The anti-apoptotic activity of melatonin in neurodegenerative diseases

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Abstract

Melatonin plays a neuroprotective role in models of neurodegenerative diseases. However, the molecular mechanisms underlying neuroprotection by melatonin are not well-understood. Apoptotic cell death in the central nervous system is a feature of neurodegenerative diseases. The intrinsic and extrinsic apoptotic pathways and the anti-apoptotic survival signal pathways play critical roles in neurodegeneration. This review summarizes the reports to date showing inhibition by melatonin of the intrinsic apoptotic pathways in neurodegenerative diseases including stroke, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, and Amyotrophic Lateral Sclerosis. Furthermore, the activation of survival signal pathways by melatonin in neurodegenerative diseases is discussed.

Keywords
Melatonin; Mitochondrial cell death pathways; Survival signal pathways; Stroke; Alzheimer’s disease; Parkinson’s disease; Huntington’s disease; Amyotrophic Lateral Sclerosis

1. Introduction

1.1. Melatonin may be beneficial in treatment of neurodegenerative diseases

Melatonin (N-acetyl-5-methoxytryptamine) is a natural hormone secreted by the pineal gland. In clinical use for many years, melatonin is safe and well-tolerated even at high doses [1] and easily crosses the blood-brain barrier. Besides being used to increase sleep efficiency, treat jet lag, improve the cardiovascular system [2], and as an anti-aging drug [3–5] and a dietary supplement and cancer-protective hormone [6], intensive research in the past roughly 10 years has indicated melatonin’s beneficial effects in experimental models of neurodegenerative disorders. Brain oxidative damage has been implicated as a common link in the pathogenesis of such diseases. This small amphiphilic molecule acts as a free-radical scavenger, and its broad spectrum of antioxidant activities in many central nervous system neurodegenerative diseases [7] has been well-documented and reviewed [8]. There is growing evidence that its anti-apoptotic effects play an important role in neurodegeneration as well. This review will summarize the anti-apoptotic activities of melatonin via the inhibition of intrinsic apoptotic pathways and the activation of survival signal pathways in stroke, Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), and Amyotrophic Lateral Sclerosis (ALS).
1.2. The intrinsic and extrinsic apoptotic pathways in neurodegenerative diseases

Two types of cell death occur in neurodegeneration: apoptosis and necrosis. Apoptosis (also called programmed cell death) occurs naturally under normal physiological conditions and in a variety of diseases while necrosis is caused by external factors, such as infection, toxins, or trauma. Apoptosis is a feature of both acute and chronic central nervous system neurodegenerative diseases. There are two major apoptotic signaling pathways: extrinsic and intrinsic. The extrinsic apoptotic pathway (death receptor pathway) is initiated by death receptors (e.g. CD95/APO-1/Fas; TNF receptor) on the surface of the cells, involving caspase-8/Bid and caspase-10 activation [9,10]. Since there have been no obvious reports of the involvement of extrinsic pathways in the neuroprotection of melatonin, this review focuses only on the intrinsic pathway (the mitochondrial pathway) [11] (Figure 1).

Pro-apoptotic mitochondria molecules cytochrome c, Smac (second mitochondrion-derived activator of caspase)/Diablo, AIF (apoptosis inducing factor), and Endo G (endonuclease G), when released into the cytoplasm from mitochondria, induce both caspase-dependent and -independent mitochondrial death pathways in neurodegenerative diseases (Figure 1) [12–19]. The release of cytochrome c is pivotal in the activation of caspases [20]. During the progression of neurodegenerative diseases, once cytochrome c is released, it binds to Apaf-1 and dATP, which stimulates the activation of caspase-9, and then in turn cleaves the key effector caspase-3 and two other effectors, caspase-6 and -7 [12,14–18,21–24]. In addition, DNA-repairing enzyme poly (ADP-ribose) polymerase (PARP) is cleaved [21], and transcription factors such as NF-κB [5,25–27], TNFα-induced activator protein-1 (AP-1) [28,29], and p53 [30,31] are activated. Nuclear condensation and DNA fragmentation are induced, as shown by terminal deoxynucleotidyl transferase-mediated DNA nick-end labeling (TUNEL)-positive cells, Hoechst 33342 stain, PI (propidium iodide) and DAPI staining, as well as DNA ladder. These events ultimately cause neuronal cell death [13]. Other mitochondrial factors include mitochondrial permeability transition pores (mtPTP) and mitochondrial membrane potential (ΔΨm). mtPTP represents a multi-protein complex including inner and outer membrane components. The pores regulate transport of ions and peptides into and out of mitochondria. The activation of the permeability transition and in irreversible opening of mitochondria pores is a major step in the development of neurodegeneration [32–34]. ΔΨm reflects performance of the electron transport chain and can indicate a pathological disorder. The dissipation of ΔΨm and concomitant neuronal death have been reported in experimental models of neurodegeneration [14,18,34–36].

