Review Article

Melatonin and Its Agonist Ramelteon in Alzheimer's Disease: Possible Therapeutic Value

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Alzheimer's disease (AD) is an age-associated neurodegenerative disease characterized by the progressive loss of cognitive function, loss of memory and insomnia, and abnormal behavioral signs and symptoms. Among the various theories that have been put forth to explain the pathophysiology of AD, the oxidative stress induced by amyloid β-protein (Aβ) deposition has received great attention. Studies undertaken on postmortem brain samples of AD patients have consistently shown extensive lipid, protein, and DNA oxidation. Presence of abnormal tau protein, mitochondrial dysfunction, and protein hyperphosphorylation all have been demonstrated in neural tissues of AD patients. Moreover, AD patients exhibit severe sleep/wake disturbances and insomnia and these are associated with more rapid cognitive decline and memory impairment. On this basis, the successful management of AD patients requires an ideal drug that besides antagonizing Aβ-induced neurotoxicity could also correct the disturbed sleep-wake rhythm and improve sleep quality. Melatonin is an effective chronobiotic agent and has significant neuroprotective properties preventing Aβ-induced neurotoxic effects in a number of animal experimental models. Since melatonin levels in AD patients are greatly reduced, melatonin replacement has the potential value to be used as a therapeutic agent for treating AD, particularly at the early phases of the disease and especially in those in whom the relevant melatonin receptors are intact. As sleep deprivation has been shown to produce oxidative damage, impaired mitochondrial function, neurodegenerative inflammation, and altered proteosomal processing with abnormal activation of enzymes, treatment of sleep disturbances may be a priority for arresting the progression of AD. In this context the newly introduced melatonin agonist ramelteon can be of much therapeutic value because of its highly selective action on melatonin MT_1/MT_2 receptors in promoting sleep.

1. Introduction

Alzheimer's disease (AD), a major age-associated neurodegenerative disease, is characterized by progressive loss of cognitive function, loss of memory, impaired synaptic function, and a massive brain cell loss that ultimately results in premature death. Although the exact cause of the disease is under intense investigation, the prevailing hypothesis proposes that the deposition of amyloid β-protein- (Aβ-)containing senile plaques and of intracellular neurofibrillary tangles major etiological factors in AD [1]. Deposition of amyloid plaques causes cell death by inducing mitochondrial dysfunction and oxidative stress [2]. Aβ deposition initiates the flavo-enzyme-dependent increase of hydrogen peroxide (H_2O_2) and lipid peroxides that increase free radical generation [3, 4]. Neural tissues of AD patients exhibit increased levels

of end products of peroxidation such as malondialdehyde, 4-hydroxynonenal, or carbonyls. Though Aβ contributes directly or indirectly to neuronal degeneration, its potential to cause AD depends on individual's susceptibility to Aβ-mediated toxicity [5].

Mitochondrial dysfunction plays an important role in AD and the link among impaired mitochondrial function, tau phosphorylation, and Aβ amyloidosis is increasingly recognized as a major phenomenon in AD physiopathology [2, 6, 7]. Aβ accumulation and neurofibrillary tangles composed of tau protein induce functional deficits of the respiratory chain complexes thereby resulting in mitochondrial dysfunction and oxidative stress (the "Aβ cascade hypothesis of AD"). It is interesting to note that women are more vulnerable to AD than men, presumably because the mitochondria are protected by estrogens against Aβ toxicity [8].

Indeed, aging and neurodegenerative diseases are accompanied by abnormal levels of oxidation of proteins, lipids, and nucleic acids [9–11]. Mechanisms such as chronic inflammation associated with the release of cytokines and trace element neurotoxicity have also been suggested as possible contributory factors underlying the physiopathologic events of AD [12–14]. Membrane disruption and induction of apoptosis by caspase enzymes have also been implicated [15].

In addition to cognitive and memory dysfunction, sleep-wake and other circadian rhythm dysregulation, are commonly seen in AD [16–19]. These circadian rhythm disturbances are associated with disturbed melatonin rhythmicity and decreased circulating and brain melatonin levels [20–22]. It is hypothesized that the decreased levels of melatonin, in fact, could contribute to the pathophysiology of AD in view than melatonin combines chronobiotic with effective antioxidant, anti-inflammatory, and antifibrillogenic properties [23].

Among the factors known to suppress the production of melatonin by the pineal gland, hypoxia deserves to be considered [24]. Reduced production of melatonin has been reported to occur in other ischemic conditions such as coronary artery disease or severe congestive heart failure [25–27]. Hypoxia may play a role in the pathogenesis of AD as it can induce formation of Aβ [28–30]. The role of hypoxia in potentiating AD is supported by the observation that patients suffering from cardiorespiratory disorders, cerebral ischemia or stroke are much more susceptible to development of dementias including AD [31]. It is remarkable that the daily administration of melatonin reduces the hypoxia induced Aβ generation in the rat hippocampus [32].

