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Review

New perspectives in melatonin uses

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ABSTRACT

This review summarizes the metabolism, secretion, regulation and sites of action of melatonin. An updated description of the melatonin receptors, including their signal transduction mechanisms, distribution and characterization of receptor genes, is given. Special emphasis is focused on the clinical aspects and potential uses of melatonin in the sleep-wake rhythms, in the immune function, in cancer therapy, in neuroprotection against oxidative damage and antioxidant activities in different tissues. Finally, combined effects of melatonin with other drugs are discussed.

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Melatonin (MEL) (N-acetyl-5-methoxytryptamine) was discovered about fifty years ago and is a compound synthesized by the pineal gland in the human brain. It is also produced in retina, thymus, bone marrow, respiratory epithelium, skin, lens, intestine and in other sites [1,2]. Pineal MEL passes freely through membranes and distributes in all body compartments, whereas retinal MEL apparently acts locally within the eyes [3]. MEL participates in the regulation of important physiological and pathological processes. It is considered a hormone that regulates the circadian day–night rhythm and seasonal biorhythm by the classical chronobiology. MEL has been characterized as an effective synchronizing agent in several physiological and pathological conditions, such as in maternal-fetus entrainment [4] and in dissociated circadian rhythms induced by a short light–dark cycle [5]. In addition, modulation of immune defense responses, body weight and reproduction, tumor growth inhibition and anti-jetlag effects have been recognized [6]. There is also evidence that MEL could act as a potent

Abbreviations: AANAT, N-acetyltransferase; AD, Alzheimer disease; AFMK, N^1-acetyl-N^2-formyl-5-methoxykynuramine; AMK, N^1-acetyl-5-methoxykynuramine; CAT, catalase; CNS, central nervous system; ETC, electron transport chain; GI, gastrointestinal; GPCR, 7-transmembrane G-protein-coupled receptor; GPx, glutathione peroxidase; GR, GSH reductase; GSH, glutathione; GSSG, oxidized GSH; HIOMT, hydroxyindole-O-methyltransferase; IFN-α, interferon alpha; IFN-γ, interferon gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; LOX, lipoxygenase; MDM2, murine double minute-2; MEL, melatonin; MEN, menadione; mtDNA, mitochondrial DNA; NAS, N-acetylserotonin; NAT, N-acetyltransferase; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson disease; PLA2, phosholipase A2; QR2, quinone reductase 2; RNS, reactive nitrogen species; ROS, reactive oxygen species; SCN, suprachiasmatic nucleus; SOD, superoxide dismutase; Th1, Type 1 helper T lymphocytes; Th2, Type 2 helper T lymphocytes; TNFα, tumor necrosis factor alpha.

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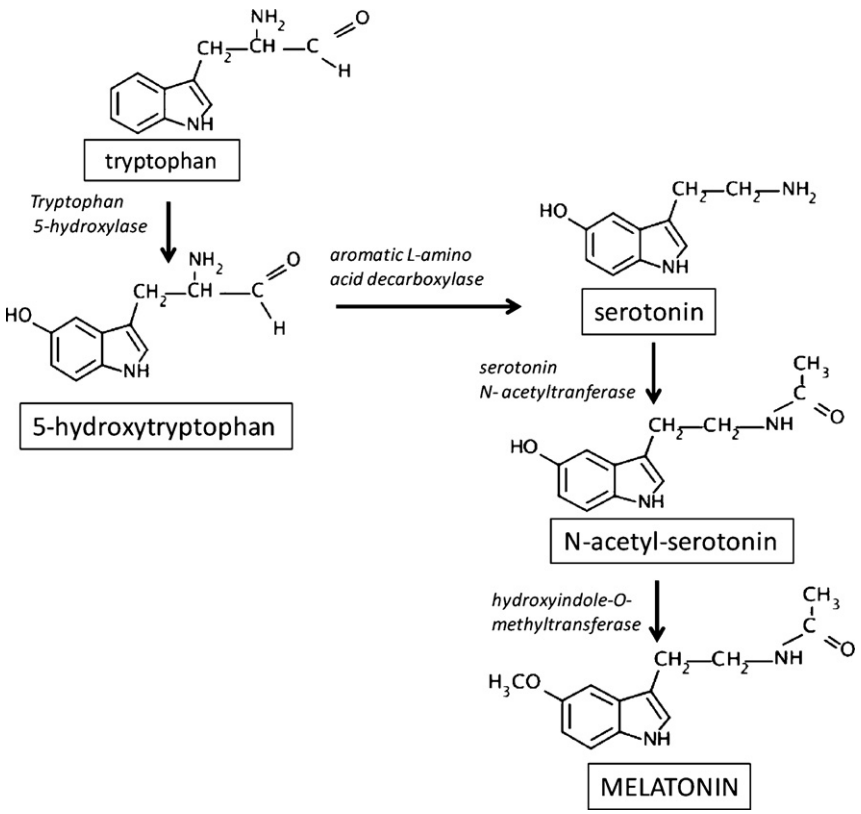

Fig. 1. Metabolic pathway of melatonin synthesis.

direct antioxidant, as a chemotoxicity reducing agent and a putative anti-aging substance [7].