Caspase-1 activation is an early event in neurodegenerative diseases [24,37]. Caspase-1 activator Receptor interacting protein-2 (Rip2) stimulates caspase-1 to activate IL-1β by truncating the pro-inflammatory cytokine. The release of mature IL-1β indicates caspase-1 activation [38]. The inhibition of pro-IL-1β cleavage and mature IL-1β secretion are associated with inhibition of apoptosis in neurodegeneration [16,18]. Rip2 upregulation has already been reported in AD [39], HD [40], and stroke [16].

Bcl-2 family members include pro-apoptotic molecules (Bax, Bak, Bok, Bad, Bid, Bik, Blk, Hrk, BNIP3, and BimL) and anti-apoptotic molecules (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1). Bcl-2 family proteins participate in the modulation and execution of cell death [31] and can preserve or disrupt mitochondrial integrity by regulating the release of cytochrome c/Smac/AIF/Endonuclease G [41,42]. Cytosolic Bax translocates to mitochondria upon death stimulus [23,43], promoting cytochrome c release [43]. Besides the involvement of the Fas/Caspase-8/Bid cascade, Bid also mediates cytochrome c release while binding to both pro-apoptotic members (e.g. Bax) and anti-apoptotic members Bcl-2 and Bcl-xL [44]; moreover, cleavage of Bid by caspase-8 and caspase-1 mediates the mitochondrial damage [45,46]. Bax mediates cell death with mitochondrial permeability transition [11]. Bcl-2 and Bcl-xL bind to Apaf-1, inhibiting the association of caspase-9 with Apaf-1 [47].
Prostate apoptosis response-4 (Par-4) induces mitochondrial membrane permeability changes and promotes mitochondrial dysfunction [48]. Par-4 increases the secretion of Amyloid beta (Aβ) and neuronal degeneration [49]. Par-4 levels are augmented in AD patients [50] and in models of stroke [51]. RNAi knockdown of Par-4 inhibits neurosynaptic degeneration in ALS-linked mice [52]. Par-4 interacts with Bcl-2, caspase-8, and PKCζ, thus inhibiting NF-κB-dependent survival signaling [53].

The MAPK family includes three members: extracellular signal regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK), and c-Jun NH(2)-terminal kinase (JNK). Another kinase is MAP kinase kinase (MEK). JNK pathway has been observed in neurodegenerative diseases mostly by activating apoptosis [54,55] and partly by inhibiting cell death [56]. DNA damage causes the JNK activation which contributes to the mitochondrial transduction of Bax [57,58]. The absence of JNK causes a defect in the mitochondrial death signaling pathway, including the failure to release cytochrome c [57]. Moreover, SP600125, a JNK inhibitor enhances the activation of JNK pathway and attenuation of apoptosis by through protection of mitochondrial dysfunction and reduction of caspase 9 activity in PC12 cells [59].

