an elevated lipid peroxidation in the brains of AD patients (86, 87), also showing effects of the disease on DNA oxidation and protein reorganization in the brain cortex, with typical abnormalities in cytoskeletal architecture (88, 89).

The inflammatory component in AD significantly contributes to cell stress by promoting microglial activation with the resultant generation of inflammatory cytokines and neurotoxic free radicals (90); however, inflammatory manifestations in AD typically lack in some features such as neutrophil infiltration and edema, whereas other characteristics including acute-phase proteins and cytokines accumulation can be identified.

The neurotoxic effect has been ascribed to the intracellular formation of i) neurofibrillary tangles (NFT), that are histopathological lesions consisting of hyperphosphorylated microtubule-associated protein tau at the cytoskeletal level, and ii) senile plaques, derived from the extracellular accumulation of soluble beta-amyloid peptides (A-beta) in arterial walls of cerebral blood vessels (91).

Tau protein promotes microtubules assembly and stabilization; it also takes part in the formation and maintenance of the axonal structure (92). Hyperphosphorylated tau protein reduces the ability to stabilize microtubules, leading to disruption of the cytoskeletal arrangement and neuronal transport (93). In AD, the cytoskeleton is abnormally assembled into NFT, and impairment of neurotransmission occurs. It is widely accepted that hyperphosphorylation of the tau protein is due to an imbalance between the activities of protein kinases and protein phosphatases, suggesting that these proteins could serve as therapeutic targets for AD.

Amyloid-beta1-42 peptide (A-beta1-42), which is a fragment derived from proteinases-induced cleavage of amyloid precursor protein (APP), is believed to play a major role in promoting neuronal degeneration. Actually, although the mechanism underlying A-beta neurotoxicity remains to be fully elucidated, there is increasing evidence that A-beta induces mitochondrial dysfunction, triggers apoptosis, and increases the intracellular levels of calcium and ROS in the AD brain, thus leading to a series of events which destroy adjacent neurons (94, 95) .

The involvement of ROS in neuronal death associated to AD led to investigate the effects of antioxidants in preventing or delaying the sequence of events causing neuronal destruction. Among these, melatonin has been deeply evaluated, due not only to its potent activity as free radical scavenger, but also to the finding that lower levels of endogenous melatonin were observed both in serum and in CSF from AD patients compared with that in age-matched control subjects (96). Moreover, it has been observed that the concentration of melatonin in CSF decreases with the progression of AD neuropathology (97), and that melatonin levels both in CSF and in postmortem human pineal gland are already reduced in AD subjects manifesting only the earliest signs of AD neuropathology, in absence of any sign of cognitive impairment (98-100). As a consequence, the determination of CSF concentration of melatonin has been proposed as an early marker for the detection of AD (101).

The antioxidative protection against A-beta of melatonin has been confirmed by Pappolla *et al.*, which showed that co-incubation of both murine neuroblastoma (N2a) and pheochromocytoma (PC12) cells with A-beta-peptides and melatonin greatly reduced the degree of A-beta-induced lipid peroxidation, thus greatly increasing the survival of the cells (102). *In vitro* studies demonstrated that melatonin could efficiently protect from alterations in neurofilaments hyperphosphorylation and accumulation induced by calyculin A (CA), through not only its antioxidant effect but also its direct regulatory effect on the activities of protein kinases and protein phosphatases (103). A recent *in vivo* study by Yang *et al.* (77) confirmed that administration of melatonin intraperitoneally for 9 consecutive days before injection of calyculin A in seventy-eight male Sprague–Dawley rats could prevent calyculin A-induced synaptophysin loss, memory retention deficits, as well as hyperphosphorylation of tau and neurofilaments.

Furthermore, supplementation with melatonin by prior injection and reinforcement during haloperidol administration significantly improved memory retention deficits, arrested tau hyperphosphorylation and oxidative stress, and restored Protein phosphatase 2A activity (104).

Melatonin decreased not only oxidative stress and tau hyperphosphorylation, but also reversed Glycogen synthase kinase 3 (GSK-3) activation, thereby showing that melatonin's actions exceeded its antioxidant effects, and also interfered with the phosphorylation system, especially stress kinases (103). Tyrosine kinase (trk) receptors, representing an additional important elements of the phosphorylation system and neurotrophins, were also shown to be affected by oxidotoxins, including A-beta. In neuroblastoma cells, melatonin was capable of normalizing trk and neurotrophin expression (105).