1. Structure, metabolism and secretion

MEL is an indoleamine containing two functional groups, which are decisive not only for the receptor binding but also for its amphiphilicity giving to the molecule the capacity to enter any cell compartment or body fluid. Due to its extensive solubility in lipids, MEL easily passes by diffusion from the peripheral circulation to other fluids or cells. In serum, 70% of the MEL is bound to albumins and the remaining 30% diffuses to the surrounding tissues [8].

Tryptophan and serotonin are precursors of MEL. Two well characterized enzymes participate in its synthesis: N-acetyltransferase (AANAT), which converts serotonin to N-acetylserotonin (NAS), and hydroxyindole-O-methyltransferase (HIOMT), which converts NAS to MEL (Fig. 1). The regulation of MEL synthesis is controlled by the light–dark cycle, acting through neural activation of the anterior hypothalamus *via* the axons of retinal ganglion cells running in the optic nerves and forming the retino-hypothalamic tract. Suprachiasmatic nucleus (SCN) is connected with the pineal gland through paraventricular nuclei and preganglionic sympathetic neurons. Norepinephrine released from postganglionic sympathetic fibers at pinealocyte membrane stimulates its adrenoceptors leading to cAMP formation as well as other second messengers, which stimulate the expression and activity of AANAT, the first-rate limiting enzyme in MEL production [9]. In non-mammalian vertebrates, this enzyme appears to be directly controlled by circadian clock genes in the pineal gland [8].

The secretion of MEL has a typical diurnal rhythm. At night the synthesis and secretion of MEL are stimulated, reaching a peak value (80–150 pg/mL) between midnight and 3 a.m., while its concentration during the day is low (10–20 pg/mL) [10]. Once synthesized in the pineal gland, MEL is secreted into the blood and cerebrospinal fluid and reaches other body fluids such as bile, cerebrospinal fluid, saliva, semen, ovarian follicular fluid and amniotic fluid. Small amounts of unmetabolized MEL are excreted in the urine.

The half life of MEL in the serum has been calculated to be in the range 30–50 min [11]. The ability to synthesize MEL is rather constant in a given person, but there is marked variability among individuals. There is evidence that MEL levels decrease with increasing age in mammals, including humans. The serum level of MEL is very low in the first weeks of postnatal life, without diurnal variation. At six months of life the typical diurnal rhythm of secretion appears, reaching the maximum levels between the third and sixth years of life. A marked decrease in MEL secretion has been noted during sexual maturation. At 40–50 years, a noticeable decrease in daily MEL synthesis has been observed and after the 70 years of age the diurnal rhythm of secretion practically disappears in most individuals [10]. A seasonal variation in the synthesis of MEL in humans seems to exist, the levels being higher in winter than in summer [12].

The catabolism of MEL was thought to be almost exclusively done by the hepatic P450 monooxygenases, followed by conjugation of the resulting 6-hydroxy-melatonin to give the main urinary metabolite 6-sulfatoxy-melatonin. This might occur with the circulating hormone. In contrast, in the central nervous system (CNS) the oxidative pyrrole-ring cleavage predominates and no 6-hydroxy-melatonin was detected after MEL injection into the cisterna magna, which may be important because much more MEL is released *via* the pineal recess into the cerebrospinal fluid than into the circulation. The primary cleavage product is N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK). Several different reactions lead to the same product, AFMK, and this pathway contributes to about one third of the total catabolism. AFMK is converted into N^1-acetyl-5-methoxykynuramine (AMK). AFMK and AMK form metabolites by interaction with reactive oxygen and nitrogen

species. Some other metabolites have been detected, but apparently in minor quantities [13].