1.3. The Survival signaling pathways in neurodegenerative diseases

During the progression of neurodegenerative diseases, the survival signaling cascades are activated by neuroprotective agent [60] including the phosphoinositol-3 kinase (PI3K)/Akt pathway, the Bcl-2 pathway, the NF-κB pathway, as well as the MAPK pathway (Figure 1). AKT (v-Akt Murine Thymoma Viral Oncogene)/PKB (Protein Kinase-B) has been identified as an important mediator of neuronal cell survival that helps counteract apoptotic stimuli. PI3K/Akt pathways play essential roles in neuronal cell survival. PI3K is activated and the membrane phospholipid phosphatidylinositol-3,4,5-trisphosphate is generated, which in turn recruits Akt to the membrane, where it becomes phosphorylated. Once Akt is activated, it phosphorylates survival-mediated targets including Bcl-2 family members, thereby promoting cell survival and inhibiting apoptosis [61]. The anti-apoptotic Bcl-2 family encodes Bcl-2, Bcl-xL, and Bfl-1 (A1) [62]. These anti-apoptotic proteins repress mitochondrial death pathways through heterodimerization [62]. Depletion of the endogenous neuroprotective Bcl-2 family signals directly contributes to neuronal loss in neurodegenerative diseases [62]. NF-κB (Nuclear-Factor kappa B) is an inducible transcription factor that exists in several dimeric forms, with the p50/p65 heterodimer predominant [63]. The NF-κB pathway induces the expression of stress proteins, antioxidant enzymes, and calcium-regulating proteins. The activation of NF-κB not only induces apoptotic signaling [5,25,26,64] but also has been known to activate survival signals in neurodegeneration [27]. Additionally, the phosphorylation of Raf-1, MEK1/2, and ERK1/2 has been reported in neurodegeneration [65]. The JNK pathway also is involved in neurodegenerative diseases by inhibiting cell death [56].

2. Melatonin in neurodegenerative diseases

2.1. Melatonin in Experimental Stroke

Animal models of stroke include global, multifocal, and focal cerebral ischemia. Focal cerebral ischemia is divided into transient (with reperfusion) and permanent (without reperfusion). Middle cerebral artery occlusion (MCAO) is the most commonly used animal model in the study of melatonin. Primary cortical neurons (PCNs) are the cells most commonly used in cellular model of stroke. The ability of melatonin to reduce infarct volume and/or inhibit neuronal cell death in experimental models of stroke has been demonstrated in different mammalian species [18,66–68], but the signaling mechanisms underlying melatonin’s neuroprotective actions remain incompletely understood. We summarize reports of neuroprotection by melatonin gained through inhibiting mitochondrial cell death pathways.
The highest levels of melatonin are found in the mitochondria [69]. Mitochondria have been identified as a target for melatonin [70,71]. Melatonin promotes mitochondrial homeostasis. Taken together, melatonin may be possible to treat neurodegenerative disorders by inhibiting mitochondrial cell death pathways [1,72–75]. We screened a library of 1040 FDA-approved drugs assembled by the Neurodegeneration Drug Screening Consortium of the NINDS for their ability to inhibit release of cytochrome c from Ca++-stimulated mitochondria [76]. Melatonin occupied one of the top positions (14th) [76]. Furthermore, we and other laboratories demonstrated that melatonin has proven effective not only in the cell-free purified mitochondrial system, but also inhibits cytochrome c release in an MCAO mouse model [18,71] and in PCN [18]. Melatonin prevents the release of cell death mediator AIF from mitochondria in PCNs upon insult [71]. Thus melatonin is likely to interfere with both caspase-dependent (cytochrome c) and independent (AIF) mitochondrial cell death pathways. Proper ΔΨm is critical for appropriate cellular bioenergetic homeostasis, and dissipation of ΔΨm has been involved in stroke [18,35]. Studies in both primary striatal neurons (PSNs) [71] and PCNs [18] showed that melatonin effectively inhibited oxygen/glucose deprivation (OGD)-mediated dissipation of ΔΨm. These effects reflect the ability of melatonin to ameliorate the harmful reduction in the ΔΨm, which may trigger mitochondrial transition pore opening and the apoptosis cascade. mtPTP contributes to the pathology of ischemia. Further experiments indeed demonstrated that melatonin directly inhibits mtPTP in PSNs after OGD insult [71].

Caspase-1 plays a critical role as an apical activator in models of stroke [16]. Interestingly, melatonin inhibits OGD-induced caspase-1 activation and mature IL-1β release in PCNs [18]. In vitro and in vivo experiments have shown that melatonin prevents the activation of downstream caspase-3 in OGD-mediated PCN cell death [18], cerebral ischemia-induced mouse injury, and the MCAO rat model [18,72,77]. Other experiments demonstrate significantly fewer TUNEL-positive cells [65,78], reduced levels of cleaved PARP [65], and less DNA fragments [71] are found with administration of melatonin in the rat MCAO model. In addition, melatonin prevents brain damage, with reduced TUNEL-positive cells following transient cerebral artery occlusion (CerAO) [66] and transient MCAO model [79], as well as attenuating kainic acid-induced neuronal death, and reduces the number of TUNEL-labeled DNA breaks [80].

