Therapeutic uses of melatonin and melatonin derivatives: a patent review (2012-2014)

Abstract

Introduction

Melatonin is a neurohormone involved in the regulation of circadian rhythms, with potent antioxidant activity. Melatonin has a wide functional repertoire, with effects almost on all tissues and organs and it is mainly used as a dietary supplement for sleep regulation and re-synchronization of disrupted circadian rhythms. Melatonin has very low toxicity, but some pharmacokinetic issues, such as limited oral bioavailability and short half-life, limit its tissue availability.

Areas covered

Patents and patent applications from 2012 to 2014 (September) in which melatonin or its synthetic analogues are claimed for the prevention or treatment of pathological conditions.

Expert opinion

Melatonin is considered a valuable substance that can be safely administered for the prevention and treatment of many diverse diseases. A major trend in 2012-2014 patents is the application of melatonin in combination with other drugs. In this context, melatonin usually acts as an adjuvant to strengthen the pharmacological effects and increase the efficacy of the treatment. Combination of melatonergic activity with that of other drugs can be also seen in bitopic ligands.

On the other hand, the number of new melatonin analogs has shown a marked decrease, with claimed applications mainly as hypnotic or antioxidant agents.

Keywords: melatonin, MT_1, MT_2, antioxidant activity, radical scavenger, agomelatine, ramelteon, tasimelteon, prolonged-release formulation

Article highlights

- Melatonin is a safe molecule with different mechanisms of action, involving membrane G protein-coupled receptor activation and interaction with other cellular mediators.

- The antioxidant effect of melatonin and the related improvement of mitochondrial functionality, redox state and cellular homeostasis are at the basis of many therapeutic applications proposed for melatonin.
- Melatonin is claimed for numerous pathological conditions, spanning from CNS pathologies as diverse as sleep disturbances and stroke, to diseases affecting peripheral organs, such as diabetes, bone injury, gastric ulcer and cancer.
- Melatonin is often proposed in combination with approved drugs, acting as an adjuvant to improve the efficacy of the treatment for those cases in which the traditional treatment is poorly effective or to reduce the dose required for pharmacological treatment.
- Novel classes of melatonin-based bitopic ligands have been disclosed, which are able to interact with two different targets relevant for the pathology.
- The number of new melatonin analogs reported and patented is significantly decreased in the last three years.

1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine, Figure 1) was discovered and initially characterized in extracts of bovine pineal gland in 1958 [1]. Since then a number of studies have shed light on its distribution, effects and mechanisms of action, even if we are still far from a complete characterization of its behavior. Melatonin is widely distributed in living organisms and it has been found in vertebrates, invertebrates and also in plants.

In mammals, melatonin is mainly produced by the pineal gland following a circadian rhythm, with peak concentrations at night, being its synthesis and secretion inhibited by daylight. Numerous peripheral sites produce melatonin as well, contributing to the high local concentrations found in some tissues [2]. Melatonin is synthesized starting from the amino acid tryptophan, which is first hydroxylated in position 5 of the indole ring and then decarboxylated to serotonin. Transformation of serotonin to melatonin is achieved by acetylation of the amine (performed by the enzyme AANAT: arylalkylamine *N*-acetyltransferase), which corresponds to the rate limiting step of the biosynthetic pathway, and eventually by methylation of the 5-hydroxyl group.

The best characterized effects of melatonin are related to the entrainment of circadian rhythms and to the promotion of sleep. However, melatonin has been found to be involved in the modulation of a number of physiological and pathophysiological conditions, with effects produced in a large number of tissues [3]. On the basis of the beneficial effects demonstrated in experimental models, melatonin has been proposed for the treatment of a variety of pathological conditions, spanning from disrupted circadian rhythms and insomnia, to neurodegenerative diseases, stroke, diabetes and malignancies [4]. A number of clinical trials have been set up to evaluate its efficacy, alone or in combination with other drugs, in different clinical settings [5].

Numerous physiological effects of melatonin are mediated via interaction with the MT_1 and MT_2 G protein-coupled receptors which are widely expressed in the central nervous system (CNS), with highest density observed in the hypothalamic suprachiasmatic nucleus, the body master clock [6]. The distribution of MT_1 and MT_2 receptors has been also studied in peripheral sites, where they are

often co-expressed, sometimes exerting opposing effects, as observed at vascular level [7]. Moreover a number of cytosolic binding sites have been described for melatonin, such as the calcium-calmodulin complex and the MT_3 binding site, characterized as a quinone reductase 2 enzyme [8]. Melatonin has also shown receptor-independent actions, mainly related to its radical scavenging and antioxidant activities. Indeed, melatonin is able to promote the synthesis of a number of antioxidant enzymes, with mechanisms that still need to be characterized, and to regulate the redox state of the cell. At high (pharmacological) concentrations melatonin also exerts a direct radical scavenging action on oxygen- and nitrogen-based radicals [9].

Melatonin is available as a dietary supplement in many countries and it is mainly used to alleviate the symptoms of disrupted circadian rhythms, such as in case of jet lag, or to promote sleep induction. It is also available in a prolonged-release formulation for the treatment of primary insomnia [10]. Melatonin has the remarkable advantage of an extremely safe toxicological profile, but it is also characterized by poor pharmacokinetic properties, mainly a low oral bioavailability, due to high first pass metabolism, and a short plasma half-life. For this reason, a number of melatonin analogs have been synthesized, allowing at the same time to acquire information on structure-activity relationships and to obtain compounds selective for its binding sites [11, 12]. Some of these compounds became important pharmacological tools to investigate the role of receptor subtypes. Currently three MT_1/MT_2 nonselective melatonin receptor agonists have gained marketing authorization [13]. Ramelteon is approved for insomnia therapy, tasimelteon for the treatment of non-24-hour sleep-wake disorders in blind people and agomelatine, having also $5HT_{2c}$ antagonist activity, for the treatment of major depression. Another compound, β-methyl-6-chloro-melatonin (TIK-301) received an orphan drug designation status for circadian rhythm sleep disorders in blind people in 2001. In the last decades, parallel to the investigation on the pharmacological effects of melatonin and its derivatives, a number of patents have been deposited describing the application of these compounds in different pharmaceutical, cosmetic and dietary fields. This review reports the patents and patent applications related to the therapeutic applications

of melatonin and its analogs claimed from 2012 to September 2014. They have been grouped in different paragraphs, according to the pathology or the body district involved in the therapeutic application. In each paragraph the description of the patents is preceded by a brief introduction in which the relevant scientific information about the role of melatonin in the field is summarized.