With this background, the replacement of brain melatonin levels has been suggested as a way arresting the progress of AD and for correcting the circadian and sleep-wake disturbances associated with the disease. As melatonin is a short-lived molecule having a limited duration of action (half life = 0.54-0-67 h [33]), analogs with a high affinity for melatonin receptors and a longer duration of action have been synthesized with a potential therapeutic efficacy to treat insomnia and psychiatric disorders like depression and bipolar affective disorder [34]. Ramelteon was the first

of these molecules approved by the U.S. Food and Drug Administration to be used in the treatment of insomnia [35] and its potential use in AD together with that of melatonin is discussed in this review article.

2. Melatonin in AD

Melatonin is synthesized both in the pineal gland and in a number of peripheral organs and tissues by a process starting with tryptophan conversion to serotonin (reviewed in [36]). Serotonin is then acetylated to form N-acetylserotonin by the enzyme arylakylamine N-acetyltransferase while N-acetylserotonin is converted into melatonin by the enzyme hydroxyindole-O-methyl transferase [37, 38]. Once formed melatonin is not stored within the pineal gland it diffuses into the capillary blood and the cerebrospinal fluid (CSF) [39, 40]. CSF melatonin values are nearly 30 times higher than those in the blood; thus, the brain tissue has a higher melatonin concentration than any other tissue in the body [41].

Regional distribution of melatonin in different areas of the brain varies and early studies have shown that hypothalamic melatonin concentrations are nearly fifty times higher than in plasma [42–44]. While tissue melatonin only exhibits a moderate circadian variation, circulating melatonin exhibits most pronounced circadian rhythm with highest levels occurring at night and very low levels during daytime [36].

Circulating melatonin is metabolized mainly in the liver via hydroxylation in the C6 position by cytochrome P$_{450}$ monooxygenases (CYP1A2;CYP1A1) [45]. It is thereafter conjugated with sulphate to form 6-sulfatoxymelatonin (aMT6S), the main metabolite of melatonin in urine. In the brain, melatonin is metabolized to kynuramine derivatives like N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) [46, 47]. In several tissues melatonin is also nonenzymatically metabolized to cyclic 3-hydroxy melatonin [48].

Melatonin is involved in the control of various physiological functions such as circadian rhythmicity [49, 50], sleep regulation [51, 52], immune function [53, 54], antioxidant defense [55, 56], control of reproduction [57–59], inhibition of tumor growth [60, 61], and control of human mood [62, 63]. Melatonin participates in many of these functions by acting through G-protein membrane receptors, the MT$_1$ and MT$_2$ melatonin receptors [64–66]. Nuclear melatonin receptors belonging to the RZR/ROR α receptor class have also been described [56, 67, 68]. Melatonin also acts directly on the cells without the intervention of any of these receptors by binding to intracellular proteins like calmodulin [69] or tubulin [70]. In general, the free radical scavenging action of melatonin does not involve receptors except for the induction of synthesis of some antioxidant enzymes like γ-glutamylcysteine synthase that involves RZR/ROR α receptors [71].

In view of the involvement of oxidative stress in AD, melatonin represents an interesting neuroprotective agent as it antagonizes oxidative stress both in a direct and in an indirect way [55, 56, 72, 73]. In the N2a murine neuroblastoma cell model Pappolla et al. [74] first demonstrated

that coincubation of Aβ with melatonin significantly reduced several features of apoptosis like cellular shrinkage or formation of membrane blubs. In a number of studies melatonin prevented the death of neuroblastoma cells exposed to Aβ [5, 75, 76].

Several animal models of AD have been used to study the possible antioxidative and antiapoptotic actions of melatonin in arresting neuronal lesions. Okadaic acid induces physiological and biochemical changes similar to those seen in AD. Increased levels of 4-hydroxynonenal in cultured neuronal cells have been found following administration of okadaic acid [77]. After the administration of antioxidants like melatonin or vitamin C, the effects of okadaic acid on NIE 115 neuronal cells were prevented effectively [78]. Melatonin was more effective than vitamin C, since it not only prevented the free radical-induced damage with greater efficiency but also increased the activity of the antioxidant enzymes glutathione-S transferase and glutathione reductase [78].

Several studies indicate that the apoptosis of astrocytes contribute to the pathogenesis of AD (sees [79]). Astrocytes exhibit tau phosphorylation and activation of stress kinases as seen in AD pathology. They also produce apolipoprotein E4 (apoE4) that aggravates Aβ neurodegenerative effects [80, 81]. During interaction with Aβ, astrocytes lose control over NO production leading to the neurotoxic peroxynitrate formation. By treating the C6 astroglioma cells with melatonin, the increase in NO production induced by Aβ was effectively prevented [82].

3. Molecular Mechanisms of Melatonin's Anti-Amyloid Actions

Melatonin not only reduced apoptosis but also exerts its antiamyloid actions through additional mechanisms. One of them is by preventing Aβ-induced mitochondrial damage and disruption of respiration. Melatonin administration prevented Aβ action on mitochondrial DNA proteins and level of lipid peroxidation [75]. In this aspect it is interesting to note that melatonin's metabolite AFMK also offered protection from Aβ-induced mitochondrial oxidative stress [83] although a higher concentration was needed.