2. MEL receptors

Most actions of MEL are mediated by membrane receptors and nuclear sites corresponding to orphan members of the nuclear receptor superfamily RZR/ROR. Three subtypes of mammalian MEL receptors have been proposed and cloned. Two of these, MT1 and MT2, are members of the 7-transmembrane G-protein-coupled receptor (GPCR) family. These two receptors are classified as unique subtypes based on their molecular structure and chromosomal localization. Both of them belong to the class A group of rhodopsin-like GPCRs. They are formed by 350 and 362 aminoacids and their calculated molecular weights are 39 and 40 kDa, respectively. The gene *MTRN1A* for the MT1 receptor is located at position 4q35-1 and the gene *MTNR1B* for the MT2 receptor at 11q21-22. They share 60% homology. MT1 has two potential glycosylation sites in the N-terminal region and MT2 has one potential glycosylation site in the same region. Both receptors involve signaling through inhibition of cAMP formation and protein kinase A activity, and effects on phospholipase A2 and C, calcium and potassium channels [14]. MT3, the third receptor, is an enzyme identified as quinone reductase 2 (QR2). Little information exists on nuclear MEL receptors. Another MEL-related receptor, named *GPR50*, has also been found in different species including humans [15]. Mel_{1c}, the first type of MEL receptor discovered, is a receptor subtype expressed in non mammalian species [16]. There is some evidence that MEL receptor expression exhibits circadian variation [17].

3. MEL and antioxidant properties

There is a large body of evidence that MEL is a major scavenger of both oxygen and nitrogen-based reactive molecules. MEL provokes this effect at both physiological and pharmacological concentrations. Several of its metabolites can also detoxify free radicals and derivatives [18]. The indolamine eliminates the decomposition products of peroxynitrites, including free hydroxyl radicals and nitrogen dioxide radicals and the carbonate radical in the presence of physiological CO_2 concentration [19]. MEL induces the synthesis of another intracellular antioxidant, glutathione (GSH), in rabbits that exhibit diabetes-induced oxidative stress [20]. The benefit of antioxidant properties of MEL has been shown in patients with rheumatoid arthritis (RA) [21], females with infertility [22] and in elderly patients with primary essential hypertension [23]. However, there is no agreement about the beneficial effects of MEL on RA. Instead, MEL has been reported to be a RA promoter due to its capability to act as an immunoenhancing agent and stimulator of proinflammatory cytokine release [24,25]. With regards to the enzymes of the antioxidant system, MEL regulates the expression of several genes such as those of superoxide dismutase (SOD) and glutathione peroxidase (GPx). The hormone influences the enzyme activity and cellular mRNA levels of these proteins under physiological and oxidant conditions [26]. The MEL concentration to up-regulate the enzyme gene expression corresponds to the physiological night time peak of plasma MEL, whereas higher concentration does not affect the gene expression. The antioxidant effect of low concentrations of MEL seems to be indirect *via* up-regulation of GPx gene expression and antioxidant activity, while at high concentration the effect is attributed to direct radical scavenging actions. It has been proposed that the ability of MEL to up-regulate the antioxidant enzyme involves both membrane and nuclear receptors [27]. It appears that the stimulation of GPx by MEL is the most consistent effect, while the effect on other enzymes is tissue-specific or conditional.

4. MEL and mitochondria

The lipophilic nature of MEL favors that the indolamine crosses cell membranes to easily reach subcellular compartments including mitochondria. MEL interacts with lipid bilayers and stabilizes mitochondrial inner membranes, which may improve the electron transport chain (ETC) activity. At a concentration of 1 nM, MEL increases the activity of the complexes I and IV in rat liver mitochondria, while 10–100 nM MEL stimulates the activity of those complexes in brain mitochondria. When a dose of cyanide decreases the complex IV activity by 50%, MEL counteracts this inhibition. In contrast, when the complex IV is totally inactivated by cyanide, MEL is unable to counteract this inhibition, independently of its concentration. The previous data suggest that the regulation by MEL on the activities of complexes I and IV does not only rely on its antioxidant properties. MEL has a high reduction potential, which suggests that MEL might interact with the components of the electron transport chain increasing the electron flow, and consequently, the ATP production [28].

As known, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are synthesized as subproducts of the mitochondrial electron transport chain, although the main sources of •NO are the reactions catalyzed by nNOS (neuronal nitric oxide synthase) and the iNOS (inducible nitric oxide synthase). Moderate levels of •NO are considered favorable for mitochondrial function, whereas high •NO concentrations produce severe ETC dysfunction, blocking the respiration in extreme conditions of inflammation [29]. MEL seems to protect proteins of the ETC and mtDNA from ROS/RNS-induced oxidative damage. The hormone limits the loss of intramitochondrial GSH, improves the electron transport chain activity and reduces mtDNA damage. It apparently increases the expression and activity of complex IV and the activity of complex I, which improves mitochondrial respiration and increases ATP synthesis [28].