With regard to the anti-inflammatory effects of melatonin, the most important feature is its inhibition of mitochondrial iNOS expression (106, 107). Antioxidant and anti-inflammatory properties of melatonin are relevant in mitochondrial physiology, and they may play a neuroprotective role in neurodegenerative disorders (108). Melatonin supplementation in patients with Alzheimer disease significantly slows down the progression of cognitive impairment and decreases brain atrophy (109). In a study of 14 patients at various stages of AD, melatonin supplementation for 22–35 months improved sleep and

significantly reduced the incidence of "sundowning." Furthermore, patients experienced no cognitive or behavioral deterioration during the study period (110).

Melatonin has shown to reduce the generation of A-beta-peptide (111, 112) and also thereby secondarily reduce neuronal death more efficiently than other antioxidants (113). This effect is secondary to the inhibition of the proteolytic processing of soluble derivatives of amyloid precursor protein (sAPP), as shown by *in vitro* experiments (114, 115).

Melatonin displays its antifibrillogenic activity even in the presence of the profibrillogenic apolipoprotein E4 (apoE4), and antagonizes the neurotoxic, synergistic potentiation between A-beta protein and and apoE4 or apoE3 (116). ApoE4, which aggravates A-beta effects, is also produced by astrocytes.

The anti-amyloidogenic properties of melatonin in AD have been also observed in transgenic mice: melatonin not only inhibited amyloid plaque deposition, but also improved learning and memory deficits in an APP695 transgenic mouse model of AD (117). However, the antifibrillogenic effect was not observed when the treatment was started in old transgenic mice (118), confirming that, after numerous amyloid plaques have been formed and neuronal damage has progressed, melatonin is no longer capable of efficiently antagonizing amyloid deposition and amyloid-dependent damage.

Counteraction by melatonin against A-beta-induced apoptosis has been repeatedly demonstrated in a number of cellular models of AD including mouse microglial BV2 cells, rat astroglioma C6 cells, and PC12 cells (119-121). Studies in transgenic AD mice and cultured cells have suggested that administration of melatonin prevented the A-beta-induced up-regulation of apoptosis-related factors such as Bax, and suppressed caspase-3 activity (117, 119, 122, 123). Experiments in mouse microglial BV2 cells *in vitro* showed that melatonin also decreased caspase-3 activity, inhibited NF-κB activation, and reduced the generation of A-beta-induced intracellular ROS (124). In addition, *in vivo* observations showed that melatonin-treated animals had diminished expression of NF-κB compared to untreated animals (125). Moreover, melatonin inhibited the phosphorylation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase via a PI3K/Akt-dependent signaling pathway in microglia exposed to A-beta1-42 (126). Taken together, the above-mentioned evidences suggest that melatonin may provide an effective means of treatment for AD through its antiapoptotic activities.

Interestingly, the neuroprotective and antiamyloidogenic properties of melatonin are not mediated by MT1 and MT2 melatonin membrane receptors: experimental studies with MT1 and MT2 receptors agonists without antioxidant properties showed no effects on neuroblastoma cells and primary hippocampal neurons (111). Nevertheless, MT1 and MT2 expression in the human brain appears to be altered by pathological conditions such as AD, as observed in postmortem brain from AD patients (127). The role of MT2 receptors in hippocampal synaptic plasticity and in memory processes is also suggested by the fact that transgenic mice deficient in MT2 receptors demonstrated deficient hippocampal long-term potentiation.

Administration of melatonin to AD patients has been found to improve significantly sleep and circadian abnormality and generally to slow down the progression of the disease (128, 129). Jean-Louis *et al.* reported the effects of melatonin administration in two patients with AD (130). Melatonin enhanced and stabilized the circadian rest-activity rhythm in one of the patients along with some reduction of day time sleepiness and mood improvement. The results of this study are not statistically significant because of small sample size. Therefore, the supplementary use of melatonin in AD patients cannot be recommended based on the results of this study.

5.2. Melatonin in aging and Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, occurring most commonly in the elderly. It is believed to affect approximately 1% of the population over 55 years of age (131). PD is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (132). This depletion of neurons presents clinically with severe motor symptoms including uncontrollable resting tremor, bradykinesia, rigidity and postural imbalance. Pathologically, PD is characterized by loss of pigmented neurons and gliosis, most prominently in the substantia nigra pars compacta and locus ceruleus (LC) and by the presence of fibrillar cytoplasmic inclusions, known as Lewy bodies. These Lewy bodies (LB) are concentric eosinophilic cytoplamic intraneuronal inclusions with peripheral halos and dense cores, whose presence is essential for the pathological confirmation of PD.