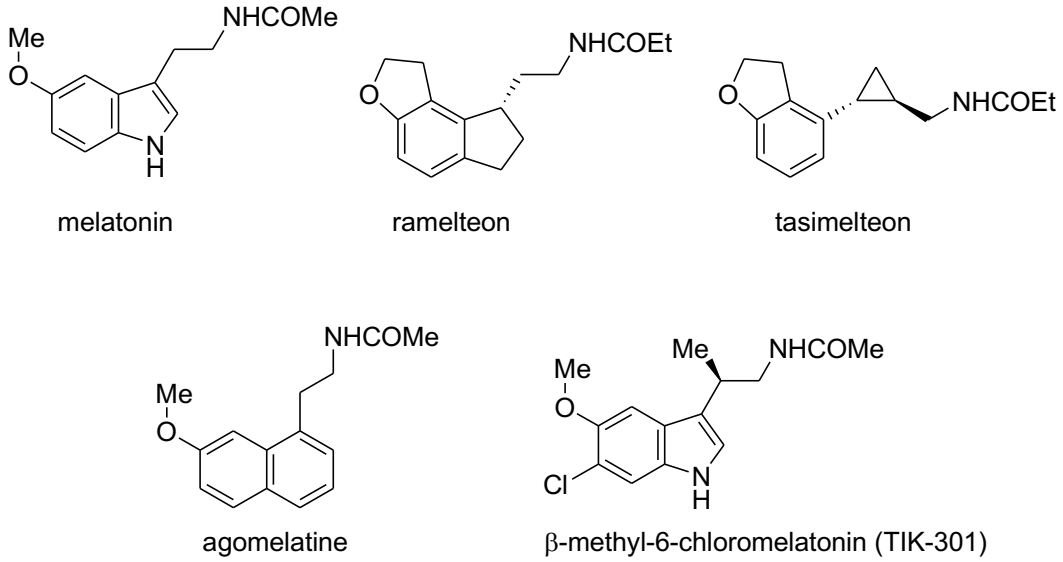


Figure 1. Melatonin and approved melatonergic derivatives.

2. Central nervous system disorders

There is a long-standing interest in the potentially beneficial use of melatonin and its derivatives for the treatment of several CNS diseases, mainly sleep disturbances and depression. Sleep disorders are common (approximately one third or one fourth of the western population suffers from primary sleep disorders) and can lead to significant disability with social and economic implications. These disturbances have a myriad of causes, one of which is disruption of the normal circadian sleep-wake cycle. In humans, as in other animals, circulating levels of melatonin are highest during the night and correlate with the habitual hours of sleep. Accumulating evidence suggests that melatonin may regulate the circadian clock located in the suprachiasmatic nucleus of the hypothalamus. Such studies have engendered great interest in the potential use of exogenous melatonin as a sleep-

promoting agent and to treat the disoriented circadian clock in cases such as jet-lag, shift-work, in profoundly blind subjects, and in individuals with delayed or advanced sleep phase syndromes.

2.1 Insomnia

The limited efficacy of melatonin for insomnia may partly be attributed to its short half-life, and solutions to this problem can be sought by developing prolonged-release formulations of the natural hormone, by using melatonergic drugs with longer half-life, or providing combinations with clinically well-proven sedating agents.

A number of patents are directed to novel combinations of melatonin with sedating compounds and to their use for improving the health of human subjects. For example a recent patent application provides combinations of sedating antihistamines (i.e. doxylamine) with certain indole-based dietary supplements such as L-tryptophan, 5-hydroxytryptophan and melatonin, optionally combined with one or more vitamins such as B3 and B6, and one or more minerals such as calcium and magnesium. These compositions are claimed as effective formulation for the treatment of insomnia and other sleep-related problems including shift-work disorders and jet lag [17].

Another patent application reports a pharmaceutical formulation to treat insomnia consisting of melatonin and a well-known hypnotic sedative, such as zopiclone, zolpidem, zaleplon or older benzodiazepine drugs. Melatonin reduces the time of sleep onset caused by the second compound, potentiates its hypnotic effect and allows to decrease the dosage of the hypnotic to initiate and maintain sleep. These novel formulations could present favorable safety profiles in comparison with common hypnotics, whose typical side effects, including cognitive impairment, psychomotor impairment, dependence, tolerance, hangover, rebound insomnia, etc., are both dose- and time-dependent [18].

In another patent application, sleep promoting amounts of astaxanthin (ranging from about 0.1 to 60 mg/kg body weight) and melatonin (0.1-40 mg/kg/bw) are administered in conjunction to promote sleep in animals, like cats and dogs, and to improve their quality of life. The pharmaceutical

composition can further comprise an amount of zinc ranging from about 10 to 100 mg/kg/bw. [19] Studies on forty eight dogs showed that the combined administration of melatonin, astaxanthin and zinc provided beneficial effects compared to the use of melatonin alone.

Related to this one, is another recent patent application claiming compositions and methods useful for enhancing cognitive functions and preventing or treating cognitive decline in humans and animals through the administration of a combination of melatonin, one or more carotenoids and optionally zinc [20]. Experimental trials (attention task tests) evidenced that the administration of melatonin, astaxanthin and zinc improves the cognitive functions of dogs (i.e. less errors in learning some tasks).

Some patents claim the usefulness of melatonin or melatonin analogues in combination with herbal extracts to treat sleep disorders. For instance, a composition of melatonin mixed with medlar, tuckahoe and hawthorn extract powders is proposed to relieve sleep disorders [21]. In another patent application the composition obtained by adding melatonin or a melatonin analogue to one or more herbal extracts (such as *Petroselinum neapolitanum*, melissa, tuber fleeceflower, chamomile, passion flower) seems to have better anti-insomnia effects than a common melatonin preparation [22]. A nutritional health-care product containing melatonin, barley grass, medlar, calcium hydrophosphate, chrysanthemum powder, and vitamins B1, B6, and B12, has been claimed to improve sleeping quality and suitable to treat intractable insomnia [23].

Another patented formulation containing tryptophan, melatonin, vitamin B3 and B6 and optionally tyrosine, vitamin B12 and γ-aminobutyric acid is designed to induce a deep state of relaxation [24] and a number of different delivery systems are proposed. The rationale for this composition is related to the beneficial pharmacological effects produced by all these substances, either directly (such as the sleep promoting hormone melatonin and the inhibitory neurotransmitter GABA), or indirectly by increasing brain levels of serotonin, a neurotransmitter which promotes calm and a sense of well-being in humans. Indeed, tryptophan in the brain is converted into serotonin, vitamin B3 is able to inhibit the production of the liver tryptophan pyrrolase and to promote the production

of 5-hydroxytryptophan. The formulation can also include vitamin B6 involved in the activation of the decarboxylating enzyme converting 5-hydroxytryptophan into serotonin. However, experimental data on the efficacy of this combination of substances are not reported in the patent.

