Review

Olfactory neuronal precursors as a model to analyze the effects of melatonin in Alzheimer's disease.

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disorder of great importance affecting millions globally and its prevalence will triple in the following decades. Therefore, analysis and identification of substances which can effectively reduce the pathological process of this disease in different study models are crucial. Melatonin works as a multitasking substance and some of its activities could be used to target the neurodegenerative process of AD. These include, but not limited to, its potent antioxidant activity, regulation of sleep-wake rhythms (important for the consolidation of memory and cognition) and its action as a neurotrophic growth factor that promotes differentiation and neuronal proliferation. To evaluate the effects of melatonin at cellular level in AD, it is essential to have a study model that reflects the pathological process occurring in the CNS. In this, review we summarize the potential use of olfactory neuronal precursors derived from olfactory neuroepithelium directly obtained from patients for such purposes.

Key words: Melatonin, Alzheimer's disease, olfactory neural precursors, olfactory neuroepithelium, neurodegenerative diseases.

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1. INTRODUCTION

Melatonin is a primary secretory product of pineal gland in mammals and was isolated and structurally identified in 1958(1). It is also synthesized in a number of extrapineal sites. These include skin, retina, intestine, platelets, testis, and bone marrow that contain the enzymatic machinery necessary for melatonin production (2–7). Melatonin is an indolamine synthesized from tryptophan taken up from circulation and transformed to serotonin; then, serotonin is converted into melatonin by a two-step enzymatic process (8). Melatonin production in pineal is influenced by signals from the suprachiasmatic nucleus in the hypothalamus, which in turn receives signals from the retina in response to changes of illumination. Thus, melatonin production is higher at

night, in the absence of light, with maximum plasma levels at around 03:00–04:00 a.m., whereas diurnal plasma levels are low or undetectable (7, 9). Once synthesized in pineal gland, melatonin is released into the bloodstream, hence blood concentrations are indicative of its current synthesis rate (10). Melatonin is involved in physiological processes, such as the circadian rhythms, especially of core temperature and sleep-wake rhythms. In humans, melatonin is commonly considered to be a sleep-promoting agent. The circadian organization of other physiological functions depends also on melatonin signaling, for instance immune, the antioxidant defenses, homeostasis, and glucose regulation (7). Also, melatonin has functions like neurotrophic factors that promote survival, neuronal proliferation, and differentiation(11).

One of great healthcare challenges is Alzheimer's disease, (shortened to "Alzheimer's" or "AD"). AD is defined as a multifactorial and irreversible neurodegenerative pathology that mainly affects aged population. It is the main cause of dementia, representing around 60% of cases. This disease causes memory loss and cognitive impairment, generating enormous social and economic burden (12). In 2019, 50 million people worldwide were living with dementia and this number is expected to triple by 2050 (13). AD has a slow and progressive development, such that the prevalence increases with age, with 34.6% of people aged 85 or older (14). The hallmark of AD is the formation of amyloid plaques and neurofibrillary tangles. Amyloid plaques are extracellular accumulations of amyloid-ß peptides (Aß40 and Aß42) as a product of enzymatic cleavage of amyloid precursor protein. This accumulation activates microglia, which, in their attempt to clear the amyloid-ß, damages neural components. Neurofibrillary tangles are helical filaments of hyperphosphorylated tau protein that accumulate inside cells, causing disorganization of the cytoskeleton, alteration of cellular functions, and damage of axons and dendrites (15, 16). The etiology of AD is more complex than the mere presence of these markers. Synaptic dysfunction, neuroinflammation, oxidative stress, and neuronal destruction are processes that coexist at the same time or that are a consequence of one another, and together form part of the global deterioration present in AD.

In this review, we will briefly discuss the role and use of melatonin as a sleep modulator, antioxidant, and as a neuroprotective agent for the maintenance of memory and the stimulation of neurogenesis in AD. We will also describe the attributes that qualify the olfactory neural precursors (ONPs) cultured *in vitro* as a model that reflects biological mechanisms taking place in the CNS, and their potential use in the study of neurological processes with a better approach. We propose *in vitro* cultured ONPs obtained from neuropsychiatric patients through exfoliation of the ONE as a model to study pharmacological responses of melatonin, as well as in combination with other psychotropic substances. The use of this model also allows progress towards personalized medicine, in which individualized treatments can be offered depending on the pharmacological responses of each patient.

