

Alzheimer's Disease: Focus on the Neuroprotective Role of Melatonin

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Abstract

Alzheimer's disease (AD) is characterized by a progressive loss of memory and cognitive function, and by behavioural and sleep disturbances, including insomnia. The pathophysiology of AD has been attributed to oxidative stress-induced amyloid- β protein ($A\beta$) deposition. Abnormal tau protein, mitochondrial dysfunction and protein hyper-phosphorylation have been demonstrated in neural tissues of AD patients. AD patients exhibit severe sleep-wake disturbances and these sleep disturbances are associated with rapid cognitive decline and memory impairment. Optimally effective management of AD patients will likely require a drug that can arrest $A\beta$ - induced neurotoxic effects and can also restore the disturbed sleep-wake rhythm, with improvement in sleep quality. In this context, the pineal hormone melatonin has been demonstrated to be an effective antioxidant that can prevent $A\beta$ - induced neurotoxic effects through a variety of mechanisms. Sleep deprivation itself produces many effects including oxidative damage, impaired mitochondrial function, neurodegenerative inflammation, altered proteosomal processing and abnormal activation of enzymes. In addition to treating the signs and symptoms of AD, treatment of sleep disturbances may also be necessary for preventing and arresting AD progression. Besides melatonin, a number of melatonergic agonists such as ramelteon, agomelatine and tasimelteon are now used clinically

for treating insomnia and other sleep disorders. Their use may also be beneficial in treating Alzheimer's disease.

Keywords: Alzheimer's disease; Melatonin; Amyloid- β protein; Insomnia; Anti-oxidant; Sleep

Introduction

Age-associated neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD) are attributed to increased oxidative damage of the central nervous system [1-5]. Because of its high rate of oxygen consumption and high content of polyunsaturated fatty acids, the brain is easily susceptible to increased oxidative stress. Increased protein, lipid and DNA oxidation has been demonstrated in brain samples of AD patients [6]. Protein carbonyl 3, 3'-dityrosine and 3-nitrotyrosine levels in post-mortem brain samples of AD patients exhibit increased oxidative and nitrosative protein modification in the hippocampal and neocortical regions [7].

Oxidative damage is related to other primary cytopathological features of AD, including mitochondrial demise and amyloid- β ($A\beta$) protein. In addition to age, the other major risk factor for AD is gender. The incidence of AD is higher in women than in men, and this has been attributed to increased mitochondrial toxicity induced by $A\beta$ proteins [8]. Increased $A\beta$ levels trigger all of the most important pathological features of AD, including tau hyper-phosphorylation, formation of neurofibrillary tangles, synaptic dysfunction, and neuronal cell death [9]. Abnormal accumulation of an $A\beta$ protein results in the formation of toxic oligomers that cause synaptic damage, alterations in glutamate receptors, circuit hyperexcitability and alterations in signalling pathways related to synaptic plasticity [10]. Recent studies implicate mitochondrial pathology as an important contributory factor in the late onset forms of AD [11].

Many of the current drugs used in the treatment of AD improve symptoms but do not have any significant disease-modifying effects. It is estimated that the cost of care to the average AD patient family exceeds \$ 90,000 per year [12], while the societal cost exceeds \$ 100 billion per annum [13, 14]. From these figures it is evident that there is an urgent

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need for speedy implementation of preventive treatment programs for arresting the progress of this disease and other neurodegenerative diseases. In the last 15 years, rapid developments have taken place on the role of the pineal hormone melatonin in arresting the neurotoxic effects induced by A β in animal studies. Melatonin has been shown to antagonize A β -induced effects such as increased oxidative stress, increased lipid peroxidation, formation of protein carbonyl products, and mitochondrial pathological mechanisms [15]. Thus, melatonin has substantial potential to prevent or delay AD progression, thereby reducing patient symptomatic burden, family expense, and societal cost. Additionally, its capacity to improve sleep disorders can improve AD symptoms and potentially reduce AD neuropathology, as discussed below.

Molecular Pathophysiology of Alzheimer's Disease

In Alzheimer's disease there is progressive loss of cognitive function, with other neurobehavioral abnormalities like agitated behaviour and profound sleep disturbances.

