CHAPTER FOUR

Melatonin and healthy aging

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Abstract

Preservation of a robust circadian rhythmicity (particulsarly of the sleep/wake cycle), a proper nutrition and adequate physical exercise are key elements for healthy aging. Aging comes along with circadian alteration, e.g. a disrupted sleep and inflammation, that leads to metabolic disorders. In turn, sleep cycle disturbances cause numerous pathophysiological changes that accelerates the aging process. In the central nervous system, sleep disruption impairs several functions, among them, the clearance of waste molecules. The decrease of plasma melatonin, a molecule of unusual phylogenetic conservation present in all known aerobic organisms, plays a particular role as far as the endocrine sequels of aging. Every day, the late afternoon/nocturnal increase of melatonin synchronizes both the central circadian pacemaker located in the hypothalamic suprachiasmatic nuclei as well as myriads of peripheral cellular circadian clocks. This is called the "chronobiotic effect" of melatonin, the methoxyindole being the prototype of the endogenous family of chronobiotic agents. In addition, melatonin exerts a significant cytoprotective action by buffering free radicals and reversing inflammation via down regulation of proinflammatory cytokines, suppression of low degree inflammation and prevention of insulin resistance. Because of these properties melatonin has been advocated to be a potential therapeutic tool in COVID 19 pandemic. Melatonin administration to aged animals counteracts a significant number of senescence-related changes. In humans, melatonin is effective both as a chronobiotic and a cytoprotective agent to maintain a healthy aging. Circulating melatonin levels are consistently reduced in the metabolic syndrome, ischemic and non-ischemic cardiovascular diseases and neurodegenerative disorders like the Alzheimer's and Parkinson's

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diseases. The potential therapeutic value of melatonin has been suggested by a limited number of clinical trials generally employing melatonin in the 2–10 mg/day range. However, from animal studies the cytoprotective effects of melatonin need higher doses to become apparent (i.e. in the 100 mg/day range). Hence, controlled studies employing melatonin doses in this range are urgently needed.

1. Introduction

Aging is the leading risk factor for a variety of chronic diseases, including cancer, metabolic, cardiovascular, and neurodegenerative diseases, which result in poor quality of life and an increase in morbidity and mortality worldwide. Among several other comorbidities, the aged population is more prone to suffer from coronavirus infection and the association of aging with a higher vulnerability to SARS-CoV-2 infection is a subject of major importance in COVID-19 pandemic (Esteve, Permanyer, Boertien, & Vaupel, 2020). Chronic, endemic disorders such as the metabolic syndrome (MS) and Alzheimer's disease (AD) are at the core of pathological aging and their prevention is presently a fundamental goal for governmental and non-governmental health organizations. The prevalence of MS varies from 15% to 30% depending on the region of the world considered (O'Neill & O'Driscoll, 2015). An increase of 1.5 to 2.5 times in cardiovascular mortality occurs when MS is present. Likewise, the global prevalence of dementia among people aged $\geq$60 years is 5% to 7% (Prince et al., 2013). In 2010, approximately 35.6 million people lived with dementia, and this number is expected to double every 20 years. These are a few examples that the aging of the world population is a recognized challenge for current health system.

Several interrelated processes, such as circadian desynchronization, free radical-mediated damage, mitochondrial dysfunction, low degree of inflammation ("inflammaging") and immunosenescence have been identified as major pathophysiological mechanisms in aging (Welz & Benitah, 2019). Environmental and nutritional factors related to different lifestyles are variables of significant importance for healthy aging. In this context, melatonin, an unusual phylogenetically conserved compound present in all known aerobic phyla, has a promising significance. Melatonin is the prototype of the drugs that influence the circadian apparatus (i.e. chronobiotics) (Arendt & Skene, 2005; Dawson & Armstrong, 1996) and plays a major function in the coordination of circadian rhythmicity. In addition, melatonin is a potent cytoprotective agent having strong antioxidant, anti-inflammatory and immunoregulating activity (Cardinali, 2019b). Because of these properties

melatonin has been advocated to be a potential therapeutic tool in COVID 19 pandemic (Cardinali, Brown, & Pandi-Perumal, 2020; Reiter, Ma, & Sharma, 2020).

The pineal gland is the demonstrable source of circulating melatonin in humans, the decrease in plasma melatonin being one of the characteristics of the advancing age (Claustrat & Leston, 2015). This Chapter is focused on the use of melatonin as a chronobiotic and cytoprotector to contribute to healthy aging.

2. Melatonin as a chronobiotic

Organisms populating the Earth are under the steady influence of daily and seasonal changes resulting from the planet's rotation and orbit around the sun. This periodic pattern is most prominently manifested by the light–dark cycle and has led to the establishment of endogenous circadian timing systems that synchronize biological functions to the environment. This is the basis of predictive homeostasis evolving as an adaptation to anticipate predictable changes in the environment, such as light and darkness, temperature, food availability, or predator activity (Riede, van der Vinne, & Hut, 2017). Therefore, the circadian clock is one of the most indispensable biological functions for living organisms that acts like a multifunctional timer to adjust the homeostatic system, including sleep and wakefulness, hormonal secretions, immune function and various other bodily functions, to the 24-h cycle.