The neuroprotective role of melatonin is also mediated through the enhancement of the PI3-K/Akt survival pathway [77,81] and JNK pathway [56] and restores reduced phosphorylated Akt in a model of mouse intraluminal MCAO [77]. Melatonin protected neuronal cells from damage by enhancing the activation of Akt and its downstream target Bad, without affecting the expression of 14-3-3, which acts as an anti-apoptotic factor through interaction with Bad, thus mediating anti-apoptosis signals in a rat MCAO model [78]. Furthermore, in the same model, melatonin inhibits apoptotic signals by preventing the injury-induced decrease of phosphorylation of Raf-1, MEK1/2, and ERK1/2 and the downstream targets, including Bad and 90 ribosomal S6 kinase [65]. Melatonin effectively attenuated ischemic brain injury via the Bcl-2-related survival pathway by increasing the expression of Bcl-2 [82] and Bcl-xL [56] in the ischemic brain. Furthermore, related to melatonin, the constitutive activation of NF-κB under physiological conditions protects neurons against physiological injury [27].

Intervention studies have identified a battery of approaches with potential benefits in reducing neuronal death in stroke patients, including antioxidant treatment. Clinical data report some alteration of the melatoninergic system in human stroke. Based on its lack of toxicity, melatonin may eventually be included in human stroke treatment.
2.2. Melatonin in Alzheimer's disease

AD, the most common neurodegenerative disease with progressive loss of memory and deterioration of comprehensive cognition, is characterized by extracellular senile plaques of aggregated beta-amyloid (Aβ), and intracellular neurofibrillary tangles that contain hyperphosphorylated tau protein. Aβ and tau therefore represent important therapeutic targets. The early phase of AD is treatable by inhibitors of β and γ secretase, which degrade amyloid precursor protein (APP) to produce β-amyloid peptide [83] and the late phase is amendable to treatment strategy by preventing or reversing tau phosphorylation [84,85]. Mild cognitive impairment (MCI) is a transition stage between the cognitive decline of normal aging and the more serious problems caused by AD. Many people with MCI eventually develop AD. Studies show that melatonin levels are lower in AD patients compared with that in age-matched control subjects [86–88]. The great advance has been currently conducted in studies of protection against AD by antioxidant melatonin inhibiting Aβ-induced toxicity [25,89–93] and attenuating tau hyperphosphorylation [85,94–99]. Besides the antioxidant properties, the antiamyloidogenic properties of melatonin for AD have been studied [100,101]. Melatonin improved learning and memory deficits in an APP695 transgenic mouse model of AD in vivo [89]. In vitro experiments showed that Aβ−treated cultures exhibited characteristic features of apoptosis, and melatonin attenuated Aβ-induced apoptosis in a number of cellular models of AD including mouse microglial BV2 cells, rat astroglia C6 cells, and PC12 cells [25,89–91,102].

It is known that melatonin scavenges oxygen and nitrogen-based reactants generated in mitochondria, and mitochondria play a critical role in the neuroprotective function of melatonin in AD. As listed in Table I, studies in transgenic AD mice and cultured cells have suggested that administration of melatonin inhibited the Aβ-induced increase in the levels of mitochondria-related bax [25,92]. Furthermore, melatonin prevented upregulated expression of Par-4 and suppressed Aβ-induced caspase-3 activity [92]. Another experiment in mouse microglial BV2 cells in vitro showed that melatonin also decreased caspase-3 activity, inhibited NF-κB activation, and reduced the generation of Aβ-induced intracellular ROS (reactive oxygen species) [25]. Additionally in vivo observations showed that melatonin-treated animals had diminished expression of NF-κB compared to untreated animals [26]. Melatonin treatment significantly decreased the number of TUNEL-positive neurons along with improving spatial memory performance in cognitively impaired, ovariectomized adult rats [89] and Alzheimer-like tau hyperphosphorylation in wortmannin-induced N2a cells [94].