Takeda Pharmaceutical describes a series of tricyclic melatonin receptor agonists structurally related to ramelteon (Figure 1), in particular indeno[5,4-*d*][1,3]oxazole derivatives (Figure 2), with improved agonist activity or pharmacokinetic properties in comparison with melatonin [25]. Compound **1** exhibited IC_{50} values of 0.030 nM and 0.049 nM on human MT_1 and MT_2 melatonin receptors respectively, and its in vitro metabolic clearance is much lower than that of melatonin against oxidative metabolism in the presence of rat and human hepatic microsomes. These compounds are claimed to be useful for the prophylaxis or treatment of diseases related to the action of melatonin such as sleep disorders, depression, anxiety or bipolar disorder, but no clinical data are reported.

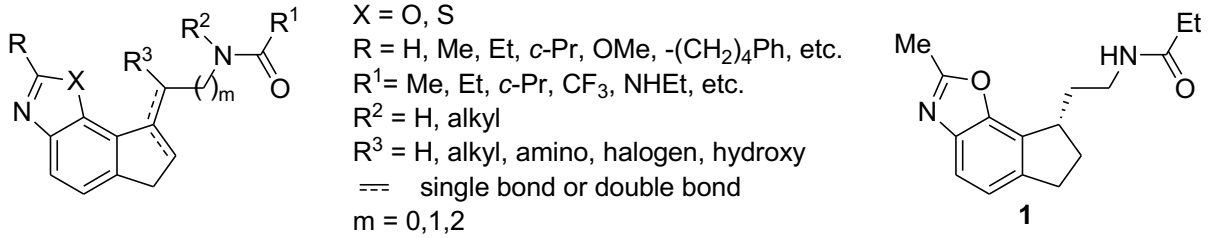


Figure 2. Indeno[5,4-*d*][1,3]oxazole derivatives.

Tasimelteon (Figure 1) is another melatonin receptor agonist with longer half-life than melatonin, patented by Vanda Pharmaceuticals. In a Phase III, multi-center, placebo-controlled, 4-week trial evaluating 322 patients with chronic insomnia, tasimelteon improved sleep latency, sleep efficiency and wake after sleep onset [27]. Phase II and III studies have also been conducted in a model of transient insomnia associated with shifted sleep and wake time, showing the ability of tasimelteon to dose-dependently advance plasmatic melatonin rhythm. Taken together, the results of these studies demonstrate the versatility of tasimelteon to treat symptoms of both transient insomnia and chronic primary insomnia.

2.2 Disrupted circadian rhythms

A new method-of-use patent for tasimelteon and its metabolites was issued by Vanda Pharmaceuticals in the treatment of Non-24-Hour Sleep-Wake Disorder (N24HSWD) a chronic, circadian rhythm disorder resulting from the misalignment of the endogenous body master clock to the 24-hour day, disrupting the sleep-wake cycle [28]. N24HSWD is an orphan indication affecting the majority of totally blind individuals and it is estimated that approximately 80,000 Americans and 140,000 people in Europe suffer from this disorder. The efficacy of tasimelteon was determined in two clinical studies involving totally blind patients with N24HSWD. The SET (Safety and Efficacy of Tasimelteon) Phase III placebo controlled trial (84 patients) evaluated the use of 20 mg of tasimelteon for at least 12 weeks. The primary endpoints for this study were entrainment of the melatonin rhythm to the 24 hour clock, calculated by various measures, the most common of which includes assessing a melatonin metabolite in the urine, and clinical responses. After 4 weeks, more patients receiving tasimelteon were entrained (20%) compared to those receiving placebo (2.6%). The RESET (Randomized withdrawal study of the Efficacy and SafEty of Tasimelteon) trial (n = 20), designed to demonstrate the maintenance effect of 20 mg/day of tasimelteon in the treatment of blind individuals in N24HSWD, involved patients who received tasimelteon for 12 weeks and became entrained. During the withdrawal period of the trial, which lasted 8 weeks, 90% of patients who used tasimelteon (n = 9/10) remained entrained compared with 20% of patients randomized to receive placebo (n = 2/10).

Tasimelteon is the first treatment approved by the U.S. Food and Drug Administration (FDA) for N24HSWD, and in April 2014 Vanda Pharmaceuticals launched the drug on the U.S. market under the brand name Hetlioz®.

2.3 Depression

Different studies in humans have shown that melatonin treatment not only improves the total sleep time but also can decrease depressive symptoms, documenting the close association between sleep and mood disorders. Therefore, some patent applications report the combined use of melatonin and antidepressants and/or antipsychotics to treat depressive disorders. An invention describes a pharmaceutical composition for treating and/or preventing depressive disturbances containing a selective serotonin reuptake inhibitor (10-30 mg) and melatonin (3-8 mg). It is also said that this composition has a markedly positive impact on the often disrupted sleep–wake rhythm of depressed patients, without affecting daytime vigilance [29]. In another invention an integrated therapy is proposed involving haloperidol (0.375-0.5 mg, 3 times a day for two weeks), paroxetine (20-30 mg in the morning for 3 months) and melatonin (1.5-3 mg, administered 20 minutes before sleeping for 7-10 days) after determining the blood serotonin/melatonin ratio. The proposed combination provides effective reduction of depressive symptoms and suicidal behavior [30].

2.4 Neuroprotection

In addition to regulating circadian rhythms, melatonin has recently attracted interest for its neuroprotective potential. Melatonin treatments have been shown to ameliorate different CNS diseases in experimental models, such as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, ischemic injury, neuropsychiatric disorders and head injury [31]. Although multifactorial processes are involved, free radical generation and oxidative damage have been extensively investigated and validated as important contributors to the pathophysiology of acute CNS injury. Melatonin is a highly lipophilic molecule able to reduce oxidative stress through its free radical scavenging effect and by indirect enhancement of antioxidative defense systems [32]. Some patent applications claim the use of melatonin, alone or in combination, in the prevention and therapy of neurodegenerative pathologies. A Canadian patent application proposes the combined use of melatonin and at least seven other natural products (including phosphatidylserine, phosphatidylcoline, quercetin, astaxanthin, *R*-alpha lipoic acid, N-acetylcysteine, taurine, L-

glutamine, carnitine, D-ribose, creatine, epigallocatechin gallate, ginkgo leaf extract, curcumin or L-glycine) for the treatment of traumatic brain injuries such as concussions [33]. The administration of these combinations to subjects with concussive brain trauma reduces the duration and severity of symptoms. It is reported that these compounds can act synergistically to reduce inflammation, improve cellular energy production, promote circulation and capillary integrity, limit oxidative stress and augment healing of damaged cervical musculature and connective tissue.