2. THE USE OF MELATONIN IN ALZHEIMER´S DISEASE AS A MODULATOR OF SLEEP DISORDER

Melatonin is an important molecule for sleep modulation and it is essential to mention that sleep is primarily involved in the processing of cognition, memory formation, neurogenesis, and synaptogenesis (17–19). Therefore, duration and efficiency of sleep play an important role in cognitive performance and memory capacity in older adults (20). Lower sleep quality is associated with a higher risk of memory impairment and poorer cognitive performance (21). Sleep disorders are particularly prevalent in patients with dementia or cognitive impairment; it has been reported

that up to 60% of them have some kinds of sleep disturbance. Patients with AD and people with mild cognitive impairment (MCI), a stage that precedes dementia, present similar rates of sleep disorders (22) which may appear even before memory and other cognitive deficits are detected. Moreover, sleep disturbances in AD are associated with disruptive behavior and aggressiveness (23). Notably, there is association between sleep latency (time taken to fall asleep), brain amyloid-β deposition determined by Positron Emission Tomography, and lower volume in frontal areas of the brain(24, 25).

Importantly, AD patients show diminished secretion of melatonin, which correlates with rets-activity rhythm disorder (26). Concentrations of total melatonin are also decreased in the cerebrospinal fluid in AD (27) and the expression of melatonin receptors are also impaired in certain brain areas (28). There is evidence that melatonin administration may be beneficial in different settings for individuals with AD. Treatment with extended–release melatonin for six months has been shown to improve sleep efficiency in moderate and mild AD, particularly in those suffering from insomnia and these AD patients also showed significant improvement on assessment of activities of daily living and self-care. In addition, levels of cognition in patients who received treatment with melatonin were stable, whereas in the placebo treated patients, their cognition levels continued to deteriorate. The benefit is greater in the subgroup with insomnia, in which a significant cognitive improvement was observed (29). This greater effect in the subgroup with insomnia may be attributed to a better sleep quality, which is essential for memory and cognition. Other studies have found no effect of melatonin on improving sleep quality or decreasing agitation in AD patients; however, in these trials, melatonin was administered for a shorter period (30). Likewise, the effect of melatonin in patients with MCI has been studied. Dietary melatonin therapy in these patients for six months augmented hippocampus and lamina cribosa thickness volume compared with the placebo group. In addition, the amount of total-tau in the cerebrospinal fluid decreased significantly compared with the group without melatonin treatment (31).

Clinical improvement reported in patients with AD that underwent treatment with melatonin may be due to the role of melatonin in neuronal plasticity. Melatonin in rat hippocampal organotypic cultures increases formation, growth, and maturation of new dendrites (32). This phenomenon may establish important synaptic connections between neurons, which could translate into a reduced clinical deterioration in AD. Collectively, these observations suggest a close relationship between memory, learning, sleep quality, and melatonin, that it is worth continuing to study in AD. Although randomized clinical trials are the best tool to evaluate medical treatments, it is important to highlight the relevance of translational research to obtain answers prior to conducting clinical studies. In this regard, the ONPs as a study model has a significant potential importance.

3. THE USE OF MELATONIN AS AN ANTIOXIDANT IN THE TREATMENT OF ALZHEIMER'S DISEASE

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS), reactive nitrogen species (RNS), and antioxidant defenses. A more contemporary definition describes oxidative stress as "a disruption of redox signaling and control" associated with cell damage (33). ROS/RNS include superoxide radical anion ($O2^{-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), nitric oxide (NO), and peroxynitrite ($ONOO^{-}$). The contribution of oxidative stress in the pathogenesis of AD has been shown in different studies (34, 35), forming part of the global

neurodegenerative process of this disease. The basic mechanisms attributed to the induction of oxidative stress include the deposit of amyloid-β and tau protein, mitochondrial damage, and metal accumulation. In turn, oxidative stress can increase the production of amyloid-β and promotes the phosphorylation of tau, causing a vicious cycle in the progression of the disease (36, 37). Neuroinflammation, mediated by microglia and astrocytes, is also involved in the production of reactive species and proinflammatory components (37).