MEL also regulates the GSH redox status in isolated brain and liver mitochondria when it is disrupted by oxidative stress. The indolamine increases the mitochondrial GSH content, decreases oxidized GSH (GSSG) and hydroperoxide levels and stimulates the activity of GPx and GSH reductase (GR), which are two enzymes involved in the GSH-GSSG balance [28]. Petrosillo et al. [30] have demonstrated that micromolecular concentrations of MEL prevent calcium dependent cardiolipin peroxidation in mitochondria, thus protecting against the induction of mitochondrial permeability transition and cytochrome c release.

It is of interest that MEL acts as an antiapoptotic agent in mitochondrial ROS/RNS- mediated cell death, but it also has proapoptotic effects in several tumor cell lines such as in MCF-7 breast cancer cells [31], which suggests that MEL has a potential use to kill cancer cells preserving normal cells.

A direct relationship between the indolamine and aging has not been proven but certain properties of the hormone indicate its potential benefit in the elderly. Data obtained in senescence-accelerated mice have demonstrated that chronic administration of MEL does not reduces respiratory function, decreases the neural and hepatic peroxidation products and the protein carbonyl content in brain [32].

5. MEL and immune system

A link between MEL from the pineal gland and the immune system in different species, including humans, has been documented [33]. The immune system is modulated by different environmental signals being light one of them. Although most of the light received by the retina goes to the visual cortex, another pathway from the retina relays to the SCN, which forms part of the hypothalamic

region in the brain [34]. The hypophysis and the pineal gland are also involved in neuroendocrine changes induced by the light. MEL is one of the neuroendocrine hormones that are sensitive to changes in circadian rhythm. Since MEL is produced not only by the pineal gland, but also in the retina and in other parts of the body, the immune system might be affected by MEL originated in different organs. Besides, human peripheral blood mononuclear cells synthesize important amounts of MEL, which indicates a potential intracrine and paracrine role of MEL in immune regulation. Membrane receptors have been found in T and B lymphocytes. MEL also stimulates cytokine production by human peripheral blood mononuclear cells through nuclear receptors [35]. Although the mechanism by which MEL enhances the immune response is not clear, it is thought that it may increase phagocytosis and antigen presentation. In addition, MEL induces T cell differentiation toward the type 1 helper T cells (Th1) phenotype and the activation of the immune system by the indolamine has been shown to be mediated by the regulation of gene expression of cytokines in the spleen, thymus, lymph nodes and bone marrow [36]. Natural killer cell activity and production are apparently increased by MEL administration either in humans or mice. Although many studies have implicated MEL as a positive regulator of immune system, other reports have also suggested that MEL could act as an anti-inflammatory agent through inhibition of immune responses. The anti-inflammatory action is considered to be, at least in part, due to the induction of type 2 helper T lymphocytes (Th2) producing interleukin (IL)-4 and inhibiting Th1 function [37]. MEL has pleiotropic effects on different steps of inflammation. It has a pro-inflammatory role at an early phase through activation of pro-inflammatory mediators such as phosholipase A2 (PLA2), lipoxygenase (LOX) and cytokines such as IL-1 and tumor necrosis factor alpha (TNFα). Contrarily, when the inflammatory process proceeds toward the chronic phase, MEL has a negative effect. The antagonist role of MEL on chronic inflammation is due to downregulation of mediators such as PLA2, LOX and cytokines, induction of a survival pathway in leukocytes and blocking of oxidation by its antioxidant properties [38].

MEL could also play a role in the influence of seasonal changes on the immune function. In healthy volunteers during autumn/winter season, the production of IL-6, interferon alpha (IFN-α) and interferon gamma (IFN-γ) was higher. The seasonal changes in immune functions appear to be mediated by alterations in duration of MEL secretion. In this sense, changes in mood and behavior such as in the seasonal affective disorder have been associated with seasonal changes in cytokines like IL-6, IFN-α or the balance between Th1 and Th2 responses [36].

6. MEL and the gastrointestinal (GI) tract

MEL is also produced in high amount in the enteroendocrine cells of GI mucosa. The amount of MEL in the gut is about 400 times larger than the content of MEL in the pineal gland [39]. MEL production in the GI tract does not follow a circadian rhythm as occurs in the pineal gland, but it responds to the periodicity of food intake rich in tryptophan [40,41]. After a pinealectomy, serum levels of MEL do not maintain a rhythm according to the light–dark cycle, but values are not too low at daytime because of the contribution of MEL by the extra-pineal sources, mainly the GI tract [9].