On the other hand, melatonin may also activate the survival signal pathways. One such pathway is the Bcl-2 pathway, which stabilizes mitochondrial function by anti-apoptotic Bcl-2 family modulators. It has been reported that Bcl-2 expression was enhanced by melatonin concomitant with inhibition of Aβ-induced cell death [25] (Table II). Another experiment demonstrated that melatonin inhibited the phosphorylation of NADPH oxidase via a PI3K/Akt-dependent signaling pathway in microglia exposed to Aβ1–42 [93] (Table II). Taken together, the above evidence suggests that melatonin may provide an effective means of treatment for AD through its anti-apoptotic activities.

It has been reported that administration of melatonin significantly delays the development of the signs of AD, prevents cognitive impairment, and ameliorates sundowning in AD patients [103–107]. In addition, light therapy or music therapy related with levels of melatonin may have effect on AD patients [108,109]. On the contrary, some researchers report that the impact of melatonin would be relatively less in late stage of AD or fails to improve sleep or agitation, therefore melatonin is not an effective soporific agent in patients with AD [110,111].

Human trials in the relatively small scale suggest that melatonin can improve MCI [112,113]. However how melatonin affects disease initiation or progression of the neuropathology and if...
the anti-apoptotic activity of melatonin is driving its function, remains to be answered. On the other hand, controversy reports suggest insufficient evidence to support the effectiveness of melatonin for managing cognitive impairment with low success rate [114]. Further clinical phase II trial of the effect of melatonin on cognitive function in MCI patients is undergoing (ClinicalTrials.gov Identifier: NCT00544791).

2.3. Melatonin in Parkinson’s Disease

PD is the second most common neurodegenerative disease, affecting ~1.8% of people over 65 years old [115]. PD is characterized by a progressive loss of dopaminergic neurons and dopamine in the substantia nigra and striatum. Oxidative stress and free radicals from both mitochondrial impairment and dopamine metabolism are considered to play critical roles in the etiology of PD. Additionally, neurodegeneration occurs in PD, at least in part, through the activation of the mitochondria-dependent apoptotic molecular pathway [17]. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been reported to cause parkinsonism via its neurotoxic form, 1-methyl-4-phenylpyridinium ion (MPP+), which inhibits mitochondrial complex I of the mitochondrial respiratory chain. MPP+ has been as a commonly used experimental model of PD [54,116].

As shown in Table (I) and Figure 1, melatonin prevents H$_2$O$_2$-induced mitochondrial calcium overload, ΔΨm depolarization, opening of mtPTP, avoidance of ROS formation, as well as blocked MPT-dependent cytochrome c release in rat astrocytes, a model of PD [117]. Also in the same model, melatonin inhibited MPT-dependent activation of caspase-3 [117]. This conclusion is further supported by the finding that melatonin suppressed 3-morpholinosydnonimine-induced caspase-3 activation in dopaminergic neurons [118] and diminished the activation of caspase-3 enzyme activity in both MPP(+) treated SK-N-SH cultured cells [54] and cerebellar granule neurons (CGNs) [116]. In addition, melatonin exerted neuroprotective effects against MPP+ induced apoptosis by inhibiting the calpain/cdk5 signaling cascade in CGNs [116]. Mounting evidence indicates that melatonin blocks the MPT-dependent apoptotic fragmentation of nuclear DNA in rat astrocytes [117], rat mesencephalic cultures [119], and mouse striatal neurons [120]. Other experiments also indicate MPTP-induced mouse brain cell DNA fragmentation in vivo [121], 6-hydroxydopamine-induced DNA fragmentation in neuronal PC12 cells [122], and MPP(+) mediated cleavage of DNA fragmentation factors in SK-N-SH cultured cells in vitro [54]. The JNK pathway is involved in PD by activating apoptosis [54,55] and transcription factors play a role in PD, as shown in two experiments demonstrating the action of melatonin to inhibit JNK signaling cascade [54, 55] and diminish the induction of phosphorylation of c-Jun in MPP(+) treated [54] and 6-hydroxydopamine-induced SK-N-SH cultured cells [54,55]. To date, there are no reports of the activation of survival pathways by melatonin in PD.