The strong antioxidant property of melatonin has been extensively described, as well as its role as an endogenous protector against highly toxic ROS/RNS. Melatonin achieves its antioxidant activity in different ways. It directly eliminates free radicals through a process known as the antioxidant cascade of melatonin, which generates different metabolites that also serve this purpose (38). This cascade reaction makes melatonin highly effective in protecting organisms from oxidative stress, since a single molecule of melatonin scavenges up to 10 ROS/RNS (39). Indirectly, melatonin scavenges free radicals by stimulating antioxidant enzymes such as glutathione peroxidase and glutathione reductase, and by suppressing the prooxidant enzyme nitric oxide synthase. In animal models, administration of melatonin decreases the production of ROS in the hippocampus, induced by injections of amyloid-ß peptides (40). Melatonin treatment on rat organotypic cultures counteracted dendritic collapse induced by okadaic acid, a pro-oxidant chemical mimicking alterations found in AD, such as oxidative stress and tau hyperphosphorylation (41). Furthermore, cells incubated with melatonin attenuated the induced oxidative stress, as well as GSK-3 overactivation and tau hyperphosphorylation (42). Despite this evidence, continued study of melatonin and its direct effects as antioxidants in AD, as well as the utility of cellular models such as the ONPs, is needed to obtain more information on this topic.

4. THE INFLUENCE OF MELATONIN ON NEUROGENESIS AND ITS UTILITY IN THE TRATMENT OF ALZHEIMER'S DISEASE

Neurogenesis is the formation of *de novo* neurons, characteristic of a developing brain. The idea of the existence of neurogenesis and the production of new neurons during adulthood had been rejected for a long time. However, this paradigm has changed from the second half of the last century when Altman *et al*. reported neurons and neuroblasts stained with thymidine H3 (a marker associated with proliferation) in some brain structures of adult rats (43, 44). This discovery was a breakthrough in neuroscience. Since then, numerous studies have shown that neurogenesis occurs mainly in two areas within the mammalian CNS: the anterior part of the subventricular zone, along the lateral ventricles, and in the subgranular zone of the dentate gyrus in the hippocampus (45, 46). Now it is widely accepted that the adult brain continues neurogenesis throughout life. However, this activity decreases with aging (47). Neurogenesis is a multistage process, which includes proliferation, differentiation, migration, axonal and dendritic targeting, and synaptic integration (48). Therefore, it is expected that incorrect functioning of any of these stages can cause the dysregulation of this process.

Melatonin can influence the neurological processes of neural stem cells (NSC). In NSC derived from the mouse embryo striatum, melatonin facilitates neural differentiation without affecting astroglia (49). Likewise, melatonin stimulates NSC proliferation during hypoxia and promotes differentiation into neurons through the MT1 receptor (50). It is also known that adult mouse precursors of the subventricular zone express melatonin receptors. These cells increase their proliferative activity when they are treated with melatonin and this effect is reversed with luzindole, a competitive antagonist of melatonin receptors (51). Melatonin also has an important

role in the survival of NSC. Song *et al*. reported that melatonin protected NSC against LPS-induced inflammatory stress in mouse embryo models (52). Additionally, melatonin exerts important effects on dendritogenesis (53). In an organotypic model of adult rat hippocampus, treatment with melatonin stimulated the formation of new dendrites, increasing their thickness, length, and complexity (32). All these influences are of great medical importance, since they offer new opportunities for therapeutic approach in neurodegenerative diseases.

4.1 Neurogenesis, an affected process in Alzheimer's disease.

Diverse evidence has suggested an impaired adult neurogenesis (47) and increased neurodegenerative diseases with aging. The decline of neurogenesis in AD suggests it could be an early risk factor to disease progression. However, Moreno-Jiménez *et al.* (54) by using postmortem human hippocampal tissue, identified the persistence of adult hippocampal neurogenesis (AHN), during both physiological and pathological aging and provided evidence that the number and maturation of these neurons progressively declined as AD advanced. Using immature neuronal marker doublecortin (DCX), they showed the persistence of AHN in aging. They observed a moderate decrease in DCX+ cells levels as age increased from 40 to 90 years in neurologically healthy subjects and that DCX+ cells levels from individuals of any age are increased when compared to those of AD patients. These results provide evidence for impaired neurogenesis as a potentially relevant mechanism underlying memory deficits in AD. Similarly, the results obtained by Tobin *et al*. suggest a possible association between neurogenesis and the degree of cognition due to the decrease of DCX+ cells levels in MCI (55).

In contrast, there has also been evidence of increased protein markers of immature neurons in the hippocampus of individuals with AD, suggesting an increased neurogenesis. This evidence leads to the idea that the increase in the production of new cells is a mechanism to replace the neuronal loss caused by the degenerative process of AD (56). It is important to note that the samples from the mentioned studies are from postmortem brains, causing possible changes in the tissue that could lead to confounding effects. Therefore, research is required where live neuronal lineage cells can be evaluated to reduce the changes caused by cell death processes.