Despite volumes of studies undertaken on AD, its etiology remains unknown. Many mechanisms including chronic inflammation associated with cytokine release, trace element neurotoxicity and free radical generation have each been suggested as possible contributory factors underlying the etiology of AD. The deposition of amyloid plaques in AD causes cell death by induction of oxidative stress, a primary pathogenic mechanism of AD. A β protein deposition initiates flavo-enzyme dependent increases in H₂O₂ and lipid peroxides that increase free radical generation [16]. This increased β -amyloid protein - induced oxidative stress in conjunction with decreased neurotrophic support [17] are the major determinants of AD. Studies undertaken in post-mortem brain samples obtained from AD patients have shown extensive lipid, protein and DNA oxidation [7]. Neural tissues of AD patients exhibit increased levels of peroxidation end-products such as malondialdehyde (MDA), 4-hydroxynonenal, carbonyls and other species [18]. Though A β contributes directly or indirectly to neuronal degeneration, its potential to cause AD depends on an individual's susceptibility to A β -mediated toxicity.

Mitochondrial Involvement in Alzheimer's Disease

Mitochondria are not only the major source of reactive oxygen species (ROS) but also the primary target of attack by ROS and reactive nitrogen species [19]. Damage to the mitochondrial respiratory chain can cause breakdown of the mitochondrial membrane proton potential, opening of the mitochondrial permeability transition pore (mtPTP) and

consequent induction of apoptosis, leading to further generation of free radicals and maintaining a vicious cycle that ultimately results in cell death by either necrotic or apoptotic processes [20]. Findings from a number of studies reveal the involvement of mitochondrial ROS production and subsequent mitochondrial abnormalities in the pathophysiology of AD [21]. Mitochondrial superoxide (O₂⁻) production plays a critical role in the pathological events that follow elevations of A β peptide levels. The histopathological changes of AD, namely extracellular accumulation of oligomeric and fibrillar A β peptides and intracellular neurofibrillary tangles induce functional deficits of the mitochondrial respiratory chain complexes and, thereby, result in mitochondrial dysfunction and enhanced oxidative stress [22].

Amyloid β Peptide and Mitochondrial Interaction as Triggering Events for Alzheimer's Disease Pathogenesis

The amyloid cascade hypothesis proposed by Hardy and Selkoe (2002) suggests the faulty metabolism of amyloid precursor protein as the initiating event in the pathogenesis of AD [23]. Whether extracellular A β - induced mitochondrial damage is the initial triggering event in AD is a subject of debate since A β appears within the mitochondria long before the appearance of extracellular A β deposits. The enzymes that transfer A β to mitochondria have been identified as a complex of translocases of both the outer membrane (TOM) and inner membrane (TIM) [24]. The intracellular presence of A β is one of the major reasons for the reduction of oxygen consumption caused by the electron transport chain, since A β diminishes the enzymatic activity of respiratory chain complexes III and IV [23]. Several other neurotoxic mechanisms, such as the formation of ionic channels that allows increased calcium uptake by mitochondria and mtPTP opening with subsequent inhibition of respiratory complexes [25], have been proposed for A β - induced neurotoxicity that is mediated through intramitochondrial interactions. The fact that A β can accumulate both intracellularly and intramitochondrially has been demonstrated by Rosales-Corral et al (2012) [26], in which intracerebral injection of fibrillar A β caused accumulation of A β both intracellularly and intramitochondrially, deep within the cristae, thereby supporting other investigators' views of intramitochondrial accumulation of A β . In addition to inhibiting mitochondrial respiratory complex activities in AD patients, disturbances in mitochondrial dynamics have also been demonstrated. This includes disturbances in mitochondrial structure, impairment of dynamin - related peptide (Drp1) and imbalances in fission and fusion, with consequent neuronal damage and synaptic loss [27]. Significant disturbances of mitochondrial proteins and lipids also occur following intramitochondrial accumulation of A β , which in turn causes functional impairment of mitochondria

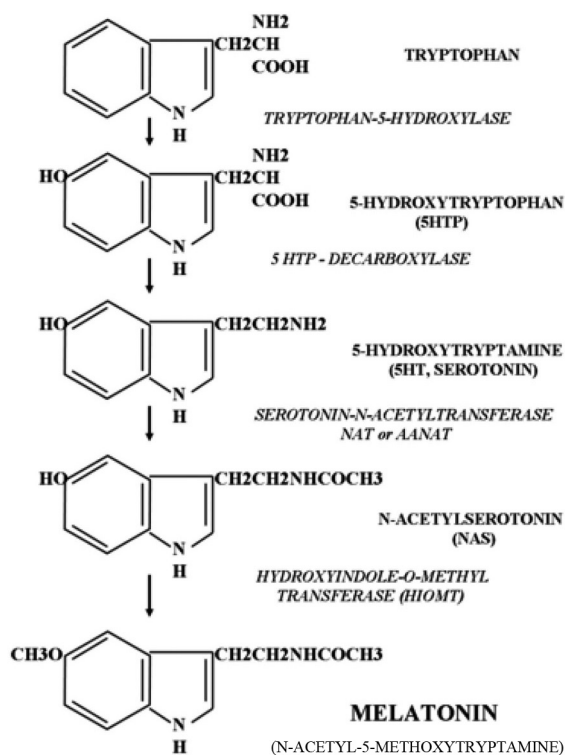


Figure 1. Biosynthesis of melatonin.

in AD [26].