The exact etiology of PD remains to be fully elucidated, but the key theories propose either an environmental or a genetic (133) origin, or a combination of both. Clinical, epidemiological and experimental studies support the potential role of many different environmental toxicants in the development of PD such as pesticides and herbicides (rotenone, paraquat, heptachlor, dieldrin), metals (manganese, iron, copper), synthetic drug products (MPTP) and plant-related food and natural products (cycads, beta-carboline alkaloids) (134-136).

Research data have clearly indicated that during PD, SN dopaminergic neurons are subject to oxidative and nitrosative stress, and mitochondrial dysfunction, proteasome inhibition and protein aggregation. leading eventually to cell death (137, 138). But the precise relationship among these pathways has not been completely defined. Oxidative stress (reactive oxygen species such as the superoxide radical, hydroxide radical, and semiquinone radicals) has been implicated in the progressive degeneration of dopaminergic neurons, which in turn is one of the principal causes of PD. Moreover, brains from PD patients show evidence of elevated oxidative damage to DNA (139), lipid peroxidation and oxidative modification

of proteins (140), decreased levels of reduced glutathione (GSH) and increased monoamineoxidase (MAO) activity (141), that indicate reduced antioxidant defense mechanisms (137). Dopamine oxidation by MAO leads to the formation of ROS (142) and, if not effectively detoxified by glutathione, hydrogen peroxide might potentially induce the generation of highly reactive hydroxyl radicals in the presence of excess iron via the Fenton reaction.

There are several neurotoxin-based models that have been important in studying the mechanisms of PD pathogenesis. These include the neuronal oxidotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (143), which produced symptoms very similar to PD after injecting in a mice, cats and primates. After administration, MPTP crosses the blood-brain barrier and is metabolized into the 1-methyl-4-phenylpyridinium ion (MPP+), by monoamine oxidase type B. MPP+ is selectively taken up by dopaminergic neurons. MPP+ toxicity is believed to result from the mitochondrial inhibition of complex I leading to oxidative stress (144), depletion of NAD and ATP, and apoptosis (145, 146). Exposure to MPTP results in nigrostriatal dopaminergic degeneration with 50-93% cell loss in the SNpc and more than 99% loss of dopamine in the striatum (147).

In *in vivo* model, rotenone acts as a specific inhibitor of complex I enzyme in the mitochondrial respiratory chain (134), thus inducing the formation of superoxide radicals, with consequent impaired tissue utilization of oxygen, depletion of the cellular energy and acute cell death. Like MPP+, rotenone is able to damage striatal DA terminals after chronic infusion in the jugular vein for 1–5 weeks, thus reproducing many of the PD symptoms including hypokinesia, rigidity and behavioral changes. Currently, the only therapies approved for the treatment of PD are agents that attenuate the symptoms of the disease.

Protection by melatonin was demonstrated in a variety of experimental PD models. MPTP-induced stress was antagonized by melatonin at the levels of mitochondrial radical accumulation, mitochondrial DNA damage as well as breakdown of the proton potential (148). Lewy bodies, which are considered cytopathologic markers of parkinsonism, comprise abnormal arrangements of tubulin and microtubule-associated proteins, MAP1 and MAP2. Melatonin effectively promotes cytoskeletal rearrangements and was assumed to have a potential therapeutic value in the treatment of parkinsonism, and, perhaps, generally in dementias with Lewy bodies (149). In unilaterally 6-OHDA injected-hemi-parkinsonian rats, protective effects by melatonin were also attributed to normalizations of complex I activity (150). Suppression of NO formation and scavenging of reactive nitrogen species by melatonin and its metabolite AMK should additionally support cell survival, along with other protective effects, such as upregulation of the antioxidant enzymes Cu,ZnSOD, MnSOD, GPx, which has been demonstrated in cultured dopaminergic cells (151). However, while complex I inhibition is a plausible cause of neurodegeneration in the toxicological animal models, it would be of particular importance to know whether mitochondrial dysfunction is relevant in the PD patient. In fact, recent investigations did not reveal any differences in complex I, II/III and IV activities in mitochondria from platelets (152). However, this does not entirely exclude striatal mitochondrial dysfunction in advanced stages of PD, because of an impairment by iron-mediated oxidative stress.