5. COGNITIVE EFFECTS OF MELATONIN AND ITS IMPORTANCE IN ALZHEIMER'S DISEASE

Neural damage in AD initially begins in areas of the brain involved in memory and cognition, including the entorhinal cortex and hippocampus (57). Thus, early symptoms of AD can be manifested as difficulty to remember recent conversations, names, or events, progressing to severe cognitive impairment (58). Melatonin has important effects on cognition and memory maintenance. Melatonin prevents the impairment of memory and place learning in rats that previously submitted to global ischemia. Furthermore, the indolamine reduces pyramidal neuronal death in the hippocampus (59). In a mouse model of multiple sclerosis, melatonin improves memory defects, upregulates expression of the synapse-associated synaptophysin and postsynaptic density protein 95 genes in the prefrontal cortex, and increases cAMP-response element-binding protein (CREB) (60). Moreover, chronic administration of melatonin attenuates alterations in the levels of proinflammatory proteins present in aged mice, suggesting an anti-inflammatory role, which may lead to the prevention of memory decline that occurs in aging (61).

It has been observed that treatment with melatonin improves memory deficits and regulates CREB/BDNF signaling and cholinergic transmission in the prefrontal cortex of an AD mouse model (62). Additionally, prophylactic administration of melatonin reduces AD neuropathology and associated cognitive deficits in the AβPP(swe)/PS1 mouse for AD (63). In patients with MCI, treatment with fast-release melatonin at bedtime for 9 to 18 months and 15 to 60 months exhibited significantly better performance in Mini-Mental State Examination and the cognitive subscale of the Alzheimer's disease Assessment Scale. Furthermore, abnormally high depression scores decreased in melatonin-treated patients, concomitantly with the improvement in wakefulness and the quality of sleep (64, 65).

Overall, these studies highlight the advanced role of melatonin in limiting the main symptoms manifested in AD, which are cognitive deterioration and all the clinical consequences that arise from it. Hence, it is worth continuing to study these implications in AD and other neurocognitive disorders.

6. OLFACTORY NEURONAL PRECURSORS AS A STUDY MODEL FOR NEURODEGENERATIVE DISEASES

A great challenge that has been faced over the years to study cellular mechanisms that involve brain processes, such as the study of neurological and neuropsychiatric diseases, is the availability of a model that matches the disease of interest. The ONE, also known as olfactory epithelium, is an extensively studied tissue whose characteristics could potentially make it a surrogate model of the CNS. This tissue is a pseudostratified columnar epithelium, localized in the posterodorsal nasal fossa, which covers the inferior portion of the cribriform plate and the superior and middle nasal turbinate. It is constituted by bipolar olfactory sensory neurons (OSNs), microvillar cells, sustentacular cells, basal cells, Bowman's glands, and axon elongations (66, 67) (Figure 1).

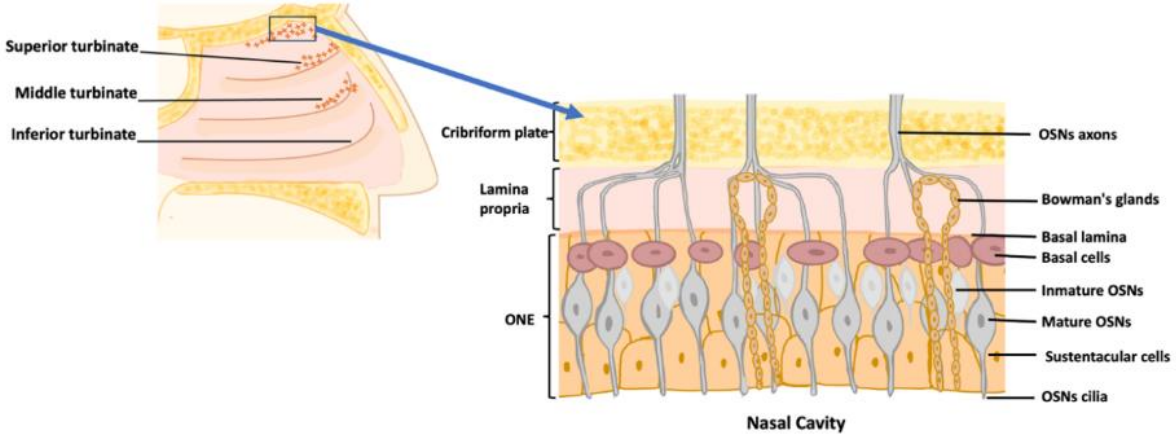

Fig. 1. Location and conformation of olfactory neuroepithelium (ONE).