Research in animals and humans has shown that only a few environmental cues, like light–dark cycles, are effective entraining agents ("zeitgebers") for the circadian oscillator. An entraining agent can actually reset, or phase shift, the internal clock. Depending on when an organism is exposed to such an entraining agent, circadian rhythms can be advanced, delayed, or not shifted at all. Therefore, adjusting the daily activity pattern to the appropriate time of day involves a rhythmic variation in the influence of the zeitgeber as a resetting factor (Duffy, Zitting, & Chinoy, 2015).

In mammals, the circadian system is composed of many individual, tissue-specific cellular clocks. To generate coherent physiological and behavioral responses, the phases of this multitude of cellular clocks are orchestrated by a master circadian pacemaker residing in the suprachiasmatic nuclei (SCN) of the hypothalamus (Buijs et al., 2017). At a molecular level, circadian clocks are based on clock genes, some of which encode proteins able to feedback and inhibit their own transcription. These cellular oscillators

consist of interlocked transcriptional and post-translational feedback loops that involve a small number of core clock genes (about 12 genes identified currently) (Welz & Benitah, 2019). The negative and positive transcriptional/translational feedback loops to form the core clockwork, have been characterized in rodents by transgenic gene deletion methodology. Clock gene expression oscillates because of the delay in the feedback loops, regulated in part by phosphorylation of the clock proteins that control their stability, nuclear re-entry, and transcription complex formation (Welz & Benitah, 2019).

As noted, the trillions of cellular clocks in primates are synchronized by a few thousand neurons located in the SCN. It is remarkable that such a small group of neurons display the properties of a central clock. Indeed, these "neuronal oligarchies," like the human ones, control trillions of cells in the body by: (a) taking control of the major communication channels (the endocrine and autonomic nervous systems); (b) concentrating the relevant information in a private way (i.e., light information arriving via the retino-hypothalamic tract) (Cardinali, 2017). Thus, it is not surprising that anatomical studies have showed that the SCN projects to at least three different neuronal targets: endocrine neurons, autonomic neurons of the paraventricular nucleus (PVN) of the hypothalamus, and other diencephalic structures that transmit the circadian signal to other brain regions (Buijs, Guzman Ruiz, Mendez, & Rodriguez, 2019). Through autonomic nervous system projections involving the superior cervical ganglia the SCN controls the release of a major internal synchronizer from the pineal gland, namely melatonin (Cardinali, 2017).

The current view of the physiological regulation of the circadian rhythm of sleep/wakefulness (the main circadian rhythm in the body) holds that it is regulated by two components, namely, a circadian (~24 h) component and a homeostatic component (Borbely, Daan, Wirz-Justice, & Deboer, 2016). Melatonin plays a major role in the circadian component that regulates the timing of sleep. The circadian rhythm in synthesis and secretion of pineal melatonin is closely associated with the sleep rhythm in both normal and blind subjects (Emens & Eastman, 2017). The onset of nighttime melatonin secretion is initiated approximately 2 h in advance to individual's habitual bedtime and has been shown to correlate with the onset evening sleepiness. Several studies implicate endogenous melatonin in the physiological regulation of the circadian mechanisms ruling sleep propensity (Auld, Maschauer, Morrison, Skene, & Riha, 2017; Gobbi & Comai, 2018).

Aging has been associated with a significant reduction in sleep efficiency and continuity and coincides with a significant reduction in amplitude of the melatonin rhythm and consequently of many circadian rhythms in the body (Duffy et al., 2015). Increase in early morning awakenings and difficulty in falling sleep have been frequent reported in the elderly. An impaired melatonin secretion is associated with the sleep disorders that are encountered in elderly insomniacs. Indeed, aging may be a process resulting from the relative circadian desynchrony produced by melatonin deficiency and melatonin can be effective for improving the quality of life in the elderly via its recognized chronobiotic capacity (Cardinali, 2019b).

Melatonin plays a major function in the coordination of circadian rhythmicity. Melatonin secretion is an "arm" of the biologic clock in the sense that it responds to signals from the SCN and that the timing of the melatonin rhythm indicates the status of the clock, both in terms of phase (i.e., internal clock time relative to external clock time) and amplitude. From another point of view, melatonin is also a chemical code of night: the longer the night, the longer the duration of its secretion. In most mammalian species, this pattern of secretion serves as a time cue for seasonal rhythms (Clarke & Caraty, 2013).

Pineal melatonin production is controlled by a complex neural system originating in the hypothalamic PVN and terminating in the high levels of the thoracic spinal cord—the superior cervical ganglion sympathetic system. The postganglionic sympathetic nerve terminals of the superior cervical ganglion release norepinephrine into the pineal gland that triggers melatonin synthesis by its interaction with β- (mainly) and α-adrenoceptors on the membrane of pineal cells. Melatonin, due to its high diffusibility, is not stored inside the pineal and is released as soon as it is produced (Tan, Xu, Zhou, & Reiter, 2018). The structures which regulate circadian rhythms have been described as the SCN-melatonin loop (Tan et al., 2018). This loop includes melanopsin-containing retinal ganglion cells, the retino-hypothalamic tract, SCN, PVN, intermediolateral cell column, the sympathetic cervical ganglia, the pineal gland, and the melatonin rhythm which feedback impacts the SCN.