A possible involvement of melatonin in mitochondria-hyperphosphorylation-neuronal apoptosis pathway is evident. Melatonin did not only antagonize MPP+- induced cell death in cerebellar granular neurons, but also activation of Cdk5 and cleavage of p35 to p25 (153), kinases involved in neuronal function and plasticity (154). Whether or not melatonin at pharmacological concentrations in the MPP+ study influences p25 and Cdk5 activity indirectly via mitochondrial actions and/or directly by receptor-mediated signal transduction pathways, it remains to be elucidated.

Melatonin secretion patterns have been studied in patients suffering from PD. A phase advance of the nocturnal melatonin maximum was noted in L-DOPA-treated but not in untreated patients, as compared to control subjects (155). Under medication with L-DOPA, day-time melatonin was additionally increased, a finding discussed in terms of an adaptive mechanism in response to the neurodegenerative process and possibly reflecting a neuroprotective property of melatonin. In rats, fluctuations in serum melatonin levels were also related to variations in motor function and attributed to the interaction of monoamines with melatonin in the striatal complex (156). Melatonin's inhibitory effect on motor activity has been suggested as one of the possible causes for the wearing-off episodes seen during drug treatment of parkinsonism.

A double-blind, placebo-controlled, crossover study, showed the efficacy of melatonin in schizophrenic patients suffering from tardive dyskinesia, another disease where oxidative stress–induced neurotoxicity in the nigrostriatal system is apparently implicated (157).

Studies undertaken in elderly insomniacs have demonstrated that melatonin can increase sleep efficiency and decrease nighttime activity (158). Administration of melatonin in 5 mg/day for 1 week reduced the nocturnal wake time for about 20 minutes in eight patients with PD (159). In a recent double-blind, placebo-controlled study on 40 subjects conducted over 10 weeks, Dowling *et al.* (159) noted that administration of a higher dose of melatonin, 50 mg/per day, increased actigraphically scored total nighttime sleep in PD patients, when compared with 5 mg or placebo-treated patients. Subjective reports of overall sleep disturbance improved significantly with 5 mg of melatonin compared to 50 mg or placebo (159). This study may indicate that very high doses of melatonin can be tolerated in PD patients over a 10-week period as in healthy older adults.

Melatonin, with antioxidant and anti-inflammatory actions, showed marked beneficial effects

against brain mitochondrial dysfunction with age. Melatonin production decreases with the aging and numerous studies have associated this decline in melatonin levels to the increased levels of oxidative stress and age-associated degenerative changes (160, 161).

These effects, and the fact that melatonin virtually lacks toxicity even at large doses, supports its clinical use in preventing the impairments of aging.

5.3. Melatonin in Huntington disease

Huntington disease (HD) is a neurodegenerative disorder with a progressive motor impairment, cognitive decline and psychiatric disturbances (162, 163). It is a genetically programmed neuronal degeneration inherited in an autosomal dominant manner and caused by an expanded CAG triplet repeat expansion in the gene encoding the protein huntingtin (HTT), a cytoplasmatic protein whose functions are not fully understood (163). Brain area initially involved is the striatum and then the cortex.

Many evidences suggest the presence of an abnormal conformation of mutant HTT (164). The clinical course, probably resulting by neuronal deterioration dysfunction and cell death (162), is characterized by a typical motor dysfunction such as involuntary, unwanted movements that primarily involve distal extremities and facial muscles and then all other muscles, with a distal to proximal progression. If movements disorders are distinguishing, also other signs fulfill the clinical picture: unintended weight loss, sleep- and circadian rhythm disturbances, dysarthria, dysphagia, dystonia, tics, cerebellar signs, psychiatric symptoms (depression, anxiety, apathy, obsessions, compulsions, irritability, aggression, loss of interest, psychosis), autonomic disturbances, cognitive decline, muscle wasting, metabolic dysfunction and endocrine disturbances (165), suggesting neurological and non neurological-mediated mechanisms (166, 167). Cell death heavily involves striatal area (168) with pronounced loss of GABAergic medium spiny projection neurons. Also are described: atrophy of the cerebral cortex, subcortical white matter, thalamus etc. Pathognomonic of HD are intranuclear inclusion bodies, which are large aggregates of abnormal HTT in neuronal nuclei, also described in cytoplasm, dendrites, and axon terminals (168, 169). A possible molecular mechanisms involved in HD pathogenesis involved intracellular calcium overload causing mitochondrial blockade, NMDA receptor-mediated excitation and oxidative stress (110). Experimental studies also shown that antioxidant, neuroprotective and antiapoptotic activities of melatonin may have an impact in HD that needs further investigations (73).