Melatonin inhibits the formation of amyloid fibrils as demonstrated by different techniques [84, 85]. The structural analog of melatonin indole-3-propionic acid not only shares the radical scavenging activity of melatonin [86] but also exhibits similar or even higher antifibrillogenic activity [87].

Several lipoproteins can modulate fibrillogenesis [88]. Melatonin was shown to reverse the profibrillogenic activity of apoE4 and to antagonize the neurotoxic combinations of Aβ and apoE4 or apoE3 [83]. ApoE4 is also produced by astrocytes and aggravates Aβ effects showing thereby the mutual interaction of Aβ protein and apo-E4 in the astrocyte-neuron interactions [81]. The antifibrillogenic effects of melatonin and its metabolites were observed not only in vitro but also in vivo in transgenic mouse models [84, 89, 90]. Protection from Aβ toxicity was observed, especially at the mitochondrial level.

As mentioned above, chronic intermittent hypoxia has been shown to induce Aβ protein generation by upregulating the APP processing enzymes BACE and PSEN-1 [28–30]. The daily administration of melatonin (10 mg/kg) prior to a short-term hypoxia prevented the generation of Aβ protein but it did not reduce the increase of HIF-1 transcription factor induced by hypoxia [32]. Hence it was suggested that melatonin's neuroprotective effect against amyloid-β-peptide was due to its direct free radical scavenging properties actions [32].

Another manifestation of AD studied in experimental models is the expression of protein hyperphosphorylation and cytoskeletal disorganization. Calyculin A, an inhibitor of protein phosphatases (PP), was used in neuroblastoma N2 cells to examine this point. Calyculin A resulted in activation of glycogen synthase kinase 3 (GSK-3), a redox-controlled enzyme involved in various regulatory mechanisms of the cell, and the consequent hyperphosphorylation of tau [91]. Melatonin administration decreased oxidative stress and tau hyperphosphorylation and reversed GSK-3 activation showing thereby that it not only acts as an antioxidant but also interferes with the phosphorylation system, particularly stress kinases [91].

The inhibition of PP-2A and PP-1 brought about by calyculin A caused hyperphosphorylation of tau and of neurofilaments, synaptophysin loss, and spatial memory retention impairment, an effect counteracted by the administration of melatonin i.p. for 9 days before calyculin injection [92]. Melatonin also partially reversed the phosphorylation of the catalytic subunit of PP-2A at tyrosine 307 (Y307) crucial site regulating the activity of PP-2A, and reduced malondialdehyde levels induced by calyculin A [92]. Melatonin also attenuated tau hyperphosphorylation induced by wortmannin [93, 94] and isoproterenol [95].

Tyrosine kinase (trk) receptors, important elements of the phosphorylation system, as well as neurotrophins, are affected by Aβ and other oxidotoxins and melatonin normalized in neuroblastoma cells trk and neurotrophin expression [96]. Recent studies using organotypic hippocampal studies confirmed that the presence of melatonin (25–100 μM) prevented the cell damage induced by exposure to Aβ reducing the activation of GSK-3β, the phosphorylation of tau protein, and the Aβ-induced increases of TNF-α and IL-6 levels [97]. The chronobiological aspects of melatonin-Aβ interaction are underlined by a study describing the protective effect of melatonin against the circadian changes produced by Aβ_{25-35} microinjection into the suprachiasmatic nuclei (SCN) of golden hamsters [98].

4. Potential Therapeutic Value of Melatonin in AD

A number of studies in AD patients have indicated that there is a profound disturbance in sleep/wake cycle associated with the progression of the disease. Cross-sectional studies reveal that sleep disturbances are associated with memory and cognitive impairment. [16–19]. A severe disruption of the circadian timing system occurs in AD as indicated by

alterations in numerous overt rhythms like body temperature, glucocorticoids, and/or plasma melatonin [22, 99, 100]. The internal desynchronization of rhythms is significant in AD patients [101, 102].

"Sundowning" is a chronobiological phenomenon observed in AD patients in conjunction with sleep-wake disturbances, including symptoms like disorganized thinking, reduced ability to maintain attention to external stimuli, agitation, wandering, and perceptual and emotional disturbances, all appearing in late afternoon or early evening [99, 103, 104]. Chronotherapeutic interventions such as exposure to bright light and/or timed administration of melatonin in selected circadian phases alleviated sundowning symptoms like wandering, agitation and delirium and also improved sleep-wake patterns of AD patients [105].

A number of studies have revealed that melatonin levels are lower in AD patients as compared to age-matched control subjects [20–22, 106]. The decreased CSF melatonin levels of AD patients were attributed to decreased melatonin production. CSF melatonin levels decreased even in preclinical stages (Braak stages-1) when patients did not manifest cognitive impairment [107] suggesting thereby that reduction in CSF melatonin may be an early marker (and cause) for incoming AD. The decrease of melatonin levels in AD was attributed to a defective retinohypothalamic tract or SCN-pineal connections [108]. The impaired melatonin production at night correlates significantly with the severity of mental impairment of demented patients [109]. As AD patients have profound deficiency of endogenous melatonin, replacement of levels of melatonin in the brain could be a therapeutic strategy for arresting the progress of the disease. Melatonin's neuroprotective and vasoprotective properties would help in enhancing cerebral blood flow and would help to improve the clinical condition of AD patients [23].