Another patent application reports a series of novel hybrid molecules potentially useful for the relief and/or treatment of neurodegenerative diseases, obtained by linking melatonin, or its oxidation products, to a tetrahydroacridine unit via a carbamate bond [34]. Some of these compounds (Figure 3) are potent cholinesterase enzyme inhibitors (IC_{50} < 10 nM) and display high selectivity for butyrylcholinesterase, an enzyme largely produced in the brain of patients suffering from Alzheimer's disease. These derivatives are also described as antioxidants and able to prevent the aggregation of beta-amyloid, but data in this regard are not reported in the patent.

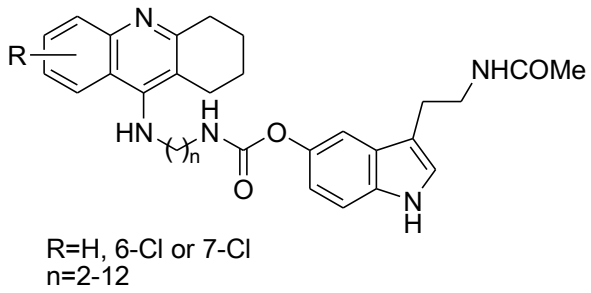


Figure 3. Hybrid melatonin-tacrine derivatives.

University of Minnesota provides compositions consisting of one or more ketone bodies (e.g., D-β-hydroxybutyrate, acetoacetate) and melatonin for treating or preventing injury and damage due to ischemia and/or reperfusion [35]. The rationale for this treatment relies on the ability of the tissues to use ketone bodies as an energy source and on melatonin antioxidant properties. The inventors report an evaluation of the metabolism and transport of [2,4-^{13}C]-D-β-hydroxyburyrate in brains

and hearts of squirrels and in vivo experiments that support the efficacy of this composition in protecting both small (rats) and large (pigs) animals subjected to a significant loss of blood.

A recent invention describes a pharmaceutical composition for preventing and treating neurodegenerative diseases containing a combination of adenosine derivatives and melatonin. 2-Chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyluronamide (Figure 4) and melatonin (weight ratio 1:1) act synergistically to suppress ischemic brain injury in rats making this composition a promising approach to treat stroke, dementia, Alzheimer's disease, Parkinson's disease, or Huntington's disease.

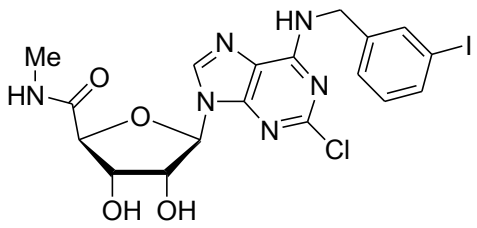


Figure 4. Adenosine derivative used in combination with melatonin as neuroprotectants.

The use of melatonin in combination with the anti-Alzheimer's agent memantine was claimed to significantly increase the effectiveness of treatment through a neurotrophic action [38].

Melatonin has been recently described as an efficient agent able to protect neuronal cells from prion-protein mediated neurotoxicity and cell death. In neuronal cells, melatonin inhibited neurotoxicity induced by the synthetic prion protein fragment PrP(106-146) via activation of autophagy signaling and regulation of mitochondrial homeostasis [39]. Moreover, melatonin induced activation of β-catenin, a protective factor against mitochondrial dysfunctions, and expression of anti-apoptotic proteins (e.g., Bcl-2), while it decreased expression of pro-apoptotic proteins. A patent application claims melatonin as part of a pharmaceutical or a food composition for preventing or treating prion diseases, given its ability to suppress prion-induced cytotoxicity without negative effects on cell survival rate [40].

Autophagy is considered of particular relevance for neurodegenerative proteinopathies, such as Alzheimer's, Parkinson's and Huntington's disease, which are characterized by the accumulation of misfolded proteins. Indeed, autophagy enhances clearance of misfolded proteins and has therefore been suggested as a therapy for proteinopathies. A patent application claims a pharmaceutical composition for the prevention or treatment of protein-aggregate inducing diseases in which the main component is melatonin, which can be combined with autophagy inducing agents [41]. According to what has been reported in this patent application, melatonin induces autophagy by activation of the mTOR (mammalian Target of Rapamycin) pathway.[42]

Another recent patent application relates to methods that make use of melatonin to reduce neurodegeneration by activating the autophagy pathway [43] through the inhibition of the expression of S6 kinase and/or mTOR, targets involved in the regulation of growth, proliferation, motility, cell survival, protein synthesis and transcription. Two trials on MDCK cells are reported in the patent, in which 1 nM melatonin induces a decrease in mRNA expression of mTOR (7.4 to 59.5%) and of S6 kinase (24.2 to 55%).

2.5 Pain

The sleep disorder can be considered as "primary insomnia" if it occurs as an independent disorder and "secondary insomnia" when it relates to another mental disorder (e.g., depression) or medical condition (e.g., pain). Several early studies in animals and humans showed that there is a circadian rhythm in pain perception. Interestingly, recent experimental and clinical trials in humans have demonstrated that melatonin has analgesic properties in chronic pain conditions (such as fibromyalgia, irritable bowel syndrome, migraine) and antinociceptive effects in animal models of acute and chronic neuropathic pain [44].

A pharmaceutical composition comprising a combination of a nonsteroidal anti-inflammatory drug (meloxicam), an analgesic adjuvant (melatonin) and an antineuritic-analgesic agent (B-complex

vitamins) has been recently claimed to show a synergistic effect for the treatment of pain of different etiologies [45]. This is supported by experiments in animal models (formalin test in rats) showing a more pronounced reduction of painful symptoms by using the above cited combination in a single dosage unit, rather than independent administrations. Among the benefits arising from this combination it should be considered a reduction in the administered doses, better compliance, faster therapeutic effect and fewer side effects.