As a result, the melatonin production, and consequently its cerebrospinal fluid (CSF) and blood levels are circadian in nature and tightly synchronized with the environmental light/dark cycle. Indeed, the circadian pineal production of melatonin is restricted to the dark phase of the light/dark cycle in all mammalian species. It is noteworthy that melatonin is always produced

during the night independent of the daily pattern of activity/rest of the species, indicating its strong relationship with the external photoperiod. Additionally, melatonin is produced during the night provided there is no light in the environment.

Light at night, mainly in the blue range, activates melanopsin-containing retinal ganglion cells, a special retinal system that inhibits the pineal sympathetic norepinephrine release and consequently decreases or abolishes melatonin production. Given the regularity of the daily melatonin production that is associated with high and low or absent blood concentrations during the night and day, respectively, melatonin is able to synchronize the circadian rhythms of several organs and their functions. In vitro experiments have shown that the artificial day and night melatonin profile can function as a pacemaker for the daily rhythmic functions of the cells (Hardeland et al., 2011).

Like the effects induced by the external zeitgeber light, effects by the internal zeitgeber melatonin on the circadian clock are time-dependent. Daily timed administration of melatonin to rats shifts the phase of the circadian clock, and this phase shifting may explain the effect of melatonin on sleep in humans. Indirect support for such a physiological role derived from clinical studies on blind subjects (who show free running of their circadian rhythms) treated with melatonin (Skene & Arendt, 2007). More direct support for this hypothesis was provided by the demonstration that the phase response curve for melatonin was opposite (i.e., about 180 degrees out of phase) to that of light (Lewy, 2010).

The direct effects in regions containing high densities of melatonin receptors, such as the circadian pacemaker SCN, or the pars tuberalis (PT), a site of particular relevance for photoperiodically controlled reproduction (Clarke & Caraty, 2013) strongly supported the premier significance of melatonin's physiological role. Although the control of circadian and seasonal rhythmicity represents melatonin's main physiological function, the actions of the methoxyindole are by no means restricted to areas of high receptor density.

The pleiotropy of melatonin has to be analyzed at different levels, from the sites of synthesis and local dynamics, distribution of receptors and other binding sites in target organs, cell-specific differences in signaling as related to the presence of G protein variants, and intracellular effects—with a particular focus on mitochondrial actions—to numerous secondary changes induced by influencing other hormones, neurotransmitters, neurotrophins and further signal molecules (Hardeland et al., 2011). In functional terms, melatonin exerts a host of effects that can be under the control of the

SCN and, in seasonal breeders, the premammillary hypothalamus and the PT, and may also have direct effects in numerous peripheral organs. In particular, melatonin is involved in sleep initiation, vasomotor control, adrenal function, antiexcitatory actions, immunomodulation including anti-inflammatory properties, antioxidant actions, and energy metabolism, influencing mitochondrial electron flux, the mitochondrial permeability transition pore, and mitochondrial biogenesis (Hardeland, 2018a, 2018b; Tan & Reiter, 2019).

The chronobiotic action of melatonin is mediated via the melatonin receptors, which have been identified both in the CNS and in the periphery (Dubocovich et al., 2010). MT_1 and MT_2 receptors all belonging to the superfamily of membrane receptors associated with G proteins (G-protein coupled receptors, GPCR) have been cloned. More recently, another member, GPR50, was included in the melatonin receptor subfamily. GPR50 shows high sequence homology to MT_1 and MT_2 but does not bind to melatonin or any other known ligand. An interesting feature of these receptors is their capacity to form homo- and heteromers between each other and also with other GPCRs (Cecon, Oishi, & Jockers, 2018) among them the serotonin 5-HT_{2C}. The heteromers display functional properties different from those of the corresponding homomers. For example, acting on the MT_2/5-HT_{2C} heteromer, melatonin binding induces the activation of Gq signaling through a transactivation of the serotonergic receptor caused by conformational changes of the MT_2, which is normally not coupled to a Gq.

Circulating melatonin is loosely bound to albumin and in the liver, it is first hydroxylated and then conjugated with sulfate and glucuronide (Claustrat & Leston, 2015). In human urine, 6-sulfatoxymelatonin has been identified as the main metabolite. In the brain melatonin is metabolized into kynurenine derivatives. It is of interest that the well-documented antioxidant properties of melatonin are shared by some of its metabolites including cyclic 3-hydroxymelatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and, with highest potency, N^1-acetyl-5-methoxykynuramine (AMK). Thus an "antioxidant cascade" is triggered by melatonin administration to experimental animals and humans (Galano & Reiter, 2018).

As mentioned, in mammals, circulating melatonin is derived almost exclusively from the pineal gland. In addition, melatonin is synthesized locally in most cells, tissues and organs, including lymphocytes, bone marrow, thymus, gastrointestinal tract, skin and eyes, where it can play an autocrine or paracrine role (Acuña-Castroviejo et al., 2014). Indeed, there is now strong evidence that melatonin is produced in every animal cell that has

mitochondria (Tan & Reiter, 2019). In both animals and humans, melatonin participates in diverse physiological functions that indicate not only the duration of the night, but also improve the elimination of free radicals and the immune response, showing relevant cytoprotective properties (Hardeland, 2018a).