The main enzymes of MEL synthesis, AANAT and HIOMT, have also been detected in GI mucosa, supporting the hypothesis that in the gut MEL is synthesized from L-tryptophan present in food [42]. The function of gut MEL is not clear yet because the intestine is not only source but also sink of MEL, brought by the circulation [43]. Decreases in motility and increases in mucosal blood flow have been observed [39,44]. It has been also demonstrated that luminal MEL stimulates duodenal HCO_3^- secretion, which suggests that MEL could be a mediator of acid-induced secretion [45]. Some of the MEL effects on the GI tract seem to be mediated by MT2 receptors [45]. However, the presence of MT1 receptor has been also demonstrated in human gallbladder epithelium from patients with cholelithiasis and gallbladder carcinoma [46]. Matheus et al. [47] have shown that MEL inhibits serotonin transporter activity in Caco-2 cells, apparently without mediation of MT1 or MT2 receptors or PKC and cAMP pathways, but the doses used were high. It is not known if this occurs under physiological conditions. MEL has also antioxidant properties in the GI system; its concentration in the human liver is suitable for prevention of oxidative damage. In our laboratory, MEL has been found to reverse the inhibition of the intestinal calcium absorption caused by menadione, apparently by antioxidant mechanisms [48].

MEL receptors have been detected in the pancreas and the stimulation of pancreatic enzyme secretion by the hormone in a dose-dependent manner has been demonstrated. MEL was also implicated in the pancreatic endocrine function. Pinealectomy produces hyperinsulinemia and increases the triglyceride content in the liver of type 2 diabetic rats [49]. Endogenous MEL has been shown to protect against pancreatitis, probably mediated by MT2 receptors [50].

7. MEL and central nervous system

MEL and melatoninergic drugs have hypnotic effects mediated through MT1 and MT2 receptors, especially those in the SCN, which acts on the hypothalamic sleep switch. They favor sleep initiation and reset the circadian clock allowing persistent sleep, a requirement in circadian rhythm sleep alterations. The action of MEL on sleep is mainly of a chronobiological nature. The hormone acts in a dual way, resetting the clock *via* MT2 receptor and suppressing neuronal firing *via* MT1 receptor. Apparently, the soporific action of MEL also involves the thalamus. MEL receptors have been found in this area and the formation of the spindles is stimulated by the hormone. Other areas of the brain seem to be involved in the transmission of MEL-dependent responses. It is uncertain to what extent the thalamus and other brain areas participate in the sleep promotion as compared to the hypothalamic *via* [13]. MEL treatment to patients with severe CNS dysfunction and MEL deficiency is not sufficient to mitigate sleep difficulties. In addition, CNS destruction causing sleep fragmentation and loss of circadian rhythmicity, allows spindle formation. MEL has a different mode of action as compared to other hypnotics such as benzodiazepins and z-drugs, which produce a more generalized CNS depression through GABA receptors. Only very high pharmacological doses of MEL can produce generalized sedative effects or even narcotic effects but mediated by other mechanisms, such as antiexcitatory suppression of calcium signaling and inhibition of neuronal NO synthase. The major obstacle for the use of MEL in primary chronic insomnia is that the half life of circulation is extremely short, 20–30 min and even less (eventually it could last up to a maximum of 45 min). MEL promotes sleep initiation but improves sleep maintenance only marginally. Part of this problem has been solved, at least in part, by drugs that prolong the release of MEL and the melatoninergic agonists with longer half-life [13].

Neurodegenerative diseases such as Alzheimer disease (AD) and Parkinson disease (PD) are age-related disorders that share mitochondrial dysfunction, oxidative/nitrosative stress and apoptosis in different areas of the brain. Patients with AD have a reduction in MEL levels both in blood and cerebrospinal fluid, which is even present in preclinical stages [51]. MEL's cognitive benefits have been demonstrated either in AD patients or in AD mice. Low levels of mRNA of SOD-1, GPx and catalase were found in hippocampus from MEL treated AD mice [7]. In PD model animals,

MEL normalizes complex I activity from electron transport chain and oxidative status in mitochondria from substantia nigra and striatum and reduces mitochondrial inducible nitric oxide synthase decreasing nitric oxide radicals and preventing the inhibition of complex IV by this radical [51]. MEL treatment improved the quality of sleep in patients with Parkinson [52]. The idea that MEL is favorable in PD patients or PD animal models is controversial. Willis et al. [53,54] showed that the light exposure at the bed time reduces the severity of PD symptoms, whereas the MEL intracerebroventricular implants increased its severity. Further studies about the use of MEL in the PD treatment are needed.