5.4. Melatonin and Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease, affecting both the first and second motoneuron. The progression of ALS is characterized by a degeneration of motor neurons associated with a dramatic demyelination in the anterior horn of the spinal cord. The etiology is only partially understood. Pathophysiologically, three major mechanisms are discussed in ALS: (a) mutations of the superoxide dismutase 1 (SOD1) gene, causing a toxic gain of function with enhanced reactivity towards abnormal substrates (tyrosine nitration), along with an impaired ability to bind zinc leading to a reduced antioxidant capacity; (b) mutations in neurofilament genes and oxidative modifications or hyperphosphorylation of cytoskeletal proteins leading to selective motor axon degeneration; (c) excitotoxicity caused by increased cerebrospinal fluid glutamate levels together with a loss of excitatory amino acid transporters (170).

There is no promising treatment available to date. The only compound used for its effects in survival time is an antiexcitotoxin, Riluzole. As the common basis of cellular and extracellular alterations in ALS seems to be oxidative stress mediated by reactive nitrogen/oxygen species, future attempts of treatment might focus on antioxidant strategies involving suppression of nitric oxide (NO) synthase.

Melatonin is an a candidate compound for neuroprotection in ALS patients, who tolerated high doses of melatonin (79). Melatonin has a unique broad spectrum of effects including scavenging of hydroxyl, carbonate, alkoxyl, peroxyl and aryl cation radicals, stimulation of glutathione peroxidase and other protective enzymes, but also suppression of NO synthase. The interference with NO metabolism has multiple consequences: down-regulation of NO formation counteracts damage by peroxynitrite-dependent radicals as well as Ca^{2+} -dependent excitotoxicity. This pleiotropy may explain, at least in part, why melatonin has been identified as a potent neuroprotectant, e.g., by attenuating oxidative damage after experimental neurotrauma (171, 172).

First of all, since melatonin appears to be free from side effects even upon long-term applications, melatonin can be used as a prophylaxis to treat those patients that are at risk of developing ALS. Such patients would be those that have been identified with the genetic marker associated with ALS, i.e., familial form of ALS, and those patients who exhibit early signs of motor neuron disease, such as impaired motor control.

Although melatonin seems to have a rapid turnover, the administration of slow release preparations maintains high plasma levels for about 6 hr. In SOD1(G93A)-transgenic mice, high-dose oral melatonin delayed disease progression and extended survival. In a clinical safety study, chronic high-dose (300 mg/day) rectal melatonin was well tolerated during an observation period of up to 2 years. Importantly, circulating serum protein carbonyls, which provide a surrogate marker for oxidative stress, were elevated in ALS patients, but were normalized to control values by melatonin treatment. This combination of preclinical effectiveness and proven safety in humans suggests that high-dose melatonin is suitable for clinical trials aimed at neuroprotection through antioxidation in ALS (48). The role of melatonin in this disease needs to be explored further conducting well-designed clinical trials.

6. MELATONIN IN DIETARY SUPPLEMENTS

Melatonin is currently marketed in several countries as a dietary supplement requiring no prescription. Commercial melatonin products are primarily synthesized from 5-methoxyindole, as natural products - extracted from bovine pineal glands - are not recommended because of the small, but significant, risk of contamination from animal CNS viruses. At present, data are too limited and/or inconsistent to recommend melatonin for any specific indication. Clinical trials have shown variable degrees of efficacy in the treatment of several pathological conditions, including circadian rhythm disorders, cancer, etc. Two meta-analyses found no significant evidence of efficacy for melatonin in improving sleep efficiency (the proportion of time in bed spent asleep) or managing secondary sleep disorders and sleep disorders accompanying sleep restriction, such as jet lag and shiftworks (173, 174). Another multi-center randomized placebo-controlled trial assessed the effects of 3-weeks prolonged-release melatonin 2 mg (PR-melatonin) versus placebo on 170 primary insomnia outpatients aged 55 years or older. PR-melatonin significantly improved quality of sleep and morning alertness compared with placebo (175). Similar results were obtained in another randomized controlled trial on 177 insomnia patients taking 2 mg modified-release melatonin or placebo (176). No adverse events were significantly more frequent with melatonin than with placebo. Adverse reactions reported in clinical trials include headache, depression, sinus tachycardia, and pruritus.