Melatonin is a powerful chronobiotic with very slight hypnotic capacity. Daily doses of 2–5 mg melatonin, timed to advance the phase of the internal clock by interaction with MT_1 receptors in the SCN, maintains synchronization of the circadian rhythms to a 24-h cycle in sighted persons who are living in conditions likely to induce a free-running rhythm (Lewy, 2010). Melatonin synchronizes the rhythm in persons after a short period of free-running. In blind subjects with free-running rhythms, it has been possible to stabilize, or entrain, the sleep/wake cycle to a 24-h period by giving melatonin, with resulting improvements in sleep and mood (Skene & Arendt, 2007). In normal aged subjects and in demented patients with disturbed synchronization of the sleep/wake cycle melatonin administration is helpful to reduce the variation of onset time of sleep. The phase shifting effects of melatonin are also sufficient to explain its effectiveness as a treatment for circadian-related sleep disorders, such as jet lag or delayed phase sleep syndrome (Burgess & Emens, 2018) (Fig. 1).

3. Use of melatonin in sleep aging

The relationship between the sleep/wake cycle and aging is bidirectional (Mazzotti et al., 2014). Inadequate sleep, both in terms of its duration and quality, can affect health and thus adversely contribute to the aging process. On the other hand, sleep/wake cycle difficulties typically increase with age as a result of flattening and misalignment of circadian rhythms like that of melatonin secretion, as well as of the sleep-disturbing influences of aging-related disorders and diseases (Hardeland, 2015). The most convincing examples are that of immunosenescence, which also affects the brain (Cardinali, Esquifino, Srinivasan, & Pandi-Perumal, 2008; Hardeland, 2018a), as well as the almost exponential increase of hydroxyl radical generation seen in the senescent brain (Poeggeler, Reiter, Tan, Chen, & Manchester, 1993). The association of age with a higher vulnerability to SARS-CoV-2 infection is a subject of major importance nowadays. Several factors, including higher stress due to social isolation, diminished melatonin levels with age, and higher exposure of individuals to light at the evening, which reduces