Interaction of A β with cyclophilin D (CypD), a Ca²⁺-associated protein found in the mtPTP, causes CypD translocation from the matrix to the inner membrane, resulting in opening of the mtPTP and consequent mitochondrial swelling, with ensuing cellular and synaptic alterations [28]. CypD deficiency has been shown to attenuate A β -induced mitochondrial oxidative stress, thereby reducing A β -related synaptic dysfunction and cognitive impairment. From this finding it is clear that the interaction of A β and Cyp D is one of the major reasons for mitochondrial pathology in AD. Thus, the mitochondrial aspect of AD with regard to A β -induced free radical generation, excitation-dependent calcium overload and its consequences for the mitochondrial membrane potential and mtPTP all must be taken into account to effectively tackle the disease with suitable drug therapy. Attenuation or prevention of increased oxidative stress seen in AD patients should be the primary goal of strategic treatment for AD.

Melatonin, Biosynthesis and Metabolism

Melatonin (N-acetyl-5-methoxytryptamine) is synthesized mainly by the pineal gland in all mammals but is also formed by many other organs and tissues in the body. It is synthesized from serotonin, and therefore ultimately from trypto-

phan. Serotonin is acetylated to form N-acetylserotonin by the rate limiting enzyme arylalkylamine-N-acetyltransferase (AA-NAT). N-acetylserotonin is then converted into melatonin by the enzyme hydroxyl-indole-O-methyl transferase. The biosynthetic pathway is given in Figure 1. Once formed, melatonin is not stored within the pineal gland but diffuses into the capillary blood and CSF. As melatonin passes through all tissues with ease and CSF melatonin values are nearly 30 times higher than those in the blood, brain tissue has higher melatonin concentrations than any other tissue in the body [29]. While tissue melatonin only exhibits a moderate circadian variation, circulating melatonin exhibits most pronounced circadian rhythms, with the highest levels occurring at night and the lowest levels during daytime in most of the age groups (10 - 70yrs).

Circulating melatonin is metabolized mainly in the liver where it is first hydroxylated at the C6 position by cytochrome P450 mono-oxygenases (CYP1A1 and CYP1A2) and, thereafter, conjugated with sulphate to form 6-sulphatoxymelatonin (aMT6S) [30]. In the brain, melatonin is metabolized to kynuramine derivatives N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK) [31]. A schematic diagram showing the metabolism of melatonin is provided in Figure 2.

Melatonin is involved in the control of various physiological functions of the body, such as co-ordination of circadian rhythms including sleep-wakefulness rhythms, sleep

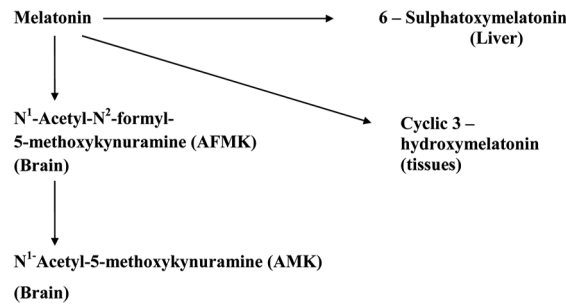


Figure 2. Schematic diagram showing melatonin's metabolic pathways in periphery and in CNS.

regulation, immune function, anti-oxidant functions, control of reproduction, inhibition of tumor growth, and the control of human mood and behaviour and these have been reviewed [32].

Melatonin participates in many of these functions by acting through G-protein membrane receptors like MT_1 and MT_2 melatonin receptors [33]. Melatonin also acts directly on cells without the intervention of any of these receptors by binding to calmodulin [34]. Although the free radical scavenging action of melatonin does not involve any receptors, melatonin's stimulatory action on the formation of the anti-oxidant enzyme γ -glutamylcysteine synthase involves RZR/ROR α receptors [35].