8. MEL and cancer

There is abundant evidence indicating that MEL is involved in preventing tumor initiation, promotion, and progression. The increased incidence of breast, endometrial and colorectal cancer seen in nurses and in other night shift workers suggests a possible link between diminished secretion of MEL and increased exposure to light during nightime [55]. This evidence and the antioxidant and anti or proapoptotic action of MEL and its relationship with the endocrine and immune systems led to examine the effect of the hormone on tumor cells. Recent human and animal studies have shown that MEL also has important oncostatic properties by altering the expression of anti-cancer cytokines IL-2 and IL-12 in human neoplasms [2]. Addition of 1 nM MEL to MCF-7 cell culture inhibits proliferation [56], increases the expression of proapoptotic proteins such as p53 and p21 [57], and reduces their metastatic potential due to increased expression of E-cadherin and β_1-integrin proteins [58]. MEL antiproliferative effects on human breast cancer cells seem to be mediated by MT1 receptor, which suggests that MEL or its analogs could be used as potential antitumoral agents [59].

The finding of MEL binding sites in human colon tissue suggested a possible role of MEL in colorectal cancer. Schernhammer et al. [60] have found increased risk of colorectal cancer in nurses subjected to shift work, which was attributed to the suppression of MEL production by nocturnal lighting. Farriol et al. [61] have demonstrated that MEL was able to inhibit cell growth in CT-26 cells, a cell line derived from a murine colon carcinoma. Although MEL had no effect on cell growth at low doses, a significant and progressive suppression of DNA synthesis was found with high doses of the indolamine. The authors concluded that MEL exerted its anti-proliferative action through a non-hormone dependent mechanism, because no receptors were involved in the cell line selected. The oncostatic effect of MEL on colon cancer was demonstrated to be mediated through MT2 receptors and through its binding to nuclear RZR/ROR α receptor [62].

9. Combined effects of MEL with other drugs

The effect of MEL in the presence of other drugs has been studied in different tissues. Several findings support the hypothesis that MEL enhances the effect of chemotherapy on colorectal carcinoma. Cerea et al. [63] evaluated the effect of the simultaneous administration of MEL and the cytotoxic drug CPT-11 in 30 patients with metastatic colorectal carcinoma. It was found that co-administration of MEL (20 mg/day at bedtime) with CPT-11 was more effective in controlling the disease than administration of CPT-11 alone. It has also been shown that 1 mM MEL potentiates flavone-induced apoptosis in HT-29 human colon cancer cells [64], by increasing the level of oxidizable substrates that can be incorporated into mitochondria in the presence of flavones.

Another relevant aspect related to the application of MEL as adjuvant in tumor therapy includes its protective effect on various organs such as heart, kidney and bone marrow in the course of chemotherapy and radiotherapy. This protective effect of MEL administration might allow treating patients more effectively using higher doses of cytostatic drugs and radiation, which by themselves could damage the organs involved [10]. Recently, it has been shown that MEL synergistically with vitamin D down-regulates Akt and MDM2 leading to growth inhibition of breast cancer cells [31].

In studies in which gastric damage was induced by indomethacin [65] or acetylsalicylic acid [66], MEL prevented or reduced the gastric mucosal lesion. In addition, the combination of MEL with anti-ulcer drugs such as ranitidine and omeprazole reduces the doses and minimizes the side effects [67].

The pathophysiology of primary headache has been partially attributed to desynchrony and dysfunction of the whole or part of the retino-hypothalamic-pineal axis [68]. The synchronizing ability of MEL together with its antioxidant and anti-inflammatory properties, make this indolamine effective to decrease the frequency or totally suppress headache of different kinds (migraine, cluster headache, *etc.*) allowing the patient to reduce the consumption of analgesics [69].

The use of MEL alone or in combination with other effective agents has been reported to diminish collateral damage in several psychiatric disorders. Anxiolytic, sedative, anticonvulsant and anti-hypertensive effects of MEL may be helpful in the treatment of depression with inhibitors of monoaminooxidase. A combination of MEL with lithium or valproate was more effective than either lithium or valproate alone in bipolar syndrome patients. MEL has also protective properties against haloperidol adverse effect in schizophrenia patients [70]. It should be noted that many of the beneficial effects of MEL are associated with the improvement of sleep disorders that are very frequent in several neuropsychiatric diseases.

The administration of MEL alone is successful in the treatment of circadian system disorders such as jet lag, shift work, some sleeps disturbances, *etc.* In these cases MEL acts as a synchronizer, entraining the human activity circadian rhythms according to a phase-response curve [71].