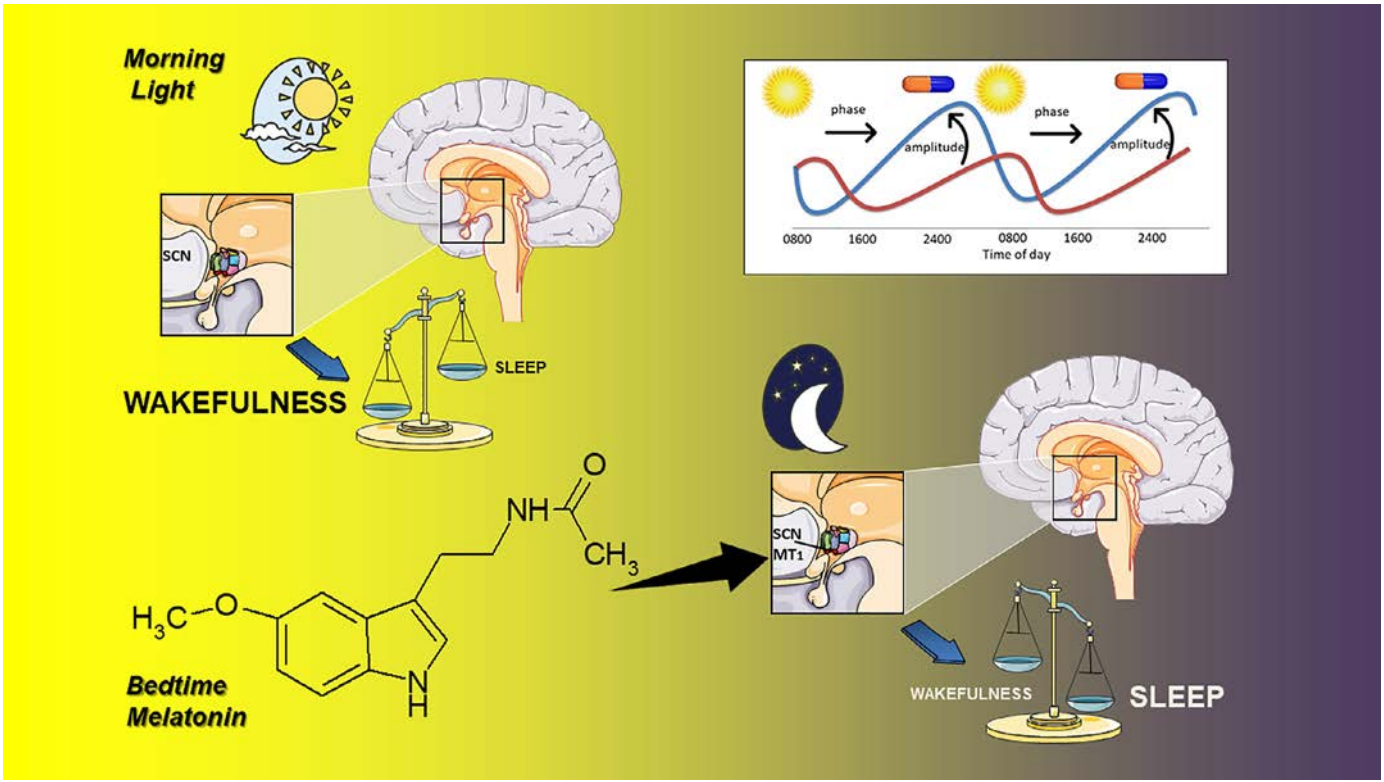

Fig. 1 Chronobiotic activity of melatonin. The combined use of melatonin and bright light to augment the amplitude and synchronize endogenous circadian rhythmicity in aging. Bright light in the morning together with melatonin at bedtime potentiate to augment the amplitude of sleep/wake cycle and other circadian rhythms. Melatonin activity is achieved via MT1 receptors located in SCN. Insert: In delayed phase sleep syndrome melatonin augments the amplitude and phase-advanced the sleep/wake rhythm.

melatonin levels and disrupts circadian rhythmicity are relevant for maintaining the circadian health in aged individuals in COVID 19 pandemic (Cardinali, Brown, Reiter, & Pandi-Perumal, 2020).

There is now considerable evidence that, with advancing age, there are increases in sleep/wake disorders. Epidemiological research has shown that more than 50% of adults above the age of 65 years suffer from some form of a chronic sleep-related complaint (Foley et al., 1995). It is important to distinguish the changes due to aging per se vs changes due to aging-related disease and secondary to the use of concomitant medications.

Several meta-analyses support the view that the chronobiotic/hypnotic properties of melatonin are useful in aged patients with primary sleep disorders to decrease sleep onset latency and to increase total sleep time, with little if any effect on sleep efficiency (Auld et al., 2017; Ferracioli-Oda, Qawasmi, & Bloch, 2013; Li et al., 2019). Several expert consensus reports also support such a role of melatonin in adult insomnia (Geoffroy, Micoulaud Franchi, Lopez, & Schroder, 2019; Palagini et al., 2020; Vecchierini, Kilic-Huck, & Quera-Salva, 2020; Wilson et al., 2010).

Many studies have shown that sleep/wake disruption is an important contributor to neuropathology. Sleep deprivation (Ooms et al., 2014) or NREM disruption (Ju et al., 2017) have been shown in healthy subjects to increase CSF levels of amyloid β $(A\beta)_{1-42}$ and $A\beta_{1-40}$, respectively. This reduction was reported to be prevented by a single night of total sleep deprivation. Similar findings were obtained in mice, in which sleep deprivation caused increases of Aβ peptides in the interstitial fluid of the brain (Kang et al., 2009). A "glymphatic" hypothesis (Iliff et al., 2012) holds that an active, lymphatic-like movements, occurs in brain extracellular space driven by perivascular astrocytes, which are strongly enriched in aquaporin-4 and by changes in the vascular lumen. Water release via aquaporin-4 would be responsible for an actively driven fluid exchange between para-arterial and paravenous spaces. The exchange of solutes between the CSF and the interstitial fluid occurs mostly during NREM sleep when the cortical interstitial space increases by >60% and provides a low resistance path for the movement of CSF and interstitial fluid in the brain parenchyma (Iliff et al., 2012). This is affected in the aging human brain. Various neurological disease states such as stroke, traumatic brain injury, and AD have been interpreted in terms of the contribution by glymphatic dysfunction (Boespflug & Iliff, 2018). The administration of melatonin to AD transgenic mice augments the glymphatic clearance of Aβ (Pappolla et al., 2018).

The prevalence of primary insomnia varies up to 10% in the general population and up to 25–30% in the elderly, for whom the treatment of insomnia is a clear medical necessity. The direct and indirect costs of insomnia represent a substantial social economic burden. Benzodiazepines (BZD) and other BZD receptor agonists (*Z*-drugs like zolpidem, zaleplon, zopiclone) are the most commonly prescribed drugs for the treatment of insomnia in the elderly. Several meta-analyses pondering the risks and benefits of these therapeutic options in older patients have reported statistically significant improvements in sleep but have also reported a statistically significant risk of life-threatening adverse events (Winkler, Auer, Doering, & Rief, 2014). In fact, these drugs are only approved by regulatory agencies for treatment for older adults no more than a few weeks in length due to safety reasons. Adverse effects have been reported in >40% of users of both BZD and Z drugs.

Health authorities in Europe are undertaking policies and recommendations to reduce the consumption of BZD and Z drugs. However, the campaigns have not been generally successful, despite national guidelines and recommendations, and the use of these drugs has continued to increase. The clearer strategy to reduce chronic BZD use is to reduce medication; abrupt cessation can only be justified if a serious adverse effect occurs during treatment. There is no clear evidence regarding the optimal rate of taper, and times vary from 4 weeks to several months.