3. Cancer and cancer treatment side effects

A number of experimental evidences support an oncostatic effect of melatonin on a variety of cancer cells and tissues, with promising results also from in vivo experiments. In some experimental conditions a pro-apoptotic activity of melatonin has also been characterized [46]. Currently, two clinical trials are ongoing to evaluate the role of melatonin in both chemoprevention and chemotherapy. The first one is testing the ability of melatonin to decrease the spread of breast cancer cells, hopefully to create an efficient cancer prevention strategy [47]. In the other clinical trial the efficacy of melatonin in lowering the incidence of developing non-small cell lung cancer recurrences or death is being evaluated, monitoring at the same time the improvements in quality of life [48]. The anticancer effects of melatonin have been related to both its antioxidant and radical-scavenging properties and to its receptor-mediated activities, mainly involving the MT_1 receptor [49]. Melatonin has also been added as an adjuvant to amplify the cytotoxic effects triggered by conventional chemotherapy. The efficacy of melatonin has been particularly evaluated in breast cancer, in which disruption of circadian rhythms and a reduced exposure to melatonin is correlated to tumor growth and progression [50]. In the case of estrogen-dependent breast cancer, experimental evidences also support an antiestrogenic effect of melatonin, which acts as a SERM (selective estrogen receptor modulator) and a SEEM (selective estrogen enzyme modulator) [51]. A

couple of patents claim the use of melatonin in the prevention and treatment of breast cancer, both in combination with other agents or in the context of bitopic ligands.

The first patent reports the combination of a hormone replacement therapy (HRT, i.e. an estrogen and a progesterone receptor-binding compound) and of melatonin to be administered at bedtime to reduce mammary cancer incidence and to treat existing mammary cancer. This combination would also alleviate the symptoms of menopause, improve sleep and well-being and reduce osteoporosis [52]. Studies on mice, reported in the patent, showed that the combined administration of HRT and melatonin produced a significant increase in latency and decrease in incidence of mammary cancer. With respect to tumor growth, the combination afforded a significant decrease in tumor weight and volume compared to control animals.

Another patent by the same research group reports hybrid compounds composed by *N*-desmethyltamoxifen or *N*-desmethyl-4-hydroxytamoxifen connected by an alkyl linker to the amide side chain of melatonin (**2**, Figure 5) [53]. These compounds bind with equal affinity to estrogen receptors and melatonin MT_1 receptors. In studies performed on mice these hybrid compounds showed anti-cancer effects on breast cancer and they did not sustain hyperproliferation of uterine tissue, as observed when tamoxifen is administered alone.

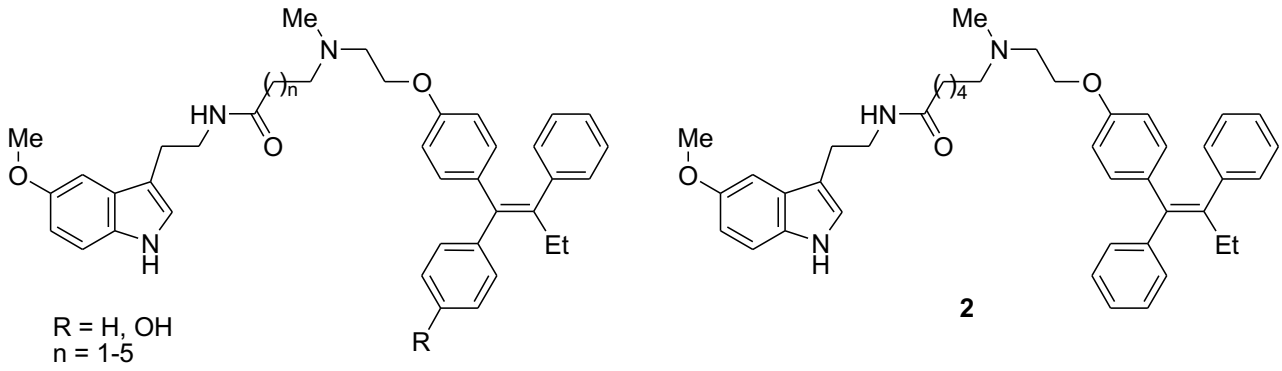


Figure 5. Melatonin derivatives reported as anti-cancer agents (**2**) or to reduce cancer treatment side effects (**3** and **4**).

Another patent stresses the importance of regulating melatonin levels for the treatment of a variety of cholangiocyte pathological conditions, in particular cholangiocarcinoma [54]. Indeed, melatonin can act as an autocrine signal regulating cell proliferation and in cholangiocarcinoma cells low levels of melatonin and of AANAT were found [55]. The patent states that diseases of the biliary tract can be treated with melatonin, a melatonin analog binding MT_1 and/or MT_2 receptors, or a melatonin signaling modulator, such as a compound modulating the expression of AANAT. This treatment can be combined with an additional therapy, such as radio-, chemo-, immuno-, gene-, or cell-based therapy. Studies performed both in vitro and in vivo on rats showed that melatonin effectively inhibits cholangiocarcinoma cell growth by modulation of the expression of CLOCK genes.

Melatonin is also described as an important option to reduce the side effects of conventional cancer treatments, which can have dose-limiting side effects and can significantly compromise the quality of life of patients. Indeed, melatonin has already shown significant protective effects over toxic manifestations induced by cytotoxic agents such as cyclophosphamide, oxaliplatin, doxorubicin and a clinical trial is actually ongoing to evaluate the effect of melatonin in improving quality of life, reducing post-operative pain and chemotherapy-induced toxicity in breast cancer patients [56]. A patent application claims the administration of melatonin or of the melatonin indole derivatives **3** (Figure 5) for the prevention or treatment of oral mucositis, in particular when caused by radio or chemotherapy [57]. Experiments on rats exposed to ionizing radiations evidenced that administration of a hydrogel formulation of melatonin in the oral cavity was able to neutralize oxidative stress, restoring proper mitochondrial function, decreasing apoptosis and increasing cell survival. Histological analysis of rat tongue showed decreased fibrosis and angiogenesis compared to untreated mice. The protective effect of a melatonin formulation was also evident on patients with head and neck cancer undergoing radiotherapy who did not require additional treatment with opioids nor had to interrupt radiotherapy.

Another patent application claims a series of vulcanized aspartic acid modified melatonin derivatives (**4**, Figure 5) to reduce toxic side effects of radio- and chemotherapy treatments [58].