Human trials for melatonin’s effect on sleep disturbances in PD shows significantly improved [123] or small improvement [124], but there is undetected differences in motor dysfunction [123].

2.4. Melatonin in Huntington’s disease

HD, a hereditary disease affecting 30,000 Americans, is universally fatal with no effective treatment. HD is characterized by movement disorder (Huntington’s chorea), cognitive deterioration, emotional distress, and dementia [125]. HD is caused by expansion of cytosine-adenine-guanine (CAG) repeats in exon 1 of the huntingtin gene [126], initially affecting the striatum and then the cortex. Since oxidative stress plays an important role in the etiology of neuronal damage and degeneration in HD [127], therapeutic strategies against HD focus on antioxidant defense.
Mitochondrial complex II inhibitor 3-nitropropionic acid can closely replicate the neurochemical, histological, and clinical features of HD and hence is used in an experimental model of HD [75,128]. So far the antioxidant melatonin has been suggested to defer the signs of HD in a 3-nitropropionic acid-induced rat animal model of HD [75] and to reduce lipid peroxidation induced by quinolinic acid (a causative agent in HD) [74]. Additionally, we report that melatonin is a remarkably potent neuroprotective agent in mutant-huntingtin (mutant-htt) ST14A cells, a cellular model of HD [76,129,130]. It protects 76.2 % of mutant-htt ST14A cell death from temperature-shift induced cell death [76]. Furthermore, melatonin prevents cell death of PCNs that have been challenged with pro-apoptotic inducer [18]. One of our compelling findings underlying the mechanism of melatonin’s action against HD is that it counters mitochondrial cell death pathways through the inhibition of the release of Smac and the activation of caspase-9 in apoptotic mutant-htt ST14A striatal cells (unpublished data). Furthermore, administration of melatonin also significantly inhibits the Rip2 upregulation in mutant-htt ST14A cells under insult (unpublished data). Thus our findings suggest that melatonin acts on initiated Rip2 (Figure 1). On the other hand, there has been no report that melatonin activates survival pathways in HD yet.

In a human study with the addition of tryptophan, albeit melatonin levels rose significantly in both of control and HD patients group, bigger increasing average mean occurs in HD patients group [131]. Moreover, the delayed onset of the diurnal melatonin rise in patients with HD in small scale has been currently reported [132]. Larger scale studies in detecting the level of melatonin in HD patients and further human trials on the impact of melatonin on HD are needed.

2.5. Melatonin in Amyotrophic Lateral Sclerosis

ALS is a fatal disease of varying etiology whose progression is characterized by a degeneration of motor neurons. Riluzole, an antagonist of the glutamate receptor, is the only approved treatment for ALS. However, it typically prolongs the patient’s life by only three months. Since the common basis of cellular and extracellular alterations in this disease seems to be oxidative stress, the strategy for the treatment of ALS therefore emphasizes antioxidant molecules.

Rival et al. report that, exactly like administration of riluzole in dEAAT1 RNAi flies, administration of the antioxidant melatonin significantly enhanced performance in a Drosophila model exhibiting remarkable similarity with some of the symptoms associated with ALS [133]. Furthermore, melatonin offers protection in human ALS. The first clinical trial of melatonin in three human ALS patients was reported in 2002 [73], and the second human trial in a group of thirty-one patients with sporadic ALS was reported in 2006 [1]. Importantly, circulating serum protein carbonyls, which provide a surrogate marker for oxidative stress, were elevated in ALS patients, but were reported to be normalized to control values by melatonin treatment in the second clinical trial [1]. In other words, reduced oxidative damage was reported in ALS trial using high-dose enteral melatonin [1]. Chronic high-dose (300 mg/day [1]) rectally-administered melatonin was well-tolerated in patients with sporadic ALS [1, 73]. Additionally, the findings from both animal models in vivo and a cellular model in vitro support the results of human trials, in SOD1(G93A)-transgenic mice, high doses of orally administered melatonin delayed disease progression and extended survival in vivo [1]. However, western blot analysis of spinal cord protein lysates in the same study found no differences in total amount or phosphorylation status of AKT or ERK ½ in SOD1(G93A)-transgenic mice with melatonin treatment compared with untreated controls [1] (Figure 1).