Sleep disturbances exacerbate memory and cognitive impairment [110]. Therefore, optimization in management of sleep disturbances is of paramount importance in treating AD patients. In an initial study on 14 AD patients with 6–9 mg of melatonin given for 2-3 year period it was noted that melatonin improved sleep quality [111]. Sundowning, diagnosed clinically, was no longer detectable in 12 out of 14 patients. Reduction in cognitive impairment and amnesia was also noted. This should be contrasted with the significant deterioration of the clinical conditions expected from patients after 1–3 year of evolution of AD [111, 112].

Several studies support the efficacy of melatonin in treating sleep and chronobiologic disorders in AD patients (Table 1). The administration of melatonin (6 mg/day) for 4 weeks to AD patients reduced nighttime activity as compared to placebo [113]. An improvement of sleep and alleviation of sundowning were reported in 11 AD patients treated with melatonin (3 mg/day at bedtime) and evaluated by using actigraphy [114]. Improvement in behavioral signs was reported with use of 6–9 mg/day of melatonin for 4 months in AD patients with sleep disturbances [115].

In a double blind study conducted on AD patients it was noted that 3 mg/day of melatonin significantly prolonged actigraphically evaluated sleep time, decreased activity in night, and improved cognitive functions [119]. In a multicenter, randomized, placebo-controlled clinical trial of a sample of 157 AD patients with sleep disturbances, melatonin or placebo was administered for a period of 2 months [120]. In actigraphic studies a trend to increased nocturnal total sleep time and decreased wake after sleep onset was noted in the melatonin-treated group. On subjective measures by caregiver ratings significant improvement in sleep quality was noted with 2.5 mg sustained release melatonin relative to placebo [120].

Negative results with the use of melatonin in fully developed AD were also published. For example, in a study in which melatonin (8.5 mg fast release and 1.5 mg sustained release) was administered at 10.00 PM for 10 consecutive nights to patients with AD, no significant difference was noticed with placebo on sleep, circadian rhythms and, agitation [124]. Although the lack of beneficial effect of melatonin in this study on sleep could be attributed to the short period of time examined, it must be noted that large interindividual differences between patients suffering from a neurodegenerative disease are not uncommon. It should be also taken into account that melatonin, though having some sedating and sleep latency-reducing properties, does not primarily act as a sleeping pill, but mainly as a chronobiotic.

Since the circadian oscillator system is obviously affected in AD patients showing severe sleep disturbances, the efficacy of melatonin should be expected to depend on disease progression. In a recent paper one of us summarized the published data concerning melatonin treatment of AD patients [125] (Table 1). Eight reports (5 open-label studies, 2 case reports) ($N = 89$ patients) supported a possible efficacy of melatonin: sleep quality improved and in patients with AD sundowning was reduced and cognitive decay showed less progression. In 6 double blind, randomized placebo-controlled trials ($N = 210$) sleep was objectively measured by wrist actigraphy and additionally neuropsychological assessment and sleep quality were subjectively evaluated. Sleep quality increased and sundowning decreased significantly and cognitive performance improved in 4 studies ($N = 143$) whereas there was absence of effects in 2 studies ($N = 67$) [125]. Therefore, the question whether melatonin has a causal value in preventing or treating AD, affecting disease progression of the neuropathology and the driving mechanisms, remains unanswered. Double-blind multicenter studies are needed to further explore and investigate the potential and usefulness of melatonin as an antidementia drug. Its apparent usefulness in symptomatic treatment, concerning sleep, sundowning, and so forth, even in a progressed state, further underlines the need for such decisive studies.

It has been shown that with degeneration of the SCN, the master body clock, there is a decrease in the expression of MT_1 receptors so that strength of melatonin as a synchronizing agent is reduced [126]. Moreover the input of neural pathways involved in entrainment (synchronization) of the central clock may become dysfunctional or less sensitive during aging and even more so in AD [127]. In a large multicentre trial only a nonsignificant trend to improvement in the circadian rhythm disturbance of AD is when treatment

TABLE 1: Clinical studies on melatonin efficacy in AD.