4 . Antioxidant action and immune system enhancement

Melatonin antioxidant activity has been widely investigated and different mechanisms seem to be involved and interrelated. Melatonin is reported to have a direct antioxidant and radical scavenging action on oxygen- and nitrogen-based radicals, usually appreciable at pharmacological doses of substance [59]. On the other hand, melatonin is also involved in the control of a number of transcription factors, such as NF-κB, AP-1 and Nrf2, leading to up-regulation of antioxidant enzymes and decreased sensitivity to oxidative stress damage. Melatonin increases the expression of superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, modulating the glutathione/glutathione disulfide cellular ratio and thus the oxidative state of the cell [60]. This antioxidant activity is at the basis of the proposed application of melatonin, alone or as an adjuvant therapy, in a number of different pathologies, spanning from neurodegenerative diseases to conditions in which ischemia-reperfusion phenomena occur, to the control of bacterial and viral infections. Additionally, melatonin exerts a stimulating effect on immune system activity, sustaining both the innate and specific immune responses [61]. The regulatory effect on the immune system is exerted at different levels, comprising receptor-mediated stimulation of immunocompetent cells, sustaining cell proliferation and modulating cytokine production.

A recent patent reports a series of indole melatonin derivatives with general formula **5** (Figure 6) having antioxidant activity and proposed for the treatment of a variety of diseases, such as emphisema, viral hepatitis, tuberculosis, psoriasis, atherosclerosis, as well as chronic inflammatory diseases [62]. Interestingly, some of these indole derivatives lack the classical substituents usually present in melatonin-receptor ligands, i.e. the acetylaminoethyl side chain and the methoxy group. The compounds were tested for their antioxidant activity in the thiobarbituric acid assay and for

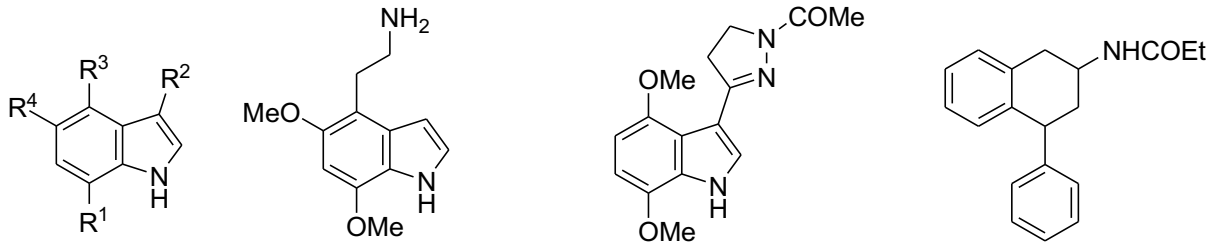
their antiproliferative activity on human keratinocytes in the MTT assay. Some derivatives (e.g., compounds **6** and **7**) showed the same antioxidant activity as melatonin and significantly higher antiproliferative activity, making them worth of particular consideration as potential anti-psoriatic agents.

In a patent application, combination of *N*-acetyl-L-cysteine (NAC), selenomethionine and melatonin is proposed for the treatment of a variety of diseases comprising cancer, autoimmune, neurodegenerative and endocrinological diseases, type 2 diabetes, fibrosis, amyloidosis, endometriosis, etc. [63]. NAC is a precursor of glutathione and is known for its antioxidant, anti-inflammatory and antiproliferative activities. The reason for the combination of NAC with selenium and melatonin relies in the purpose of increasing the efficacy of NAC through enhancement of the thiol redox system. Selenium, in its bioavailable form of selenomethionine, stimulates the activity of glutathione peroxidase and melatonin increases the expression of glutathione peroxidase and reductase. In vitro experiments supported the improved efficacy of the combination over that of NAC alone in promoting antioxidant and differentiating effects on cell lines. Additionally, the combination of the three ingredients was effective in reducing the depigmentated area in a woman affected by vitiligo, with no adverse side effects reported.

Another patent application claims melatonin as an useful treatment to counteract surgery-induced alterations of the immune and inflammatory systems. Melatonin is proposed, either alone or in combination with L-arginine and/or another compound associated with the synthesis of nitric oxide, for the prophylactic or therapeutic treatment of postoperative infectious and non-infectious complications induced by major surgical interventions, in particular for those operations including ischemia/reperfusion (I/R) of an organ [64]. Indeed, melatonin has been shown to effectively reduce tissue damage in a variety of I/R related experimental settings, comprising myocardial infarction [65], liver and renal injury and cerebral ischemia. As the treatment proposed in the patent can be applied short before the surgical intervention, it is also suitable in the case of emergency procedures. As described in the patent, in a partial hepatectomy rat surgical model, which simulates

a standard I/R situation, melatonin dose-dependently reduced damage to the liver tissue, promoted regeneration of the liver cells and increased animal survival.

Another patent application reports a traditional Chinese medicine composition, based on a number of medicinal herbs, which is added of melatonin and vitamins. This preparation is used for treating low immunity in middle-aged and elderly people and displays both short and long term effects [66].



5 **6** **7** 4-P-PDOT

Figure 6. General formula and chemical structures of melatonin indole derivatives having antioxidant activity (**5**-**7**) and of 4-P-PDOT.

5. Skin and skin annexes

The skin is one of the peripheral sites in which melatonin is produced and where its involvement in the control of the redox state of the cellular environment has been demonstrated. Melatonin has been reported to protect against oxidative stress and ultraviolet radiation-induced cellular damages of keratinocytes [67]. A number of patent applications claim the use of melatonin, alone or in combination, as an anti-radical treatment for age-related skin manifestations. A first patent application reports a pharmaceutical or cosmetic composition comprising coenzyme Q10 (CoQ10) and melatonin, or one of its derivatives indicated by general formula **3** in Figure 5, for the topical administration to the skin as an antioxidant treatment to prevent or reduce the effects of skin aging [68]. Interestingly, in vivo experiments on rats showed that the combination of melatonin and CoQ10 significantly enhanced transdermal absorption of both substances, reaching all the structures present in the skin (epidermis and derma). At the cellular level, this combination allowed to obtain

higher concentrations of CoQ10 and of melatonin in cytosol and mitochondria, compared to the administration of each substance as a single agent.

A different multi-component preparation is reported in a patent claiming the association of melatonin with another antioxidant (e.g., beta-carotene, lutein, lipoic acid) or an immunostimulant substance (e.g., ectoine, beta-glucan), or both, for the treatment of natural aging and photoaging of tissues and cutaneous annexes [69]. This preparation aims at preventing cellular damage deriving from oxidative processes in which the anti-radical activity of melatonin is sustained and integrated by the combination with the other substances, leading to an effective synergistic action. In the patent, different in vitro and in vivo tests, both on animals and on human volunteers, sustain the strong antioxidant effect of these mixtures, with the best results obtained for the triple combination of melatonin, antioxidant and immunostimulant substances. Application of this combination was able to reduce ROS presence, increase cutaneous hydration and elasticity, modulate the surface lipid film, improving the aspect of skin. Positive effects were obtained also for hair, where it produced a marked hair cycle boosting effect.