Melatonin in the Treatment of Alzheimer's Disease

Melatonin is an interesting neuroprotective agent as it displays multiple properties through which it antagonizes oxidative stress. One might classify the effects of melatonin as: 1) antioxidant, including influences on mitochondrial metabolism; 2) antifibrillogenic; 3) cytoskeletal, including the suppression of protein hyperphosphorylation. Although these actions are observed at pharmacological concentrations, the relevance of these findings can be appreciated if one considers the relatively high rates of melatonin secretion into the CSF, uptake into the brain tissue and metabolism of melatonin into other neuroprotective compounds such as the kynuramines AFMK and AMK [36]. Melatonin has been shown to prevent the death of neuroblastoma cells exposed to β -amyloid polypeptide. This was established by the pioneering work of Pappolla's research group. Using murine neuroblastoma cells (N2a), Pappola et al, 1997 [37, 38] first demonstrated that co-incubation of neuroblastoma cells with amyloid- β polypeptide and melatonin significantly reduced several features of apoptosis, including cellular shrinkage and formation of membrane blebs.

Recent studies of astrocytes show that astrocytic apop-

toxis contributes to the pathogenesis of AD. Astrocytes exhibit tau phosphorylation and activation of stress kinases, as seen in AD neuronal pathology. They also produce apolipoprotein-E4 (apoE4), aggravating β -amyloid effects [39]. The β -amyloid protein and astrocyte-neuron interaction potentiates the neurodegeneration of AD. During interaction with $A\beta$, astrocytes lose control over glial NO production, thereby forming neurotoxic peroxynitrate. Treatment of C6 astrogloma cells with melatonin effectively prevented the increase in NO production induced by $A\beta$ [40].

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 (a product of lipid peroxidation) in cultured neuronal cells (SY5Y cells) has been found following administration of okadaic acid [41]. With administration of antioxidants like vitamin C or melatonin, the effects of okadaic acid on NIE 115 neuronal cells was effectively prevented [42]. Melatonin was found superior to vitamin C in this study, since it not only prevented free radical-induced damage with greater efficiency but also increased the enzymes glutathione-S transferase and glutathione reductase, which was not evident with vitamin C [42]. In a model of APP695Tg mice, senile plaques appear in the cerebral cortex as early as at 8 months of age. These mice display behavioural manifestations and memory impairments as seen in AD patients. Administration of melatonin (10 mg/kg) alleviated learning and memory deficits and also reduced the number of apoptotic neurons [43].

Molecular Mechanisms of Melatonin's Anti-Amyloid Actions

Melatonin has not only reduced several features of apoptosis, like cellular shrinkage, or formation of membrane blebs as discussed in the earlier paragraphs, but also exerts its anti-amyloid actions through several mechanisms. $A\beta$ by in-

Table 1. Summary of Melatonin's Neuroprotective Effects in AD: Mechanisms

1. Inhibition of amyloid- β protein deposition
2. Inhibits the formation of amyloid fibrils
3. Scavenges free radicals induced by amyloid β protein
4. Inhibits lipid peroxidation reactions in neural tissues
5. Suppresses protein hyperphosphorylation
6. Activates GSK-3 (glycogen synthase kinase)
7. Prevents cytoskeletal disorganization
8. Improves sleep quality in AD patients and may thereby arrest neurodegeneration

ducing oxidative stress damages mitochondrial DNA, forms protein carbonyl, induces lipid peroxidation and impairs the mitochondrial membrane structure and respiration and breaks down the mitochondrial membrane potential. Each of these actions that produce mitochondrial dysfunction was prevented by melatonin administration. Melatonin also inhibits the formation of amyloid fibrils, as demonstrated by different techniques. Both $A\beta_{1-40}$, and $A\beta_{1-42}$ peptides were effectively inhibited [44-45]. A structural analog of melatonin, namely *indole-3-propionic acid*, not only shares melatonin's radical scavenging activity [46] but also exhibits similar or even higher antifibrillogenic activity [45-46]. Lipoproteins including cholesterol and apolipoprotein subtypes can modulate fibrillogenesis. Melatonin has been shown to reverse the profibrillogenic activity of apolipoprotein E4 and to antagonize neurotoxic combinations of $A\beta$ and apoE4 or apoE3 [47]. One other manifestation of AD as studied in experimental models of AD is the expression of protein hyperphosphorylation and cytoskeletal disorganization

driven by glycogen synthase kinase 3 (GSK-3), but melatonin also reversed GSK-3 activation, showing thereby that melatonin not only acts as an antioxidant but also interferes with the phosphorylation system, especially stress kinases [48]. Melatonin has attenuated tau hyperphosphorylation induced by wortmannin [49]. Tyrosine kinase (trk) receptors [50], important elements of the phosphorylation system and neurotrophins, are adversely affected by $A\beta$ and other oxidotoxins. In neuroblastoma cells, melatonin normalized trk and neurotrophin expression [50]. A schematic diagram showing melatonin's neuroprotective actions is presented in Table 1 and Figure 3.