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference
Open-label study	10 (6, 4) demented patients	3 mg melatonin p.o./daily at bed time	3 weeks	Daily logs of sleep and wake quality completed by caretakers	Seven out of ten dementia patients having sleep disorders treated with melatonin showed a significant decrease in sundowning and reduced variability of sleep onset time	[116]
Open-label study	14 (8, 14) AD patients	9 mg melatonin p.o./daily at bed time	22 to 35 months	Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment.	At the time of assessment, a significant improvement of sleep quality was found. Sundowning was not longer detectable in 12 patients and persisted, although attenuated in 2 patients. Clinically, the patients exhibited lack of progression of the cognitive and behavioral signs of the disease during the time they received melatonin.	[111]
Case report	Monozygotic twins with AD of 8 years duration	One of the patients was treated with melatonin 9 mg p.o./daily at bed time.	36 months	Neuropsychological assessment. Neuroimaging.	Sleep and cognitive function severely impaired in the twin not receiving melatonin as compared to the melatonin-treated twin.	[112]
Open-label, placebo-controlled trial	14 AD patients	6 mg melatonin p.o./daily at bed time or placebo	4 weeks	Daily logs of sleep and wake quality completed by caretakers. Actigraphy	The 7 AD patients receiving melatonin showed a significantly reduced percentage of nighttime activity compared to a placebo group	[113]
Open-label study	11 (3, 8) AD patients	3 mg melatonin p.o./daily at bed time	3 weeks	Daily logs of sleep and wake quality completed by the nurses.	Analysis revealed a significant decrease in agitated behaviors in all three shifts and a significant decrease in daytime sleepiness.	[117]
Open-label study	45 (19, 26) AD patients	6–9 mg melatonin p.o./daily at bed time	4 months	Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment.	Melatonin improved sleep and suppressed sundowning, an effect seen regardless of the concomitant medication employed to treat cognitive or behavioral signs of AD.	[115]
Randomized double blind placebo controlled cross over study	25 AD patients	6 mg of slow release melatonin p.o. or placebo at bed time	7 weeks	Actigraphy	Melatonin had no effect on median total time asleep, number of awakenings, or sleep efficiency.	[118]
Double-blind, placebo-controlled study	20 (3, 17) AD patients	Placebo or 3 mg melatonin p.o./daily at bed time	4 weeks	Actigraphy. Neuropsychological assessment.	Melatonin significantly prolonged the sleep time and decreased activity in the night. Cognitive function was improved by melatonin.	[119]
Randomized, placebo-controlled clinical trial	157 (70, 87) AD patients	2.5 mg slow-release melatonin, or 10 mg melatonin or placebo at bed time	2 months	Actigraphy. Caregiver ratings of sleep quality	Nonsignificant trends for increased nocturnal total sleep time and decreased wake after sleep onset were observed in the melatonin groups relative to placebo. On subjective measures, caregiver ratings of sleep quality showed improvement in the 2.5 mg sustained-release melatonin group relative to placebo.	[120]

Table 1: Continued.

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference
Open-label study	7 (4, 3) AD patients	3 mg melatonin p.o./daily at bed time	3 weeks	Actigraphy. Neuropsychological assessment.	Complete remission of daynight rhythm disturbances or sundowning was seen in 4 patients, with partial remission in other 2.	[114]
Randomized, placebo-controlled study	17 AD patients	3 mg melatonin p.o./daily at bed time (7 patients). Placebo (10 patients)	2 weeks	Actigraphy. Neuropsychological assessment.	In melatonin-treated group, actigraphic nocturnal activity and agitation showed significant reductions compared to baseline.	[121]
Randomized, placebo-controlled study	50 AD patients	Morning light exposure (2,500 lux, 1 h) and 5 mg melatonin ($n = 16$) or placebo ($n = 17$) in the evening. Control subjects ($n = 17$) received usual indoor light (150–200 lux).	10 weeks	Night time sleep variables, day sleep time, day activity, day : night sleep ratio, and rest-activity parameters were determined using actigraphy.	Light treatment alone did not improve night time sleep, daytime wake, or rest-activity rhythm. Light treatment plus melatonin increased daytime wake time and activity levels and strengthened the rest-activity rhythm.	[122]
Case report	68-year-old man with AD who developed rapid eye movement (REM) sleep behavior disorder	5–10 mg melatonin p.o./daily at bed time.	20 months	Polysomnography	Melatonin was effective to suppress REM sleep behavior disorder	[123]
Randomized, placebo-controlled study	41 (13, 28) AD patients	Melatonin (8.5 mg immediate release and 1.5 mg sustained release) ($N = 24$) or placebo ($N = 17$) administered at 10 : 00 P.M.	10 days	Actigraphy.	There were no significant effects of melatonin, compared with placebo, on sleep, circadian rhythms, or agitation.	[124]

was done using melatonin [120]. Because MT_1 receptor expression in the SCN is decreased it is certainly possible that melatonin will be ineffective as a synchronizing agent although it is possible that a higher dose of melatonin or a more potent melatonin agonist such as ramelteon may be useful. Another strategy could be exposure to bright light [128] (see below).

5. Melatonin as a Therapeutic Agent for Mild Cognitive Impairment

As outlined, melatonin acts at different levels relevant to the development and manifestation of AD. The antioxidant, mitochondrial, and antiamyloidogenic effects may be seen as a possibility of interfering with the onset of the disease. Therefore, early beginning of treatment may be decisive [129].

Mild cognitive impairment (MCI) is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia [130]. Approximately 12% of MCI converts to AD or other dementia disorders every year. Since MCI may represent prodromal A,D it should be adequately diagnosed and treated. Indeed, the degenerative process in AD brain starts 20–30 years before the clinical onset of the disease [130]. During this phase, plaques and tangles loads increase and at a certain threshold the first symptom appears. As already mentioned, CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I-II), suggesting that the reduction in CSF melatonin may be an early trigger and marker for AD. Therefore, MCI could be an appropriate moment for initiating any melatonin treatment aiming to affect progression of the disease. Studies on melatonin effect on MCI are summarized in Table 2.