The ONE is located in the nasal cavity on the underside of the cribriform plate and in areas of the middle and superior turbinate (as indicated by orange cross-outs). ONE, together with the lamina propria, make up the olfactory mucosa. ONE is composed of mature olfactory sensory neurons (OSNs) near the lumen of the nasal cavity, supporting sustentacular cells, immature OSNs, basal cells adjacent to the basal lamina, and Bowman's gland ducts. The lamina propria of the ONE has bundles of OSN axons and Bowman's glands.

There is evidence that neuronal lineage population present in the human ONE is in different states of maturation that comprise of stem cells, progenitor cells to immature and mature neurons, providing evidence of neural regeneration throughout adult life (68, 69). Basal cells localized adjacent to basal lamina can produce ONPs and then differentiate to OSNs that live for about 4 to 6 weeks (70). Furthermore, these cells express neurotrophic factor receptors on their surface, which is consistent with neural properties (68). In fact, the stimulation with neurotrophic factors makes them lose their progenitor properties, gain characteristics of mature neurons, and promote neurite formation(71). Moreover, the presence of neurotransmitter receptors for dopamine, 5-HT, and NMDA, as well as their active signaling mechanism, has been demonstrated (72). All these features qualify the ONE as a surrogated specimen of the CNS that can be helpful to describe cellular changes and neural processes involved in neurodegenerative and neuropsychiatric diseases.

The expression of specific receptors (MT1 and MT2) in ONPs makes them useful in the study of the effects of melatonin, mainly the stimulation of axonal formation and branching through a mechanism mediated through these receptors (73). ONPs also express calcium binding proteins, among which calmodulin is found, and therefore it is possible to study the calcium signaling and other mechanisms of action involved in the effects of melatonin (74).

Human ONE specimen has been obtained through various methods (postmortem samples, biopsies, and biopsies combined with laser-captured microdissections) to be processed immediately or to perform cell cultures (75–77). Recently, a new technique has been developed that facilitates and optimizes obtaining the samples of this tissue. Exfoliation is a noninvasive method, whose advantage is that it can be performed in outpatients without anesthesia. This new technic consists of obtaining cells from ONE by scraping with a tiny brush a certain area of the nasal cavity, specifically the middle turbinate. Subsequently, the sample obtained from the brush is mechanically dissociated in selective medium that enables proliferation of ONPs and restricts the growth of epithelial cells, among other cell populations (78). These processes have been illustrated in Figure 2.

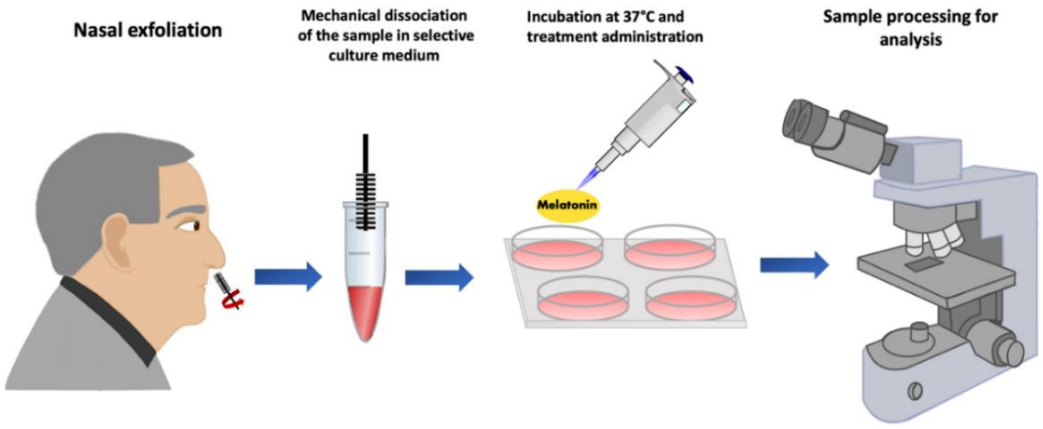

Fig. 2. Obtaining ONPs from ONE using nasal exfoliation.