As shown in Fig. 2, MEL is a pleiotropic molecule and its administration to humans and animals at both physiological and pharmacological concentrations seems to be non-toxic. Since MEL has a wide therapeutic range, it can be administered in different doses according to the expected effects. The ability of MEL to scavenge ROS is one of the explanations for the reduction of side effects produced by some pharmacological agents such as oncostatic, antinflammatory, antiepilepsic drugs [72]. In these cases, the doses of MEL range from 10 to 50 mg, much higher than physiological level, in order to counteract the high amounts of free radicals generated [73].

Finally, Table 1 shows an illustrative list of some recent *in vivo* and *in vitro* studies exemplifying the effects of the combination of MEL with other drugs. As demonstrated, MEL can be useful in the treatment of a wide variety of diseases improving their efficacy while reducing the side effects in order to enhance life quality. This broad therapeutic spectrum makes MEL a potential therapeutic tool in combination with other traditional therapies and opens a promising field of research in the pharmacological area.

10. Remarks

MEL is a hormone produced in the pineal gland mainly at night time, but is also synthesized by other tissues being the intestine the main source during the day. Its lipophilic nature makes MEL easily cross all membranes. Most of its actions are mediated by membrane and nuclear receptors. It has antioxidant and antiapoptotic properties, resulting in the improvement of mitochondrial metabolic

Table 1
Effect of MEL combined with other drugs in the treatment of different diseases. *In vivo* and *in vitro* studies.

Clinical Entity	MEL combined with other drugs or therapies	Effect	MEL dose used
Epilepsy	Phenobarbital Valproate Vigabatrin Carbamazepine Halloperidol	Suppression of seizures in refractory epilepsy in children[a] [74,75] Improvement of wake-sleep disorders[a] [76]	3 mg/day
Dementia senile	Bright light	Attenuation of aggressive behavior[a] [77]	2.5 mg/day
Depression	Fluoxetine	Improvement of subjective sleep quality[a] [78]	2 mg/day at bed time
Parkinson	Antiparkinsonian drugs: levodopa, amantadine, selegiline, pramipexol	Improvement of subjective sleep quality, sleep quantity and daytime sleepiness[a] [79–81]	5 mg/day
Alzheimer disease	Thioridazine Bright light	Increase of daytime wake time and activity levels[a] Improvement of rest-activity rhythm[a] [82,83].	3–6 mg/day, at bed time
Hepatoma cancer	Doxorubicin	Increase of apoptosis and inhibition of hepatoma cell growth[c] [84].	10^{-5} to 10^{-8} mol/L
Gastric ulcer	Omeprazol Ranitidine	Protection of the gastric mucosa decreasing the doses of these drugs[a] [67].	20 mg/kg
Chagas disease	Meloxicam Dehydroepiandrosterone Zinc	Increase of immune response altered by *Trypanosoma cruzi*[c] Decrease in parasitaemia[c] [85–87]	5 to 10 mg/kg b.w.
Tuberculosis	Isoniazid	Enhancement of mycobacterial growth inhibition[b] [88]	1.6–10 mM
Gram (−) bacterial infections	Gentamicine Tobramycin	Attenuation of nephro and ototoxicity produced by aminoglycosides[c] [89,90]	250 μg to 10 mg/kg

[a] Clinical trials or case reports.
[b] *In vitro* studies.
[c] Animal studies.