TABLE 2: Clinical studies on melatonin efficacy in MCI.

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference(s)
Double-blind, placebo-controlled, crossover study	10 (4, 6) patients with mild cognitive impairment (MCI)	6 mg melatonin p.o./daily at bed time	10 days	Actigraphy. Neuropsychological assessment.	Enhanced the rest-activity rhythm and improved sleep quality (reduced sleep onset latency and in the number of transitions from sleep to wakefulness Total sleep time unaffected. The ability to remember previously learned items improved along with a significant reduction in depressed mood.	[131]
Double-blind, placebo-controlled pilot study	26 individuals with age-related MCI	1 mg melatonin p.o. or placebo at bed time	4 weeks	Sleep questionnaire and a battery of cognitive tests at baseline and at 4 weeks	Melatonin administration improved reported morning "restedness" and sleep latency after nocturnal awakening and also improved scores on the California Verbal Learning Test-interference subtest.	[132]
Open-label, retrospective study	50 (13, 37) MCI outpatients	25 had received daily 3–9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication	9–18 months	Daily logs of sleep and wake quality. Initial and final neuropsychological assessment.	Patients treated with melatonin showed significantly better performance in neuropsychological assessment. Abnormally high. Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality.	[133]
Randomized, double blind, placebo-controlled study	354 individuals with age-related cognitive decay	prolonged release melatonin (Circadin, 2 mg) or placebo, 2 h before bedtime	3 weeks	Leeds Sleep Evaluation and Pittsburgh Sleep Questionnaires, Clinical Global Improvement scale score and quality of life.	PR-melatonin resulted in significant and clinically meaningful improvements in sleep quality, morning alertness, sleep onset latency, and quality of life	[134]
Long-term, double-blind, placebo-controlled, 2 × 2 factorial randomized study	189 (19, 170) individuals with age-related cognitive decay	Long-term daily treatment with whole-day bright (1000 lux) or dim (300 lux) light. Evening melatonin (2.5 mg) or placebo administration	1 to 3.5 years	Standardized scales for cognitive and noncognitive symptoms, limitations of activities of daily living, and adverse effects assessed every 6 months.	Light attenuated cognitive deterioration and also ameliorated depressive symptoms. Melatonin shortened sleep onset latency and increased sleep duration but adversely affected scores for depression. The combined treatment of bright light plus melatonin showed the best effects.	[105]
Prospective, randomized, double-blind, placebo-controlled, study	22 (15, 7) individuals with age-related cognitive decay	Participants received 2 months of melatonin (5 mg o.o./day) and 2 months of placebo	2 months	Sleep disorders were evaluated with the Northside Hospital Sleep Medicine Institute (NHSMI) test. Behavioral disorders were evaluated with the Yesavage Geriatric Depression Scale and Goldberg Anxiety Scale.	Melatonin treatment significantly improved sleep quality scores. Depression also improved significantly after melatonin administration.	[135]

The first report on melatonin treatment of 10 MCI patients (6 mg/day for 10 days) indicated that besides enhancing the rest-activity rhythm and improved sleep quality the ability to remember previously learned items improved along with a significant reduction in depressed mood [131]. In another double-blind, placebo-controlled pilot study performed in 26 individuals with age-related MCI, the administration of 1 mg melatonin or placebo at bed time for 4 weeks resulted in improvement of sleep and of scores on the California Verbal Learning Test-interference subtest [132].

In a retrospective study of a group of 25 MCI patients who received melatonin (3–9 mg per day) for 9 to 18 months in comparison to a similar group of 25 MCI patients who did not receive it [133], patients treated with melatonin showed significantly better performance in a number of neuropsychological tests. Abnormally high Beck Depression Inventory scores decreased in melatonin treated patients, concomitantly with an improvement in wakefulness and sleep quality. The results suggested that melatonin could be a useful add-on drug for treating MCI in a clinic environment [133]. A follow up of that study has now been completed on a group of 35 MCI patients receiving melatonin for 9 to 24 months with essentially similar results [125].

A randomized controlled trial on the effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities was published [105]. The authors concluded that light has a benefit in improving some cognitive and noncognitive symptoms of MCI which was amplified by the conjoint administration of melatonin. In other two similar studies, one of them using the prolonged release preparation of melatonin (Circadin) recently approved by the European Medicines Agency, melatonin resulted in significant and clinically meaningful improvements of sleep quality, morning alertness, sleep onset latency and quality of life in old patients with mild cognitive impairment [134, 135]. In these studies melatonin treatment also improved mood. The evaluation of the published data concerning melatonin treatment of MCI that include 5 double blind, randomized placebo-controlled trials, and 1 open-label retrospective study ($N = 651$) all agress in indicating that treatment with daily evening melatonin improves sleep quality and cognitive performance in MCI [125] (Table 2).