Melatonin has also been proposed as an ingredient of a pharmaceutical or cosmetic formulation in which it is associated to an antimicrobial agent (e.g., chlorhexidine) and/or a keratolytic agent for the prevention or the topical treatment of inflammatory dermatosis and in particular of acne [70]. In this formulation, melatonin acts as an antiseborrheic agent exerting a synergistic action with the antimicrobial substance. According to the results of studies performed on patients with mild, moderate and severe acne, the formulation comprising melatonin and chlorhexidine reduces the clinical symptoms associated to acne, is suitable for treatment in the short, medium and long term, at the same time reducing side effects, such as irritation and dryness of the skin. This topical treatment also allowed the complete healing of about 20% of the patients.

Another cream is claimed as a formulation able to delay skin aging and to produce a skin whitening effect. This formulation comprises melatonin and a number of other substances, mainly plant

extracts, and through cutaneous absorption it could reduce the melanin content of the skin and block its synthesis [71].

6. Stem cell therapy and tissue healing

Melatonin is known to promote the differentiation of mesenchymal stem cells, a process that could be exploited for the regeneration of different tissues, comprising bone, cartilage and muscle [72]. Despite the mechanisms sustaining the differentiating activity of melatonin are still to be completely elucidated, they seem to combine promotion of both receptor-dependent and receptor-independent cellular activities. In particular, melatonin effectively sustains bone health as it increases bone density and induces new bone growth by targeting many levels of bone remodeling, in which an important role seems to be exerted by the MT_2 receptor. Melatonin regulates the release of RANKL and osteoprotegerin, thus inducing osteoblastogenesis and inhibiting osteoclastogenesis [73]. In this context, the decrease in plasma melatonin levels observed in elderly people may enhance susceptibility to osteoporosis. Melatonin activity on bone remodeling can be seen as the rationale behind two patent applications claiming its efficacy in bone healing and proposing its application in combination with bone graft material. The first patent application reports that melatonin added to a bone graft or implant, preferably composed by calcium aluminate (CA), can be used in bone reconstruction procedures [74]. Melatonin can be absorbed or covalently linked to CA. The presence of melatonin accelerates bone healing by increasing osteoblast formation, while suppressing fibroblast and scar tissue formation. Furthermore, another application is proposed for these melatonin functionalized substrates that can be used as scaffolds for tissue engineering, serving as a base to graft artificial tissues for transplants.

Another patent claims a formulation for the improvement of bone recovery after bone surgery or impacting injury which comprises a blood component, such as platelet-rich plasma, and other pharmaceutical ingredients, in particular a glucocorticoid (e.g., dexamethasone) and melatonin in a

sustained release form [75]. As reported in the patent, these compounds generated a synergistic effect on human mesenchymal stem cells and induced osteoblastic differentiation more quickly than in standard differentiation medium, thus potentially increasing the rate of bone growth and healing. This composite formulation can be directly applied to the site of interest or it can be part of a structural plug, scaffold or grafting support.

Two recent Chinese patent applications claim the use of melatonin to sustain mesenchymal stem cell differentiation. In particular, one patent application discloses a melatonin synergistic extracellular matrix biomaterial for the preparation of a drug product to promote osteoblast differentiation from mesenchymal stem cells for treating bone diseases [76]. The second patent application claims the use of melatonin in the preparation of drugs for promoting mesenchymal stem cells chondrogenic differentiation for the treatment of joint and cartilage diseases [77].

Another application of melatonin in stem cell therapy pertains to the field of neurodegenerative diseases, in particular of Alzheimer's and Parkinson's diseases, and of stroke and ischemia. In this case the beneficial effects exerted by mesenchymal stem cells are thought to be related to their trophic activity, which reduces apoptotic cell death and stimulates a number of regenerative processes including angiogenesis, vasculogenesis and neurogenesis, sustained by the release of trophic factors. The application claims a method for the treatment of neurodegenerative disorders based on the administration of amniotic membrane-derived stem cells [78]. Stimulation of these stem cells, which express the MT_1 receptor, with melatonin suppressed their proliferation and enhanced differentiation, contributing to the neuroprotective effect against cell death. The combined treatment with amnion-derived stem cells and melatonin enhanced the neuroprotective effect in an in vitro experimental stroke model, compared to the treatment with unstimulated stem cells. Moreover, a significant improved neuroprotection was also obtained when stem cells and melatonin were combined at suboptimal levels.

7. Gastrointestinal tract

7.1 Gastrointestinal tract diseases

Melatonin has been reported to favor gastric ulcer healing, acting at different levels. Melatonin administration increases prostaglandin synthesis and secretion of mucosal bicarbonate, mediates the release of hormones, like gastrin and ghrelin, that stimulate gastric mucosal cell proliferation and enhances the expression of hypoxia-inducible factor and vascular-endothelial growth factor, which in turn promote angiogenesis around the site of ulceration. These actions seem to be related to membrane receptors activation, in particular the MT_2 subtype, as well as to the free radical scavenging properties of melatonin [79]. A patent application claims a dietary supplement composed by melatonin, tryptophan, methionine, betaine, folic acid, vitamins B6 and B12 for healing or regressing the symptoms of gastroesophageal reflux disease (GERD), gastritis and ulcers [80]. In this composition melatonin is assumed to exert an inhibitory action on gastric acid secretion and to promote ulcer healing by sustaining hyperemia at the ulcer margin. In a study with the said dietary supplement, oral treatment of patients affected by heartburn, regurgitation, dysphagia and chest pain led to a complete regression of symptoms in 100% of patients after 40 days of treatment. In the same condition omeprazole was effective in 65% of patients [81].

Another patent application describes a pharmaceutical composition for treating or preventing gastric and duodenal ulcers containing a proton pump inhibitor (PPI) and melatonin [82]. Indeed, melatonin has already been reported to improve the effect exerted by PPIs, accelerating ulcer healing [83]. Moreover, a clinical trial is currently ongoing, evaluating the efficacy of melatonin in combination with omeprazole in chemoprevention of esophageal adenocarcinoma in patients with Barrett's esophagus, a complication of chronic GERD [84].