The technique consists of introducing a small cylindrical brush into the nostril, spinning 3 times around the middle turbinate. Subsequently, the sample of the brush is mechanically dissociated and cultured in a selective medium to promote proliferation of ONPs. Finally, experiments such as immunofluorescence exemplified in the image, can be carried out. Additionally, these cultures can be treated with melatonin.

ONE cells have been studied in conditions such as AD, schizophrenia, and bipolar disorder (77–80). The potential of culturing cells obtained from ONE to elucidate the pathophysiology of AD was suggested at the end of the last century. Since then, it was used to study proteins differential expression and amyloid precursor protein catabolism (81–84). In the search for main AD biomarkers present in ONE, research began by studying postmortem samples. In 2009, Arnold *et al.* found higher expression of amyloid-β in the ONE from patients with a diagnosis of AD confirmed by autopsy than in those individuals without the diagnosis. Similarly, the expression of helical filament-tau was overexpressed. These pathological findings correlated with the average amyloid-β plaques and helical filament-tau found in cortical regions (85). In contrast, in a recent study carried out in 2019, no increase was found in amyloid-β and helical filament-tau expression in the ONE, and this did not correlate with brain pathology scores at autopsy (86). These two studies have contradictory findings, probably due to the cellular and molecular changes that can occur because of the time between death and sample collection. Postmortem samples also assess a heterogeneous population of cells, without distinguishing between neural lineage cells or epithelial cells. In a study where ONE was obtained by biopsy, it was reported that the expression of amyloid-β deposits was higher in cells near the ONE lumen and in the parenchyma of patients with MCI and AD. Additionally, an explant culture system was established, and it was reported that cell migration from patients with AD and MCI is decreased compared to their counterparts without cognitive impairment (87). With a different approach, Riquelme *et al*. obtained ONP derived from ONE by exfoliating the nasal cavity with the technique mentioned above. They found an increase in the levels of total-tau and phosphorylated-tau in this cell population obtained from AD patients in comparison with control patients without AD (88). All this evidence suggests that ONE and particularly ONPs reflect the cellular changes present in pathologies such as AD.

6.1. ONE as an oxidative stress model.

Primary culture of ONE will be useful in understanding the mechanism of oxidative damage in AD and may even be utilized to develop antioxidant therapeutic strategies. To assess oxidative stress in ONE, the activity of 3-nitrotyrosine (3-NT), a stable marker of oxidative damage to cell proteins, has been evaluated. 3-NT is a product of tyrosine nitration mediated by RNS and is increased in the brains of AD patients. 3-NT Immunoreactivity was localized in olfactory receptor neurons, including dendritic knobs. These results demonstrate increased oxidative stress that may contribute directly to olfactory impairment in AD patients (89). Moreover, there is also an imbalance in the antioxidant response. Increased immunoreactivity of manganese and copper-zinc superoxide dismutase's (antioxidant enzymes) has been found in the ONE of AD patients compared with age-matched controls. The enhanced production in these enzymes suggests that there is an increase in both oxidative stress and counteracting antioxidant defenses (90). Other well-known markers of oxidative stress, specifically lipid peroxidation adduct hydroxynonenal pyrrole, Nε-(carboxymethyl) lysine, and heme oxygenase-1, are significantly increased in neuronal cultures derived from ONE in AD compared with controls. These markers are also present in early pathological changes in the AD brain (91). Furthermore, ONE cells obtained from patients with AD are more susceptible to oxidative stress (H_2O_2-induced stress), producing less viability and a change in its morphology compared to their non-stressed counterpart. Lastly, treatment with PAN-811 (an ion chelator, originally used for cancer therapy) reduces cell death induced by H_2O_2, suppressed intracellular ROS accumulation, lipid peroxidation, and cell membrane damage (92). All this evidence shows that ONE is a useful model to describe oxidative processes that occur in

AD and allows us to study possible effects of substances with antioxidant properties such as melatonin.

6.2. Olfactory neuronal precursors as a study model to assess neurogenesis and the effects of melatonin.

As mentioned above, the ONE is a tissue found outside the CNS but where the neuronal populations in different stages of maturation exist, including ONPs (68, 69, 93, 94) The culture of ONPs from ONE has proven to be useful for the analysis of the processes involved in neurogenesis. Although the main topic of this review is about AD, it is important to mention that the leading reason why neurodevelopment and neurogenesis in ONPs has been studied is to explain the etiology of schizophrenia and bipolar disorder (77, 96-102). One of the most suggested models that explain the etiology of schizophrenia is the neurodevelopmental hypothesis. This hypothesis explains that developmental insults during pregnancy caused by pathologic processes, involving genetic and environmental factors, lead to the activation of abnormal neural circuits (95). Therefore, studies of neurogenesis and neurodevelopment in this tissue focus mainly on these two conditions.