Melatonin's Neuroprotective Role for Mitochondrial Homeostasis in Alzheimer's Disease

Mitochondrial abnormalities in AD have been discussed in the earlier paragraphs. Melatonin's neuroprotective role in

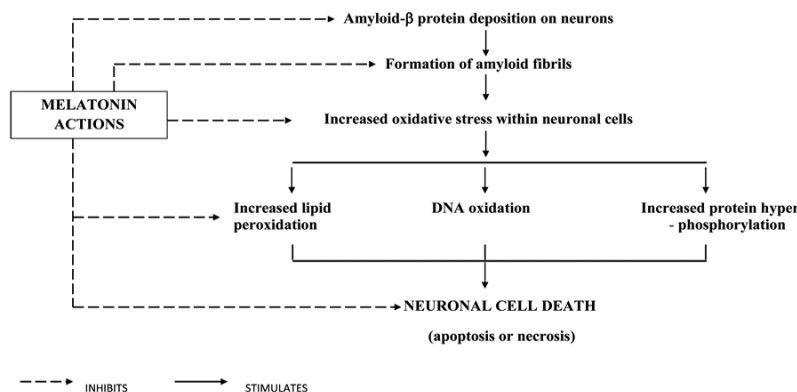


Figure 3. Pathophysiology of AD and melatonin's neuroprotective actions.

Table 2. Summary of Melatonin's Beneficial Physiologic and Metabolic Effects on Mitochondrial Dysfunction Induced by Amyloid-B in Neural Tissues

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1. prevents intramitochondrial effects of amyloid- β effects
 2. prevents opening of mitochondrial transition pores
 3. increases mitochondrial respiratory enzyme complex activities
 4. safeguards electron flow through the electron transport chain
 5. increases intramitochondrial GSH pool
 6. prevents intramitochondrial lipid peroxidation
 7. prevents mitochondrial DNA oxidation
 8. increases ATP synthesis
 9. prevents cell death by necrotic effects or apoptotic effects
-

AD not only depends upon its radical scavenging actions but also on its additional actions, including protecting the mitochondrial membranes and mitochondrial DNA from oxidative insults, stimulation of glutathione (GSH) synthesis, reduction of oxidized glutathione (GSSG) levels and maintenance of mitochondrial electron flux [20]. The electron transport chain (ETC) represents a major source of reactive oxygen species (ROS) within the cell [51]. Complex I and III of the ETC have been identified as the two principal sites of superoxide anion (O_2^-) generation [52]. While much of the O_2^- is released from complex III to either side of the inner membrane, the iron-sulfur cluster N_2 of complex I appears to be the main site of O_2^- release to the matrix [53]. The O_2^- so formed is disposed of by several pathways. A portion of it re-donates electrons to the ETC at cytochrome c [54]. Another fraction is converted into H_2O_2 and O_2 by the mitochondrial Mn-containing subform of super oxide dismutase (MnSOD) [55]. A third fraction of O_2^- combines with NO to give rise to peroxynitrite, an additional source of destructive proteins. Melatonin's mitochondrial actions take place within the organelle. Melatonin, possessing a balanced amphiphilicity, crosses the cell membranes with ease and is concentrated within subcellular compartments [56]. Melatonin's effects on electron flux have two aspects. First, it increases the activities of mitochondrial respiratory complexes I and IV in a time dependent manner [57]. This effect of melatonin, namely the improvement of electron transport capacity in mitochondria, is remarkable as these effects are observed in senescence-accelerated mice [58]. In addition to this, other processes perturbing the mitochondrial membrane potential such as calcium overload are also antagonized by melatonin. Melatonin has been shown to prevent calcium overload and counteracted the collapse of the mitochondrial

membrane potential induced by H_2O_2 [59], and also inhibited the opening of the mtPTP, thereby preventing the occurrence of apoptosis. A summary of melatonin's neuroprotective actions on mitochondrial physiology and metabolism are presented in Table 2 and Figure 4. Melatonin also activates signalling pathways, namely the Bcl-2 pathway that stabilizes mitochondrial function by antiapoptotic Bcl-2 family modulators. Melatonin enhanced Bcl-2 expression with consequent inhibition of A β -induced cell death [60]. Caspase-3 is known to be directly linked to cytochrome c release and is related to cell death in AD [61, 62], and this is down regulated by melatonin [63]. Thus, the apoptosis-inducing factors liberated during mitochondrial damage are effectively inhibited by melatonin through a variety of mechanisms, thereby preventing the neuronal cell death seen in AD.