6. Use of Melatonin Agonist, Ramelteon in AD

As AD is associated with disturbed sleep/wake rhythms and circadian rhythm disturbances, a melatonin agonist with higher affinity to melatonin MT_1 and MT_2 receptors with a longer duration would theoretically be beneficial in tackling sleep-wake and circadian rhythm disturbances. In this aspect, ramelteon, which is the first melatonin receptor agonist approved by FDA with activity on MT_1 and MT_2 receptors, should be considered [136, 137].

The chemical structure of ramelteon is: (S)-N-[2-(1,6,7,8-tetrahydro-2Hindeno[5,4-b]furan-8-yl)ethyl] propionamide. This melatonin receptor agonist has a chemical formula $C_{16}H_{21}NO_2$ with a molecular weight 259.34.

Receptor binding studies indicated that ramelteon has high selectivity for MT_1 and MT_2 receptors, with little affinity for quinone reductase 2 binding [138]. The selectivity of ramelteon for MT_1 has been found >1000-fold over that of MT_2 receptors. It is well known that melatonin exerts its hypnotic effects through the activation of the MT_1 and MT_2 melatonin receptors [139]. Although both MT_1 and MT_2 receptors are involved in the regulation of sleep, the selectivity of MT_1 receptors by ramelteon suggests that it targets sleep onset more specifically than melatonin [140]. Ramelteon has been found to have no affinity for benzodiazepine (BZP), dopamine, opiate, or serotonin receptor binding sites [138]. Hence ramelteon has advantages over other hypnotic drugs in not causing rebound insomnia, withdrawal symptoms, or dependence which is common with the activation of BZP, opiate, or dopamine receptors.

On oral administration, ramelteon is rapidly absorbed with a T_{max} of less than 1 hour [141]. The absolute bioavailability of the oral formulation of ramelteon is less than 2% (range 0.5% to 12%) [141]. It is metabolized mainly in the liver via oxidation to hydroxyl and carbonyl groups and then conjugated with glucuronide. CYP1A2 is the major hepatic enzyme involved in ramelteon metabolism. Four principal metabolites ramelteon, that is, M-I, M-II, M-III, and M-IV, have been identified [141]. Among these, M-II has been found to occur in much higher concentration with systemic concentration being 20- to 100- fold greater than ramelteon.

Ramelteon is rapidly excreted and its elimination is significantly higher in elderly than in younger adults [142]. The influence of age and gender on the pharmacokinetics and pharmacodynamics of ramelteon has been evaluated in healthy volunteers following the administration of a single dose of 16 mg of ramelteon. When compared to young volunteers, ramelteon clearance was significantly reduced in elderly volunteers and its half life significantly increased. No significant effect of gender was observed [142]. The contribution of ramelteon's metabolites on the net pharmacologic activity was also evaluated. Among the four metabolites produced, the activity of M-II was to be about 30-fold lower than that of ramelteon, but its exposure exceeds exposure to ramelteon by a factor 30. It was thus suggested that M-II may contribute to net clinical activity of ramelteon [142].

The subjective efficacy of ramelteon was evaluated in clinical trials consisting of 829 elderly outpatients with chronic insomnia; 701 patients (128 patients discontinued) were treated for a period of 5-weeks with 4 mg and 8 mg ramelteon [143]. Patients in both ramelteon groups reported significant reductions in sleep onset latency (SOL) and increases in total sleep time (TST). Continuation of this study on 100 elderly patients established the efficacy of ramelteon in improving TST and decreasing SOL [144]. A number of studies have now established the efficacy of ramelteon in treating patients with chronic insomnia [145–147].

Concerning the safety and adverse effects with ramelteon, in a double blind placebo controlled study of rebound insomnia (sleep latency after treatment discontinuation) Roth and co-workers [143] evaluated each of the 7 nights
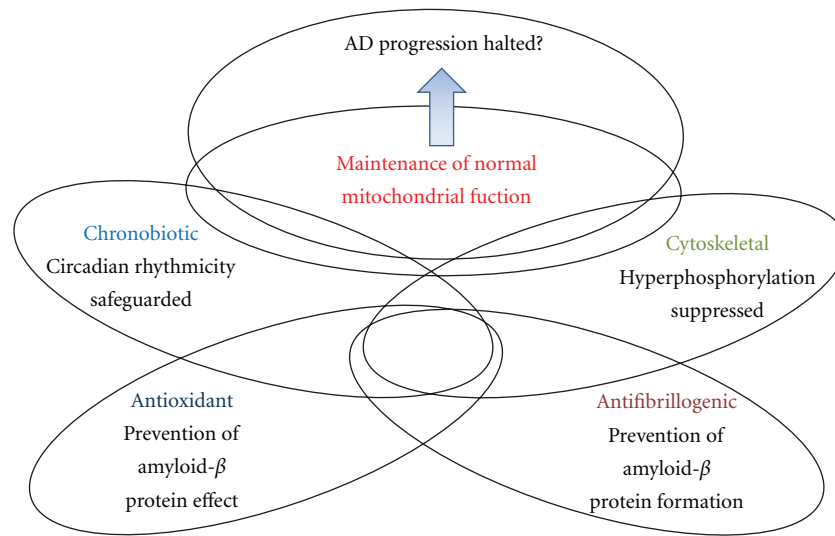
FIGURE 1: Melatonergic agonists in AD. The multiple effects of melatonin discussed in the text and the different degree of overlap (interrelations and mutual influences) are indicated by the respective intersections in the scheme.