In 1986 we demonstrated the interaction of melatonin with central BZD receptors (Acuña-Castroviejo, Lowenstein, Rosenstein, & Cardinali, 1986) and in 1997 we published the first demonstration of the reduction of BZD consumption in melatonin-treated elderly subjects (Fainstein, Bonetto, Brusco, & Cardinali, 1997). Melatonin competes with BZD and Z drugs at their site of action. A facilitator role of melatonin on GABA neurotransmission explains the anxiolytic, antihyperalgesic and antinociceptive effects of melatonergic agents (Cardinali, Golombek, Rosenstein, Brusco, & Vigo, 2016). Several clinical studies now support the efficacy of melatonin to reduce BZD use in chronically treated patients (Baandrup et al., 2018). In a pharmaco-epidemiologic study aimed to evaluate the impact of anti-BZD/Z drugs campaigns and the availability of alternative pharmacotherapy (melatonin) on the consumption of BZD and Z drugs in several European countries, the results indicated that campaigns failed unless they were associated with the availability of melatonin in the market (Clay, Falissard, Moore, & Toumi, 2013). Melatonin has thus become an effective treatment

for the preservation of a healthy sleep in the elderly. Melatonin has been used as a sleep aid for decades without any serious adverse effects being reported. Moreover, it has often been used in critically ill patients to improve sleep and wellbeing, both of which are also beneficial to SARS-CoV-2 infected patients. It is a molecule with an uncommonly high safety profile and can be administered via numerous routes including orally. It is inexpensive, stable without refrigeration, and would be particularly useful in underdeveloped countries where access to high-quality health care may be lacking.

4. Cytoprotection by melatonin in animal models of MS and AD

The cytoprotective activity of melatonin exceeds that mediated via receptors. Almost every cell in the human body contains melatonin, in quantities much higher than those circulating in blood derived from the pineal gland (Acuña-Castroviejo et al., 2014). The mitochondrial capacity to synthesize melatonin is now confirmed, but for reasons that remain unexplained, intracellular melatonin does not get to the extracellular space.

To modify intracellular melatonin levels, doses much higher than those employed as a chronobiotic are needed (Reiter et al., 2018; Venegas et al., 2013). Most studies on neuroprotective and anti-inflammatory effects in animals employ pharmacological doses, which clearly exceed the saturation of the receptor.

In both the cytoplasm and the cell nucleus, melatonin has important antioxidant and scavenging effects on free radicals, which are largely independent of receptors (Manchester et al., 2015). These effects are exerted in three ways: (a) melatonin is a free radical scavenger; (b) melatonin is metabolized to compounds with high antioxidant activity; (c) melatonin is an indirect antioxidant, which stimulates the synthesis of antioxidant enzymes and inhibits that of prooxidant enzymes. Melatonin has a proven superiority to vitamin C and E in protection against oxidative damage and in the elimination of free radicals (Galano, Tan, & Reiter, 2011). In addition, melatonin potentiates the effects of other antioxidants, such as vitamin C and Trolox. Several antiapoptotic and cytoprotective effects of melatonin are exerted under conditions of ischemia (unrelated to free radicals) and can be attributed to the stabilizing activity of the mitochondrial membrane (Reiter et al., 2018).

Melatonin is also an immunological modulator that shows proinflammatory and anti-inflammatory properties (Carrillo-Vico, Lardone, Alvarez-Sanchez,

Rodriguez-Rodriguez, & Guerrero, 2013; Hardeland, 2018a). The anti-inflammatory actions are of medicinal interest, since they are observed in high-grade inflammation such as sepsis, ischemia/reperfusion and brain injury, as well as in the low-grade inflammation seen in MS, neurodegenerative disorders and aging. Melatonin has significant anti-inflammatory properties presumably by inhibiting the binding of nuclear factor κB (NF κB) to DNA, thus decreasing the synthesis of proinflammatory cytokines, by inhibiting cyclooxygenase (Cox) (Cardinali, Ritta, Fuentes, Gimeno, & Gimeno, 1980) in particular Cox-2 (Deng, Tang, Tseng, & Wu, 2006), and by suppressing the expression of the inducible gene of nitric oxide synthase (Costantino, Cuzzocrea, Mazzon, & Caputi, 1998). In addition, other pathways of secondary signaling are involved (Hardeland, 2018a).

Treatment with melatonin of rats can reduce obesity, type 2 diabetes and hepatic steatosis. In several animal models of hyperadiposity, the injection of melatonin could normalize most of the observed alterations and correct the altered biochemical proinflammatory profile (for reference see Cardinali, 2019a). In addition, melatonin is effective in animal models of ischemic and nonischemic heart failure, an important comorbidity of MS.

Collectively, the results in animal studies indicate that the administration of melatonin effectively counteracts some of the disrupting effects seen in diet-induced obesity in animals insulin resistance, dyslipidemia and obesity, and the consequences of ischemic and non-ischemic heart disease. Interestingly, a recent study examining the subcellular distribution of the melatonin in the heart of rats indicates that at a dose of 40 mg/kg b.w, maximal concentration of melatonin was reached in the nucleus and mitochondrion. The authors concluded that doses of melatonin $\geq$112 mg/day are required for therapeutic purpose in a 70 kg adult (Acuña-Castroviejo, Noguera-Navarro, Reiter, & Escames, 2018).