Besides its well-known regulatory role on circadian rhythm, the growing body of observations from experiments with endogenously produced and exogenously administered melatonin disclosed its involvement in a broad range of biological functions, ubiquitously occurring in the body. These activities, particularly the capacity of melatonin to reduce the degree of tissue damage and limit the progression of those diseases associated with oxidative stress have been documented in a number of *in vitro* and *in vivo* studies and are being tested for their clinical implications.

In two different double-blind, randomized, parallel-group, placebo-controlled trials, Gupta *et al.* assessed the effect of add-on melatonin administration on the antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GRd) in epileptic children receiving carbamazepine or valproic acid in monotherapy, respectively. An increase in GRd activity was noted in the melatonin group as compared with a decrease of the same enzyme in the placebo group, confirming the capacity of melatonin to minimize damage caused by oxidative stress in carbamazepine/valproate-treated patients (177, 178). Melatonin also showed to improve both survival and quality of life of metastatic non-small cell lung cancer patients when concomitantly administered to chemotherapy (179). The rationale of melatonin–chemotherapy association is mainly justified by the fact that melatonin reduces chemotherapy-induced lymphocyte damage (180); furthermore, recent experimental observations have shown that the antioxidant agents may enhance the cytotoxic action of the chemotherapeutic drugs (181). Moreover, melatonin showed no benefits in surgical oxidative stress as well as rheumatoid arthritis in three different randomized placebo-controlled trials (182-184).

With specific reference to the brain, as mentioned above, the fact that melatonin readily crosses the blood-brain-barrier, coupled with its optimal safety profile at the highest dosages represent two major advantages compared to other available antioxidants. An indirect prove confirming the preventive effect of this hormone against free radicals insult is the significant presence of melatonin in several plant species known for their neuroprotective properties, which are often consumed with the food or used in the context of Chinese traditional medicine (185).

7. PERSPECTIVE

The increased prevalence of neurodegenerative diseases in developed countries and absence of effective and/or well tolerated treatments for many of them, urge further investigation to elucidate the role of free radicals in such disorders and the potential clinical role of melatonin (as well as other antioxidants) in slowing down their progression. Confirmations from large clinical trials will also be essential to identify clinically relevant concentrations of melatonin required under different pathological conditions.

Regrettably, the number of randomized controlled trials testing the effectiveness of melatonin on neurodegenerative disorders is still very low, and their quality is often poor. The limitations of interventional trials in this field are that, because diseases have a long induction period, these types of studies may not be very feasible because of high costs and excessive time required. Furthermore, although epidemiological studies clearly show a correlation between the increased consumption of food rich in antioxidants and a decreased risk of oxidative stress-induced diseases, it is often very difficult to establish whether supplementation beyond dietary intake levels is of benefit, as well as to interpret the role of the nutraceutical supplementation in the progression of the disease. As a consequence, hard endpoints (such as mortality) are rarely used. To overcome this difficulty, the development of validated biomarkers as intermediate endpoints may help to understand the complexity of degenerative diseases at their different stages.

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Abbreviations: ROS: reactive oxygen species, CNS: central nervous system, CSF: cerebrospinal fluid, NO: nitric oxide, AFMK: N^1-acetyl-N^2-formyl-5-methoxykynuramine, AMK: N^1-acetyl-5-methoxykynuramine, COX-2: cyclooxygenase 2, TNF: tumor necrosis factor, mtPTP: mitochondrial permeability transition pore, iNOS: inducible nitric oxide synthases, NSAIDs: non-steroidal ant-inflammatory drugs, AD: Alzheimer's disease, NFT: neurofibrillary tangles, APP: amyloid precursor protein, CA: calyculin A, GSK-3: Glycogen synthase kinase 3, NADPH: nicotinamide adenine dinucleotide phosphate, PD: Parkinson's disease, SNpc: substantia nigra pars compacta, LC: locus ceruleus, LB: Lewy bodies, MAO: monoamineoxidase, MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPP+: 1-methyl-4-phenylpyridinium ion, HD: Huntington disease, ALS: amyotrophic lateral sclerosis, SOD1: superoxide dismutase 1, GPx: glutathione peroxidase, GPd: glutathione reductase.

Key Words: Melatonin, Dietary supplementation, Neurodegenerative disorders, Antioxidant, Neuroprotection, Review

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