ONPs obtained from patients with schizophrenia have an increase cell proliferation with significantly more mitosis compared with controls (77, 96). It has also been reported that, initially, there is an increase in proliferation that subsequently decreases (80). This altered cell proliferation is consistent with the dysregulation of density and proportions of neuronal linage reported in postmortem ONE from subjects with schizophrenia, where immature neurons are increased and precursor cells are decreased (97). The ONE is also useful for transcriptomic analysis, where significant differences have been shown in the expression of transcript-related neurogenesis (MSI1) and cell cycle (RAD51L1, NCK2, and VIPR1) between schizophrenia and controls(96). Alterations in the function and structure of microtubules are a finding described in ONPs of patients with schizophrenia. This is important because, during neurogenesis, microtubules play an essential role, since they participate in multiple processes such as cell cycle, migration, and outgrowth of axons and dendrites. ONPs obtained by exfoliations from the ONE of schizophrenia patients have shown areas with disorganized microtubules in the cytoplasm and at cell periphery (98). In addition, the dynamics of the microtubules is altered as shown by Brown *et al*. in their experiments, where the microtubules of ONE culture from patients with schizophrenia are more stable compared to their control counterparts (99). The changes in microtubular dynamics can reshape and affect ultrastructural components of synapses. Migration, other cellular process where microtubules are involved, is also affected in ONPs from schizophrenia and AD patients (100).

Importantly, melatonin modulates the organization of main components of the cytoskeleton and, in this regard, may contribute to the structural changes that occur during neurodevelopment (101). The effect of melatonin on neurogenesis has been studied in ONPs from individuals with schizophrenia. Galván-Arrieta *et al.* reported that formation of axons in ONPs is reduced with the lower expression of melatonin receptors. When those cells were treated with melatonin, the reduction of melatonin receptors was recovered and the axonal formation and branching were increased. Moreover, melatonin causes increased levels of phosphorylated GSK3B, whose inhibition by phosphorylation during neurodevelopment triggers rearrangements in the cytoskeleton promoting axonal formation (73). Regarding actin filaments, which are part of the cytoskeleton structure, melatonin thickened actin filaments in both control and schizophrenia ONPs (102). Another characteristic observed in ONPs of patients with schizophrenia was an

increase in the evoked secretion of K+ (exocytosis); melatonin treatment modulated this process, causing a secretion back to the control level (102). Of note, brain images have shown a decreased hippocampal volume in schizophrenia, probably due to a process related to neurogenic alterations, a similar feather of AD. Therefore, the effect of melatonin on neurogenesis can be useful to reduce the hippocampal atrophy and to reestablish brain connectivity that will improve cognition in AD.

The effects of melatonin on the ONPs of patients with schizophrenia, including changes in neurogenesis, suggest that more information could be obtained through the evaluation of this hormone in ONPs of patients with AD.

Recently, our team found decreased proliferation quantified by Ki-67 in ONPs from AD compared to older adults without evidence of cognitive impairment. In addition, older adults had a lower rate of proliferation compared to young adults. These promising results suggest that, just as the regeneration of new neurons is diminished in the hippocampus, the same may be occurring in the ONE. The potential effects of melatonin on neurogenesis from ONE were summarized in the Figure 3.