Another study showed that administration of melatonin alters the expression of SOD1 in the lumbar spinal cord of neonatal rats [134]. Furthermore melatonin attenuates superoxide-induced cell death and modulates glutamate toxicity in cultured NSC-34 motoneuron cells in vitro [1]. Though evidence indicates that mSOD1-induced spinal cord motor neuron death and cultured motor neuronal cells involve apoptotic machinery [12,135–137], to date, the
neuroprotection afforded by melatonin through the inhibition of cell death pathways or activation of survival pathways remains essentially uninvestigated.

Because melatonin is neuroprotective in human, cellular, and animal models of ALS and is relatively nontoxic, it should be considered for further larger clinical trials as a novel pharmacotherapeutic agent to treat ALS.

3. Conclusion and perspective

Given the fact that vigorous research efforts to date have achieved poor results in their efforts to identify effective treatments against neurodegenerative diseases, the combination of preclinical effectiveness and proven safety of melatonin in humans, animals, and cultured cells recommends it as a particularly interesting candidate neuronprotectant in clinical trials seeking protection against neurodegeneration. Interestingly, melatonin is capable of interfering with mitochondrial cell death pathways and activating survival pathways, both of which would be useful in treating common events in stroke, AD, PD, ALS, and HD. In addition, blood concentrations of neurohormone melatonin are significantly decreased in patients with AD [87], while low levels of melatonin and a prolonged signal of melatonin are found in PD patients [138] and the delayed onset of the diurnal melatonin rise in HD patients [132]. Thus it is believed that reduced secretion of melatonin is associated with the development of neurodegenerative disease [87]. Knowing about the molecular mechanism of melatonin’s declining potency should tell us about the pathogenesis of related neurodegenerative diseases and will guide the contemplated translation to the clinic. Pharmacological strategies to enhance melatonin levels may benefit those suffering from neurodegenerative diseases.

Cell death-based therapies are becoming an active area of drug development. For a multi-drug regimen to effectively protect neurons from inappropriate apoptosis, several pathways could be coactivated, including anti-apoptotic pathways and survival pathways. This review gains deeper insights into the action mechanism of melatonin. Thus it may provide a new perspective in our understanding of the regulation of apoptotic cell death in neurodegeneration by the pharmacotherapeutic indoleamine. Besides its traditional role as an antioxidant and free radical scavenger, melatonin proved to target a variety of pathways while its systemic effect correlates with the drug’s disruption of the intrinsic mitochondrial cell death pathway, silencing of the Rip2/Caspase-1 pathway, and the activation of survival pathways. These actions may be synchronistic and complementary in models of HD. Effective treatment to prevent neurodegeneration could be achieved using a combination of melatonin and other pharmacological agents that act on different apoptosis targets.

Future therapeutic strategies could be directed at identifying and developing drugs from among the analogues of melatonin. Candidate drugs may have more powerful inhibitory effects on the mitochondrial cell death pathway and activate the survival pathway, thus slowing the progression of neurodegenerative diseases.

Acknowledgments

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References


Figure 1. Scheme of neuronprotection of melatonin
The possible inhibition of the intrinsic cell death pathway and activation of the survival pathway by melatonin is schematized.
Table I
Summary of inhibition of the anti-apoptotic cell death pathway by melatonin