Other Possible Mechanisms of Melatonin's Action in Alzheimer's Disease

Glutamate receptors, particularly NMDA receptors, are involved in the pathophysiology of AD [64]. Excessive glutamate activity resulting in excessive calcium influx activates a number of enzymes including phospholipases, endonucleases, neuronal nitric oxide synthase and proteases that can result in neuronal toxicity leading to several neurodegenerative diseases including AD [64]. Melatonin, by reducing the NMDA-induced high Ca^{2+} influx, thereby offers a neuroprotective effect. The regulation of intracellular Ca^{2+} by melatonin is explained through its action on melatonin MT2 receptors. cAMP - dependent protein kinase (PKA) is involved in the activation of calcium channels [64]. Melatonin, by acting through MT2 receptors, decreases cAMP formation and

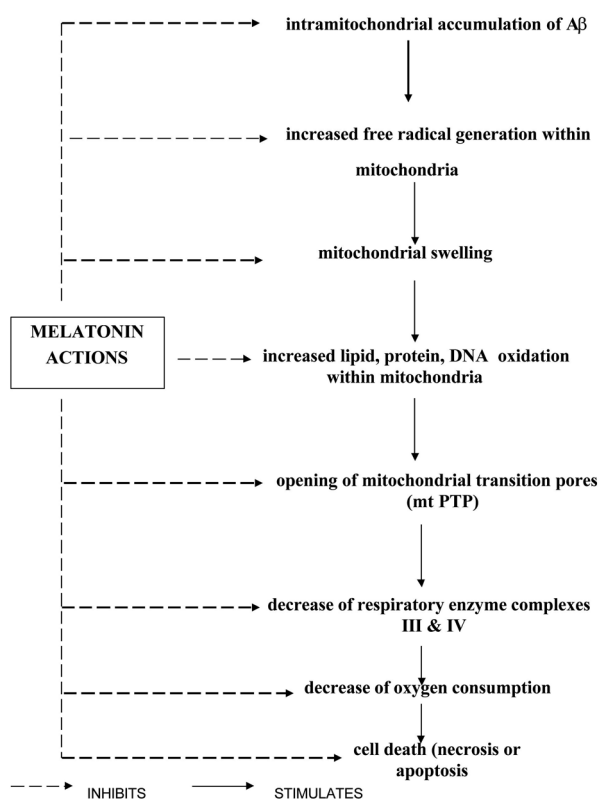


Figure 4. Melatonin's effects on mitochondrial pathophysiology in AD.

thereby blocks PKA activation [65]. Findings in AD patients reveal that both MT1 and MT2 receptors are decreased in the cortical regions of AD patients [66].

Sleep-Wake Rhythm and Circadian Rhythm Disturbances in Alzheimer's Disease

A number of studies in AD patients have shown that there are profound disturbances in the sleep-wake cycle correlating with the progression of the disease. Cross-sectional studies reveal that sleep disturbances are associated with memory and cognitive impairment. Clinical studies in AD patients favour disruption of the circadian timing system in AD since numerous overt rhythms including body temperature, glucocorticoid, melatonin and other hormonal rhythms are disturbed [67-69]. Phase differences between the rest-activity and core body temperature cycles are significantly delayed in AD patients [70]. A chronobiological phenomenon observed in AD patients in conjunction with sleep-wake disturbances is "sun downing," in which symptoms such as disorganized thinking, reduced ability to maintain attention on external stimuli, and motor disturbances like agitation, wandering, perceptual and emotional disturbances all tend to appear in the late afternoon or early evening [71]. Chronotherapeutic

procedures including exposure to bright light in selected circadian phases alleviated sundowning symptoms like wandering, agitation and delirium and also improved sleep-wake patterns in AD patients [72].

Melatonin Levels in Alzheimer's Disease

Studies of melatonin levels in AD patients reveal that they are lower in AD patients compared to age matched controls [73-76]. Decreased CSF melatonin levels in AD patients were attributed to decreased melatonin production rather than to diluting effects of CSF. CSF melatonin levels decreased even in preclinical stages (Braak stage-1) when patients did not yet manifest cognitive impairment, suggesting that a reduction in CSF melatonin may be an early marker for the first stages of AD [76, 77]. The decrease of melatonin levels in AD has also been attributed to defective retino-hypothalamic tract or suprachiasmatic nucleus-pineal connections [78]. However, decreased nocturnal melatonin levels have also been shown to correlate with the severity of mental impairment of patients with dementia [78]. Recently it has been suggested that the choroid plexus portal theory of CSF circulation offers an explanation for the neuropathology of AD. Contrary to earlier views expressed in medical text

books that the cerebrospinal fluid absorption occurs through the arachnoid granules into the superior sagittal sinus, present evidence indicates that cerebrospinal fluid is moved from the choroid fissure into the ventricular system. Understanding the circulation of CSF through these portals, combined with understanding of the consequences of deficient CSF melatonin, may provide answers to the puzzles of AD pathophysiology [79].