Concerning experimental models of AD, cell line studies regarding AD and melatonin have delineated important melatonin mediated mechanisms in AD prevention. For a comprehensive review on melatonin activity to reverse disrupted signaling mechanisms in neurodegeneration, including proteostasis dysfunction, disruption of autophagic integrity, and anomalies in the insulin, Notch, and Wnt/β-catenin signaling pathways see reference Shukla, Govitrapong, Boontem, Reiter, and Satayavivad (2017).

In transgenic models of AD the obtained results are compatible with the view that melatonin regulates Aß metabolism mainly at the initial phases of the pathological process. From the doses of melatonin used in these different transgenic models, the HED of melatonin for a 75 kg adult can be calculated

by normalization of body surface area (Reagan-Shaw, Nihal, & Ahmad, 2008). Theoretical human equivalent doses ranged from 2- to 3-orders of magnitude greater than those employed in humans.

A summary of the cytoprotective effects of melatonin in healthy aging is depicted in Fig. 2.

5. Use of melatonin in MS and AD patients

Type 2 diabetic patients have low circulating levels of melatonin (Tutuncu et al., 2005). In addition, allelic variants for melatonin receptors were associated with an increase in fasting blood glucose levels and/or an increased risk of type 2 diabetes (see for reference (Vallim, Amaral, Cipolla-Neto, & D'Almeida, 2019)) and with the polycystic ovarian syndrome (PCOS) (Song et al., 2015; Spinedi & Cardinali, 2018). Patients with coronary artery disease show a decrease in melatonin secretion and among elderly hypertensive patients, nocturnal urinary melatonin excretion was inversely associated with the non-dipper pattern of hypertensive disease (Obayashi et al., 2013). Melatonin administration ($\leq$5 mg/day) proved capable of reducing nocturnal blood pressure in hypertensives and attenuated age-dependent disturbances of cardiovascular rhythms (see for reference (Nduhirabandi & Maarman, 2018; Pandi-Perumal et al., 2017)).

Treatment with melatonin ($\leq$5 mg/day) improves MS in obese and PCOS patients as well as in bipolar and schizophrenic patients receiving second generation antipsychotics The administration of melatonin normalizes MS in elderly hypertensive patients and improves the enzyme profile in patients with alcoholic hepatic steatosis. The combination of melatonin and zinc acetate, when used alone or in combination with metformin, improved the glycemic control in type 2 diabetic patients (see for reference Cardinali, 2019a). Additional information concerning a glucose tolerance-reducing property of melatonin in humans came from the detection of melatonin receptor polymorphisms. To date, several single nucleotide polymorphisms located near or inside the gene encoding MTNR1B with an association with type 2 diabetes mellitus have been identified (see for reference Vallim et al., 2019). Some of these melatonin receptor polymorphisms are dysfunctional because of their incapability of binding melatonin, and others were found to be unable to interact with G_i proteins. Thus, the absence of melatonin signaling is presumably diabetogenic (Vallim et al., 2019).

Concerning AD, CSF melatonin levels decrease even in preclinical stages of the disease when the patients do not manifest any cognitive impairment,