<table>
<thead>
<tr>
<th>Inhibits death pathway event</th>
<th>Diseases/Models</th>
<th>Effects of melatonin</th>
<th>Species Cell line</th>
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<tr>
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<td>Rat; Mouse, PCN</td>
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<td>PD</td>
<td>Prevents cyto. c release</td>
<td>Astrocyte</td>
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<td>HD</td>
<td>Neuroprotective in HD models</td>
<td>Mu-htt ST14A [unpublished data]</td>
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<td>Attenuates cerebral ischemic injury</td>
<td>Rat</td>
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<td>Astrocyte</td>
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<td>NSC34 motoneuron</td>
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<td>Rat</td>
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<td>[18,71]</td>
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<td>Prevents DNA fragmentation</td>
<td>SK-N-SH cell; Astrocyte; mesencephalic cell; striatal neuron; mouse; PC12 cells</td>
<td>[54,117,121,122]</td>
<td></td>
</tr>
<tr>
<td>TUNEL-positive</td>
<td>Neurodegeneration</td>
<td>Reduces number of DNA breaks</td>
<td>Rat</td>
<td>[80]</td>
</tr>
<tr>
<td>Stroke/MCAO</td>
<td>Decreases TUNEL-positive cells</td>
<td>Rat</td>
<td>[65,78,79]</td>
<td></td>
</tr>
<tr>
<td>Stroke/OGD</td>
<td>Neuroprotective in PCN</td>
<td>PCN</td>
<td>[18]</td>
<td></td>
</tr>
<tr>
<td>AD/OVX</td>
<td>Improves spatial memory performance; Reduces apoptosis</td>
<td>Rat</td>
<td>[89]</td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>Protects the wortmannin-induced tau hyperphosphorylation</td>
<td>N2a cells</td>
<td>[94]</td>
<td></td>
</tr>
<tr>
<td>JNK</td>
<td>PD</td>
<td>Inhibits cell death</td>
<td>SK-N-SH cell</td>
<td>[54,55]</td>
</tr>
<tr>
<td>Inhibits death pathway event</td>
<td>Diseases/Models</td>
<td>Effects of melatonin</td>
<td>Species</td>
<td>Refs</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------</td>
<td>----------------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>Par-4</td>
<td>AD</td>
<td>Reduces Par-4 upregulation</td>
<td>Mouse</td>
<td>[92]</td>
</tr>
<tr>
<td>NF-κB</td>
<td>AD</td>
<td>Blocks Aβ25-35-induced apoptosis</td>
<td>Microglial cell; Mouse</td>
<td>[5,25]</td>
</tr>
<tr>
<td>AD</td>
<td></td>
<td>Anti-inflammatory effect on Aβ vaccination in mice</td>
<td>Mouse</td>
<td>[26]</td>
</tr>
</tbody>
</table>

Abbreviations: OVX, ovariectomized.
### Table II
Summary of activation of anti-apoptotic survival signal pathway by melatonin

<table>
<thead>
<tr>
<th>Activates element</th>
<th>Diseases/Models</th>
<th>Effects of melatonin</th>
<th>Species</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3-K/Akt</td>
<td>Stroke/MCAO</td>
<td>Restores phosphorylated Akt</td>
<td>Mouse; Rat</td>
<td>[56,77,78]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against brain injury</td>
<td>Rat</td>
<td>[81]</td>
</tr>
<tr>
<td></td>
<td>AD</td>
<td>Impairs NADPH oxidase via PI3K/Akt signaling pathway</td>
<td>Mouse</td>
<td>[93]</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Stroke/MCAO</td>
<td>Enhances Bcl-2 upregulation</td>
<td>Rat</td>
<td>[79,82]</td>
</tr>
<tr>
<td></td>
<td>AD/Aβ25-35</td>
<td>Attenuates Aβ25-35-induced apoptosis</td>
<td>Microglial cell</td>
<td>[25]</td>
</tr>
<tr>
<td>Bcl-xL</td>
<td>Stroke/MCAO</td>
<td>Elevates Bcl-xL in brain injury</td>
<td>Mouse</td>
<td>[77]</td>
</tr>
<tr>
<td>JNK1/2</td>
<td>Stroke/MCAO</td>
<td>Increases JNK1/2 phosphorylation</td>
<td>Mouse</td>
<td>[56]</td>
</tr>
<tr>
<td>ERK1/2</td>
<td>Stroke/MCAO</td>
<td>Increases ERK1/2 phosphorylation</td>
<td>Mouse; Rat</td>
<td>[56,65]</td>
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<td>Raf-1</td>
<td>Stroke/MCAO</td>
<td>Attenuates cerebral ischemic injury</td>
<td>Rat</td>
<td>[65]</td>
</tr>
<tr>
<td>MEK1/2</td>
<td>Stroke/MCAO</td>
<td>Attenuates cerebral ischemic injury</td>
<td>Rat</td>
<td>[65]</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Stroke</td>
<td>Relates with NF-κB-mediated protective signaling</td>
<td>Primary neuron</td>
<td>[27]</td>
</tr>
</tbody>
</table>