Melatonin's Potential Therapeutic Value in Alzheimer's Disease

As AD patients have a profound deficiency in endogenous melatonin production, replacement of physiological levels of melatonin in the brain may represent a kind of treatment for arresting the progress of this disease. Melatonin's vasoprotective properties could conceivably help in enhancing cerebral blood flow and could therefore help to improve the clinical condition of AD patients. Moreover AD patients show a greater breakdown of the circadian sleep-wake cycle when compared to similarly aged non-demented controls. As sleep disturbances exacerbate memory and cognitive impairment, amelioration of sleep disturbances is of paramount importance in treating AD patients [80]. In a study conducted in 14 AD patients with 6 - 9 mg of melatonin/day and follow up over a 2 - 3 year period, it was noted that melatonin improved sleep quality in most of the patients [81, 82].

Sundowning, diagnosed clinically, was no longer detectable in 12 out of 14 patients. Reduction in cognitive impairment and amnesia was noted. This should be contrasted with significant deterioration of the clinical conditions of the disease expected in patients after a 1 - 3 year evolution of AD [83]. The efficacy of melatonin in treating AD patients is supported by a number of other studies. Administration of melatonin (6 mg/day) for 4 weeks to 7 AD patients reduced night-time activity compared to placebo [83]. The improvement of sleep and alleviation of sundowning was reported in 11 elderly AD patients treated with melatonin (3 mg/day at bedtime) [72]. Significant decreases in agitated behaviour and daytime sleepiness were also noted in this study. Reduction of sun downing with the use of 3 mg of melatonin was reported using actigraphy in 7 AD patients [84]. 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 at night-time and improved cognitive functions [85]. In a multicenter, randomized, placebo-controlled clinical trial in a large sample of 157 AD patients with sleep disturbances, patients were randomly assigned to one of three treatment groups (placebo, 2.5 mg slow release melatonin or 10 mg melatonin). Melatonin or placebo was administered for a period of 2 months with actigraphic monitoring, and an increased nocturnal total sleep time and decreased waking after sleep onset was noted in

the melatonin group. On subjective measures using caregiver ratings, significant improvement in sleep quality was noted in the 2.5 mg sustained release melatonin group relative to the placebo group [86]. However in a recent study, melatonin 8.5 mg immediate release and 1.5 mg sustained release was administered at 10:00 PM for 10 consecutive nights to patients with Alzheimer's disease and no significant difference was noticed between placebo and melatonin on sleep, circadian rhythms or agitation [87]. The lack of beneficial effect of melatonin on sleep was attributed to supraphysiological doses of melatonin. Besides tackling the sleep and circadian rhythm disturbances seen in AD, melatonin can also act at multiple different levels in AD. The antioxidant, mitochondrial and antiamyloidogenic effects of melatonin can be viewed as potentially interfering with the onset of the disease. As such, melatonin has several obvious advantages over other comparable compounds, including most other antioxidants. The question of whether melatonin has therapeutic value in preventing or treating AD, affecting disease initiation or progression of the neuropathology and its driving mechanisms remains to be answered by future multicenter double-blind clinical trials. In the meantime, it is suggested that improvement of insomnia in neurodegenerative conditions and particularly in AD with melatonin represents a clinical choice that may be of help in arresting the progression of the disease as well as offering neuroprotection against the development of AD.

Use of Other Psychopharmacological Agents for Treatment of AD

In a recent review of psychopharmacological approaches to neuroprotection, one of the authors (ECL) suggested a trial of lithium (a mood stabilizer) with melatonin since this combination can potentially synergize neuroprotective effects on A β , hyperphosphorylated tau, ROS, mtPTP, and apoptosis, with lithium also potentially improving ubiquitylation [88]. It is suggested that treatments lowering plasma A β will be beneficial for reducing the risk of developing AD.

Melatonergic Agonist Ramelteon for Treatment of AD

Ramelteon is (s)-N-(2-(1,6,7,8-tetrahydro-2H-indeno(5,4-b)furan-8-yl)ethyl)propionamide. This melatonin receptor agonist has a chemical formula C₁₆H₂₁NO₂ with a molecular weight of 259.34. Its affinity for MT₁ and MT₂ receptors is highly selective, with little affinity to quinone reductase (formerly known as MT₃ receptors) [89].