of placebo run-out period. It was noted that during each of the 7 nights, patients in both ramelteon treatment groups (4 mg/day and 8 mg/day) maintained a similar or greater reduction in sleep latency from baseline as compared to those receiving placebo [143]. Withdrawal effects, as assessed by a BZP withdrawal symptom questionnaire, did not differ from the placebo group [143]. In another recent study it was noted that ramelteon did not affect alertness or the ability to concentrate, indicating no next-morning residual effects [148]. The incidence of adverse effects in ramelteon-treated patients in a 5 week study was found to be similar to that of placebo-treated patients. The adverse effects included mild gastrointestinal disturbances and nervous system effects such as dizziness, headache, somnolence, depression, fatigue, myalgia, and exacerbated eye pain [143].

Ramelteon not only has the potential in improving the sleep quality of AD and other neurodegenerative patients but can also offer neuroprotection as well in AD [149]. As ramelteon is a melatonin agonist with more potency and longer duration of action, it could act more efficiently than melatonin in its actions against neurotoxic effects involved in the pathogenesis of AD.

To what extent ramelteon reproduces the nonreceptor mediated effects of melatonin is not known. Ramelteon displays no relevant antioxidant capacity in the ABTS radical cation assay, as compared to luzindole or melatonin [150]. However, MT_1/MT_2 receptor-mediated effects on the upregulation of several antioxidant enzymes by physiological concentration of melatonin [151] such as glutathione peroxidase, glutathione reductase, γ-glutamylcysteine synthase, glucose-6-phosphate dehydrogenase, hemoperoxidase/catalase, Cu,Zn- and Mn-superoxide dismutases (reviewed in [152–155] can well give the basis for the use of ramelteon in AD. Since there are extensive data indicating a loss of melatonin receptors in AD patients, including the cerebral cortex and pineal gland (MT_1 and MT_2 receptors) [156], the hippocampus [157] and retina

[158] (MT_2 receptors) and the cerebrovascular system [159], and SCN [126, 128] (MT_1 receptors), the chances of alleviating symptoms such as sundowning and disturbed sleep by giving the MT_1/MT_2 receptor agonist may vanish in late AD patients.

In addition, it has been suggested that melatonin and its receptors participate in neurodevelopment and regulation of neurotrophic factors [160]. In vitro studies have shown that melatonin promotes the viability and neuronal differentiation of neural stem cells and increases the production brain-derived neurotrophic factor (BDNF) by acting through MT_1 receptors [161]. In mouse cerebellar granule cells in culture ramelteon increased the neural content of BDNF [162]. Therefore, if ramelteon treatment is capable of regulating brain BDNF levels, it could be used as a possible therapeutic agent in neurodegenerative diseases like AD for treating symptoms other than sleep disturbances.

7. Concluding Remarks

As AD disease involves a complex physiopathology, it has been suggested that monotherapy targeting early single steps in this complex cascade process may not be of much help [149]. Pleiotrophic drugs that can act independently by different routes including antioxidant, antiinflammatory, and antiamyloid effects would be much beneficial in the treatment of AD and other neurodegenerative disorders. Available evidence indicates suppression of GSK-3β overactivity; neuroinflammation and mitochondrial impairment are some of the combined strategies required in AD.

Melatonin is a pleiotropic molecule with antioxidant, antiinflammatory and antinitridergic properties [56, 154, 163]. It has also a role in sleep induction, and this is important in view that sleep deprivation is one of the cardinal features seen in AD and other neurodegenerative diseases. Sleep deprivation is associated with GSK-3β activation [164], altered proteosomal processing [165],

oxidative damage [166], impaired mitochondrial integrity and function [167], and neurodegenerative inflammation [168]. Therefore, improvement of insomnia in neurodegenerative conditions and particularly in AD is a good practical approach for arresting the progression of the disease (Figure 1).

Melatonin and particularly ramelteon can be greatly beneficial in preventing the insomnia-induced damage of neuronal cells and can be of therapeutic value in treating AD. Owing to its potent effect on MT_1 and MT_2 receptors, ramelteon activates sleep onset by influencing the hypothalamic "sleep switch" downstream from the SCN more efficiently than melatonin itself [35]. Multicenter, placebo-controlled clinical trials using ramelteon are needed to prove the efficacy of this drug in arresting the progression or prevention of AD or remission in the early stages of AD such as MCI.

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Conflict of Interest Statement and Disclosure Statement

S. R. Pandi-Perumal is a stockholder and the President and Chief Executive Office of Somnogen Inc., a New York Corporation. He declared no competing interests that might be perceived to influence the content of this article. All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this manuscript.

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