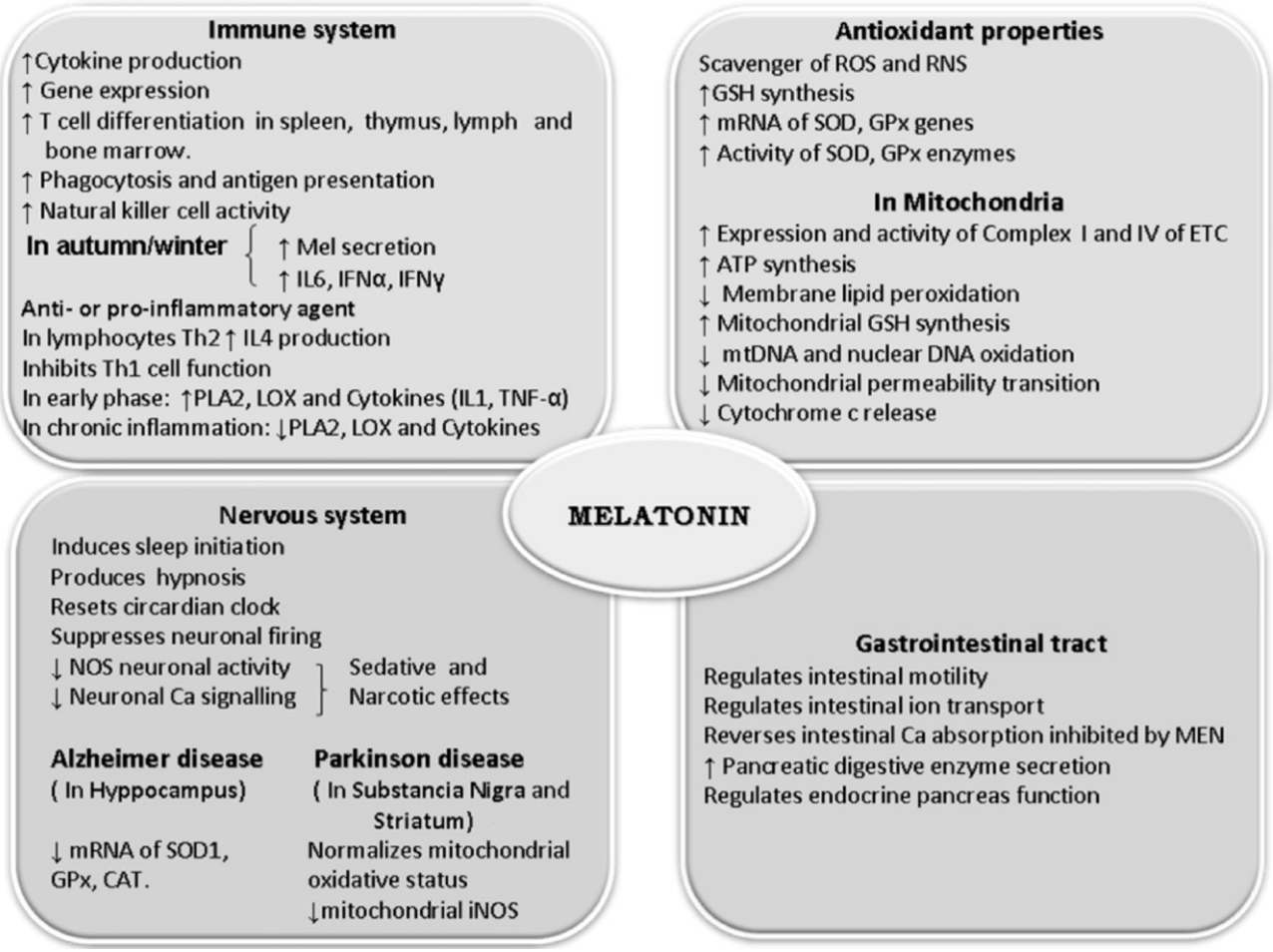

Fig. 2. Effects of melatonin on different tissues and systems. CAT: catalase, ETC: electron transport chain, GPx: glutathione peroxidase, GSH: glutathione, IFNα: interferon alpha, IFNγ: interferon gamma, IL: interleukin, iNOS: inducible nitric oxide synthase, LOX: lipoxygenase, MEN: menadione, mtDNA: mitochondrial DNA, NOS: nitric oxide synthase, PLA2: phosholipase A2, RNS: reactive nitrogen species, ROS: reactive oxygen species, SOD1: superoxide dismutase one, Th1: type 1 helper T lymphocytes, Th2: type 2 helper T lymphocytes, TNF-α: tumor necrosis factor alpha.

pathways and ATP production. A potential intracrine and paracrine role of MEL in immune regulation has been suggested although the molecular mechanisms involved in the immunoregulatory function remain to be elucidated. The action of MEL on sleep is mainly of a chronobiological nature. One of the major obstacles for the use of MEL in primary chronic insomnia is that the half life of circulation is extremely short, which has been partially solved by synthesis of drugs that prolong the release of MEL and the melatoninergic agonists with longer half-life. MEL's cognitive benefits have been demonstrated either in AD patients or in AD mice. Some approaches indicate that MEL alone or in combination with other drugs could be used to prevent tumor initiation, promotion, and progression. Anti-proliferative effects of MEL on breast and colon cancer cells have been demonstrated, but the underlying mechanisms are not clear yet. Another possible use of MEL alone or combined with other drugs would be for treatment of several psychiatric disorders due to its anxiolytic, sedative, anticonvulsant and anti-hypertensive properties. In conclusion, the spectrum of uses of MEL seems to be wide, although more investigation is needed in order to know better the molecular mechanisms and the possible side effects.

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