The selectivity of ramelteon for MT₁ receptors is 1000-fold more than its affinity to MT₂ receptors. Ramelteon is metabolized in the liver via oxidation to hydroxyl and car-

bonyl groups and then conjugated with glucuronide. CYP2A2 is the major hepatic enzyme involved in ramelteon metabolism. Four principal metabolites of ramelteon, namely M-I, M-II, M-III and M-IV have been identified [90]. Among the M-II occurs at a high concentration with its systemic level being 20 to 100-fold greater than ramelteon. A number of clinical studies of the use of ramelteon in primary insomnia in both adult and elderly patients have shown that it is very effective in reducing sleep onset latency and increasing total sleep time and sleep efficiency. Studies on the efficacy of ramelteon in treating insomnias have been published elsewhere [91-93]. As ramelteon has greater potency and affinity at melatonin receptors, it is suggested that melatonin's neuroprotective effects could be shared by ramelteon with greater efficacy and can be of value in treating Alzheimer's disease [94]. The neuroprotective effect of ramelteon in AD is inferred from the recent finding that ramelteon increased the neural content of BDNF in cultured mouse cerebellar granule cells [95]. Since neither melatonin nor its agonist ramelteon has undergone clinical trials for clinical and disease modifying neuroprotective properties in neurodegenerative diseases, ramelteon's clinical findings remain to be established in neurodegenerative disease patients [94]. Melatonin and ramelteon, because of their sleep promoting actions, may have an important role to play in the treatment of Alzheimer's disease and other neurodegenerative diseases. Of these, melatonin may be more significant because of its probable involvement in the aetiology of AD itself. Deficiency of CSF melatonin is postulated as one of the major reasons for AD neuropathology as pointed out in the earlier paragraphs.

The loss of this antioxidant in the CSF will expose vital neurons to increased oxidative stress with resultant cell destruction and the development of AD [79]. A year's melatonin intake at a dose of 9 mg/day will cost only \$ 25 and could greatly benefit the patient in slowing the progression of AD [79].

Conclusion

Although the exact cause for AD is still under intense investigation, available evidence strongly implies the deposition of amyloid- β protein and oxidative stress and their neurotoxic effects as the major contributing factors to the pathogenesis of AD. Intramitochondrial accumulation of amyloid- β protein is one of the major reasons for mitochondrial pathophysiology in AD. Inhibition of respiratory enzyme complexes, with increased electron leakage, decreased oxygen consumption and decreased ATP synthesis caused by increased oxidative stress ultimately contribute to neuronal cell death by necrosis or apoptosis. AD patients have profound sleep-wake cycle disturbances. The hormone melatonin, involved in the regulation of sleep and circadian rhythms, is greatly diminished in patients with AD. Melatonin also has been

demonstrated as an effective antioxidant both in vivo and in vitro model systems. Experimental studies using neuronal cell cultures or animal models of AD have shown that melatonin has been effective in preventing A β -induced toxic effects on neuronal cells. Melatonin also exerts its beneficial effects intramitochondrially, increasing respiratory enzyme complex activities, increasing the flow of electrons, scavenging free radicals and preventing oxidative stress-induced lipid, protein and DNA oxidation within cells and also within mitochondria. Treatment of AD patients with melatonin has clearly improved sleep quality in almost all studies undertaken and it also has been beneficial in improving cognitive functions. The preventive potential of melatonin in AD disease progression remains to be established by clinical trials. In this respect, the effect of a newly introduced sleep promoting drug, namely ramelteon, a melatonin agonist, may be of benefit since it can improve both sleep quality and may also offer neuroprotection against pathogenic proteins, free radical-induced lipid peroxidation and mitochondrial transition pore development. Multicenter placebo controlled clinical trials using ramelteon have to be undertaken to prove the efficacy of this drug in preventing or arresting the progression of AD.

Conflict of Interest

All authors of this paper declared that they have no conflict of interest. V. Srinivasan (the corresponding author) has not received any pay or salary during the last three years and has published nearly twenty review/research papers in international medical journals with an international collaborative team. Sri Sathya Sai Medical Educational and Research Foundation, A Medical Sciences Research Study Centre (founded by DR. V. Srinivasan, PhD., MAMS (Member, National academy of Medical Sciences India), has not received any research grant from any Governmental, Private, Public, National or International Funding agencies, since its inception in 2009.

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