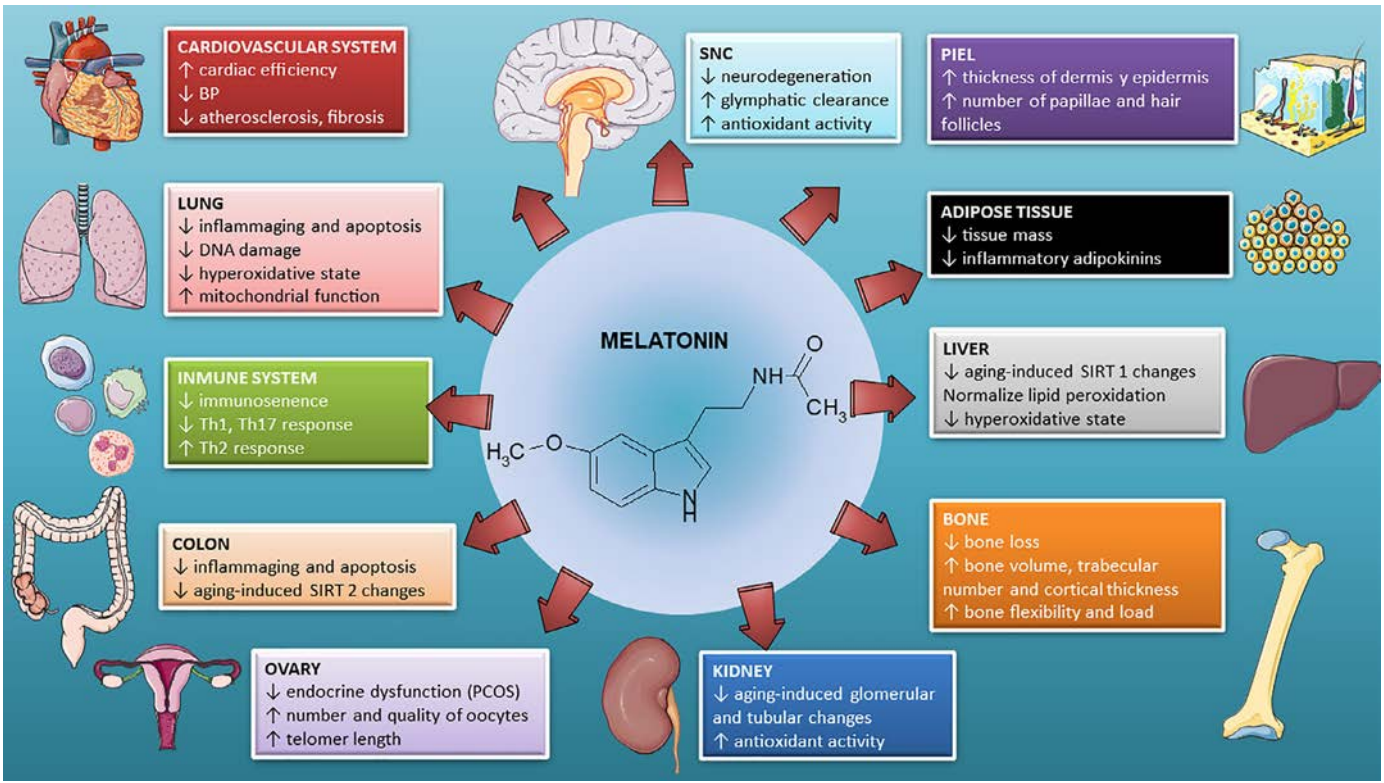

Fig. 2 Melatonin as a cytoprotector in healthy aging. The anti-aging potential of melatonin is shown in different organs.

suggesting that the reduction in CSF melatonin may be an early trigger and marker for AD (Liu, Zhou, van Heerikhuize, Hofman, & Swaab, 1999). Although it is not known whether the relative melatonin deficiency is either a consequence or a cause of neurodegeneration, it seems clear that the loss in melatonin aggravates the disease and that early circadian disruption can be an important deficit to be considered. Significant differences were observed in melatonin levels between mild cognitive impairment (MCI) and AD patients with a negative correlation between neuropsychological assessment of dementia and melatonin levels (Sirin et al., 2015).

Two meta-analyses endorsed the view that melatonin therapy is effective in improving sleep in AD patients (Xu et al., 2015; Zhang et al., 2016). Moreover, the melatonergic agonist ramelteon was reported as effective to treat delirium, an acute state of mental confusion that can lead to many adverse sequelae in intensive care unit elder patients (Furuya et al., 2012).

An analysis of published data of the use of melatonin in the early stages of cognitive decline consistently showed that administration of melatonin, every night before retiring, improves the quality of sleep and cognitive performance in this phase of the disease (see for reference Cardinali, 2019c). Patients treated with melatonin showed a significantly better performance in various neuropsychological tests. They also had lower scores in the Beck Depression Inventory concomitantly with improvement in the quality of sleep and wakefulness. Therefore, melatonin treatment can be effective in the early stages of the neurodegenerative disease (Cardinali, 2019c).

6. Conclusions

Melatonin exhibits both hypnotic and chronobiotic properties and has been used therapeutically for the treatment of insomnia related to age, as well as other primary and secondary insomnia (Leger, Laudon, & Zisapel, 2004; Zhdanova et al., 2001). Several meta-analyses and consensus reports support this role.

As discussed in this article, studies using 2–5 mg of melatonin/day are not adequate to provide an adequate comparison with data on the protection of MS or AD derived from animal studies. Hence clinical trials with doses in the 40–100 mg/day range are urgently needed.

It should be noted that melatonin is remarkably non-toxic, and its safety is very high. The lethal dose 50 for the intraperitoneal injection of melatonin was determined for rats (1168 mg/kg) and mice (1131 mg/kg), but the oral administration of melatonin (tested up to 3200 mg/kg in rats) could not be

determined and for melatonin subcutaneous injection (tested up to 1600 mg/kg in rats and mice) (Sugden, 1983). In humans, melatonin has a high safety profile and, in general, is very well tolerated [see for reference (Cardinali, 2019a)].

Unfortunately, the pharmaceutical industry is refractive to support melatonin studies because of the lack of protective patents for a natural compound. Hence, only with the involvement of governmental and non-profit organizations such a goal can be achieved. At present, the only option for the attending physician interested in the use of melatonin as a cytoprotective is the off-label indication of the drug.

In conclusion, from studies in animals, several potentially useful effects of melatonin, such as those in MS and AD, require high doses of melatonin to be evident. If melatonin is expected to be effective in improving health, especially in the elderly, it is likely that the low doses of melatonin commonly used are not very beneficial so far (Cardinali, 2019b). The question of whether melatonin has a therapeutic value in the prevention or treatment of MS or AD deserves further analysis. Multicenter double-blind studies are needed to explore and further investigate the potential and utility of melatonin. The doses of melatonin used should be re-evaluated in view of the equivalent human doses of melatonin derived from preclinical data. Unfortunately, of the 139 clinical trials related to melatonin in an initial state (recruitment and non-recruitment) listed in PubMed (ClinicalTrials.gov Search results 05/05/2020) none is directed to address this query.

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Conflict of interest

The author declares that there are no commercial or financial relationships that could be construed as a potential conflict of interest.

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