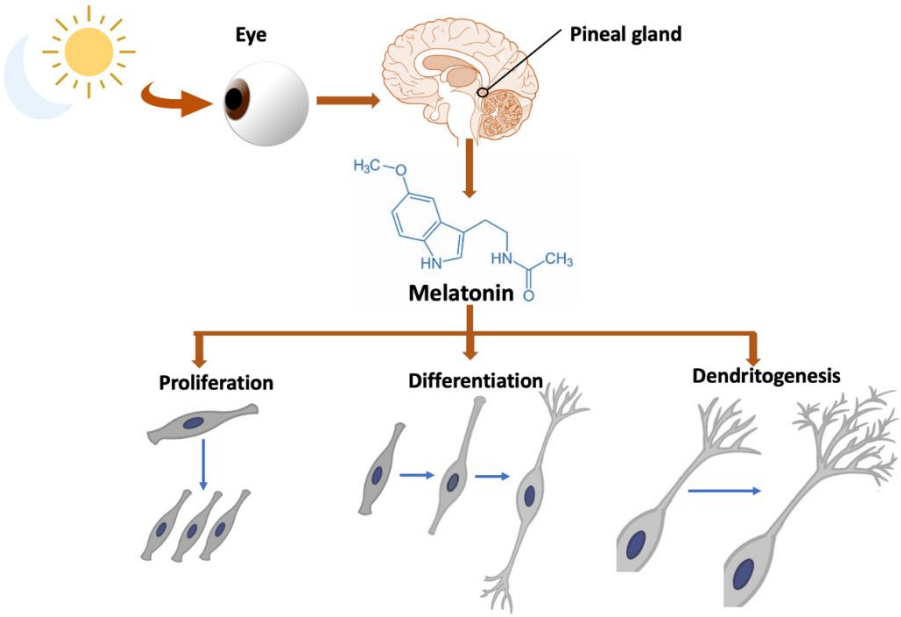

Fig. 3. Schematic representation of effects of melatonin on neurogenesis.

Changes in light stimuli to the retina translate into information for the synthesis of melatonin in the pineal gland. Melatonin induces proliferation of neuronal precursors, differentiation to mature neurons, and formation and maturation of new dendrites.

6.3. The utility of olfactory neuronal precursors to study neurogenic responses elicited by melatonin in combination with psychotropic drugs for the treatment of Alzheimer's disease.

Recent evidence indicates that ONPs like a surrogate model of the CNS are useful to study neurobiological responses elicited not only with melatonin, but also in combination with other psychotropic drugs that may contribute in the treatment of AD. Melatonin with ketamine (hallucinogen with anesthetic and antidepressant properties) in ONPs increases neurogenesis, assessed by markers of neuronal progenitors, neurogenic neurons, and proliferation, at doses that produce antidepressant effects in mice. The response to this combination of compounds is like those produced by nerve growth factors (103). With this evidence, it can be affirmed that ONPs

from ONE are a feasible method to evaluate the various processes involved in neurogenesis and the effects of melatonin, alone or in combination with psychotropic drugs, in patients suffering from diseases with alteration in the regeneration of new neurons, such as AD.

7. DISCUSSION

Fully understanding the pathophysiology underlying AD is complex, as various alterations occur, culminating in neuronal, axonal, and synaptic degeneration with the clinical consequence of cognitive impairment. Just as AD is multifactorial, finding a solution to this disease process must aim to have multiple targets. Melatonin is an extensively studied compound whose functions include not only the control of the circadian rhythms, but also relevant properties that could be useful in the treatment of AD.

First, as a regulator of the sleep-wake rhythm, melatonin may help improve sleep quality, which is disturbed in a significant fraction of AD patients. As we have mentioned before, a good sleep quality is associated with memory consolidation and learning. Therefore, we can elucidate a close relationship between cognitive deterioration and sleep disturbances, as well as the role of melatonin plays in this process. Second, oxidative stress acts as a relevant mediator of AD pathophysiology, whose damages can be counteracted by melatonin and its various antioxidant properties. Third and last, melatonin may improve processes involved in neurogenesis that include proliferation, neuronal survival, differentiation, and migration.

Although the evidence is not consistent regarding neurogenesis in AD, both of its increase and decrease reported by different studies may be part of the overall neurodegenerative process. Further studies of melatonin as a treatment in AD are required. In this review, we present a translational study model: the obtainment of ONPs derived from ONE, which has potential utility for studying processes involved in the etiology of the diseases, treatments, and personalized pharmacological responses. Translational research is an emerging discipline focused on turning observations in the laboratory and clinic into tangible health interventions, such as diagnostic methods, therapies, or other medical procedures (104). We also highlighted how the ONE was obtained directly from patients with a noninvasive and injury-free process. It is impractical to obtain the live neuronal stem cells from the CNS of AD patients. The easy availability of live ONE which have the characteristics of potential neurogenesis from the AD patients provides useful tool for AD research, given that the effects of melatonin can be evaluated in pathologies such as AD, which further implicates the potential of individualized medicine.

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AUTHORSHIP

VSM searched for the information, synthesized, and wrote the manuscript, likewise produced, and edited the figures. Dr. GBK guided the concept of the review, likewise, guided the writing, edited the manuscript, reviewed in detail and finally approved the manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest associated with this work.

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