7.2 Promotion of gastrointestinal absorption

A patent claims the use of melatonin to improve the absorption of peptides, peptidomimetics or other gastrointestinal (GI) transport protein substrates [85]. Melatonin is orally administered and it

can be given in conjunction with the substance to be absorbed as well as in a food composition. As reported in the patent, melatonin increases the transport of the substance by GI transport proteins belonging to the PTR2 family, such as PepT1. The transported substance can be a nutrient, such as an amino acid, a drug or a prodrug. Improved absorption of drugs such as beta-lactam antibiotics can allow administration of lower doses and reduce the risk of developing antibiotic-resistant microorganisms. Studies performed on dogs showed that administration of cephalexin preceded by that of melatonin led to an increased total absorption capacity for cephalexin, obtaining higher peak concentrations and a reduction of the time to achieve peak concentrations. This effect was dose-dependently related to the dose of melatonin administered.

7.3 Food intake

Melatonin participates in energy balance control. In humans it exerts hypolipidemic effects and reduces low-density-lipoprotein cholesterol and triglyceride levels in diabetic patients [86]. A Korean patent application describes a food component composition made of ascorbic acid, beta-D-glucan, hesperetin, melatonin, ginsenoside-Rb1 and ginsenoside-Rg1 able to block appetite sensation [87]. This effect is ascribed to the ability of said composition to suppress the activity of ghrelin. Another patent application reports a health functional food composed by a mixture of the same ingredients plus serotonin, alpha-tocoferol and naringenin to promote leptin secretion and suppress appetite [88].

8. Hypertension

Melatonin participates to blood pressure control in a complex manner, exerting both receptor-independent (e.g., interaction with calcium-calmodulin complex, activation and over-expression of several antioxidant enzymes) and receptor-mediated actions. The last ones can be of peripheral vascular origin or centrally determined, for example through interaction with the sympathetic

nervous system [89]. Melatonin administration has been reported to reduce hypertension both in animals and humans. However, data supporting the antihypertensive effect of melatonin on humans are somehow controversial, with positive or null effects depending on the particular clinical trial considered. An analysis of a number of clinical trials reported that melatonin is effective in combination with standard hypertensive treatment to reduce nocturnal blood pressure when administered in a controlled-release form, while it is ineffective as a fast formulation [90]. A patent assigned in the US on December 2011 reports a formulation for the prevention and treatment of symptoms of hypertension in patients resistant to classical hypertensive therapy [91].The formulation comprises an antihypertensive drug and melatonin which is expected to lower blood pressure and in particular nocturnal pressure in patients with impaired nocturnal blood pressure reduction. This treatment could also lower the cortisol production and delay peak cortisol serum concentrations, reducing the risk of ischemic attacks in the morning hours. The formulation should be administered in a controlled-release form, as the patent reports that melatonin in fast-release or controlled-release formulations exert different effects on blood pressure, cortisol levels and patient mood.

A patent application reports a pharmaceutical composition for the prevention or the treatment of arterial hypertension composed by an angiotensin converting enzyme inhibitor and melatonin [92]. This formulation is described as providing a therapeutically significant effect, with stable nocturnal blood pressure profile.

9. Diabetes

The effect of melatonin on insulin levels and glucose homeostasis is rather complex, and literature data report conflicting experimental results. Melatonin and insulin are functionally interrelated, with high plasma levels of insulin usually measured when melatonin secretion is reduced and vice versa, thus following an opposite circadian rhythm during the 24 hours. It has been demonstrated that

melatonin administration inhibits insulin secretion by involvement of both MT_1 and MT_2 receptors and that increased insulin levels reduce melatonin secretion [93]. This suggests a sort of functional antagonism of the two hormones. On the other hand, diabetic patients have low melatonin levels and studies on animals showed that while pinealectomy increases the risk to develop diabetes, melatonin exerts a diabetes-preventive effect. Interestingly, 12-h pretreatment of INS-1 pancreatic cell line and of isolated rat islets with melatonin induced an increase in insulin secretion, suggesting that melatonin could induce sensitization of pancreatic beta cells to take advantage of the morning food intake [94].

A patent application claims a method for the prevention and treatment of type 2 diabetes by administering a melatonin receptor agonist alone or in combination with one or more antidiabetic drugs (e.g., sitagliptin) [95]. When melatonin is used as monotherapy it is preferably administered 6-12 hours before the meal in a sustained release formulation. In the case of a combined therapy, both the melatonin receptor agonist and the antidiabetic drug can be administered either in an immediate-release or in a controlled-release formulation, also depending on the nature of the antidiabetic agent. Melatonin receptor agonists would potentiate the effect of the antidiabetic drug, allowing a reduction in the administered dose. As reported in the patent application, studies in mice showed that 12-h sustained-release melatonin significantly decreased blood glucose levels and increased insulin levels compared to untreated mice. When melatonin was administered in combination with sitagliptin, a marked decrease of glucose blood concentrations were observed as well.

10. Reproduction

The ability of melatonin to sustain effective in vitro embryo development has been proven for different animal species, for example mouse and sheep [96]. Melatonin, added to the culture medium, promotes the development of blastocyst and increases the rates of embryo implantation, of

pregnancies and postnatal survival of offspring. This positive effect has been ascribed to its antioxidant and free radical scavenging properties. In women, melatonin administration increases intra-follicular melatonin concentrations, reduces intra-follicular oxidative damage and elevates fertilization and pregnancy rates [97].

Interestingly, a patent application claims the topical administration of melatonin to promote the mechanism of embryo implantation and for the prevention of implantation failure in the context of assisted reproduction in both the medical and veterinary field [98]. Melatonin can be replaced by an analogue such as agomelatine, or by 6-hydroxymelatonin, serotonin and 5-hydroxytryptophan. The topical administration is carried out via endometrial irrigation or uterine or endometrial washing at the time of oocyte retrieval. In two clinical studies described in the patent application, treatment with melatonin allowed to double the number of pregnancies. In vitro studies on endometrial tissue showed that addition of melatonin to the growth medium led to a complete morphological expression of pinopodes which are essential for successful blastocyst implantation.

Melatonin has been also described as an important procontractile factor in labor. Interestingly, initiation of labor in women has a peak at night, when melatonin concentrations are highest. Melatonin receptors are expressed on myometrial cells with minimal density in nonlaboring term pregnant uterus, being upregulated as labor initiates [99]. Oxytocin receptors in human myometrium are also upregulated in late pregnancy and this supports the hypothesis that endogenous melatonin can act synergistically with oxytocin to facilitate coordinated and forceful contractions of the uterus necessary for term labor. Indeed, melatonin receptor activation leads to myosin light-chain kinase phosphorylation, increasing sensitivity to oxytocin and myometrial contractility. Additionally, melatonin promotes the synthesis of myometrial gap junction proteins, improving intercellular communication and facilitating synchronized contractions during labor. On the other hand, expression of melatonin receptors prematurely in the myometrium of pregnant women may contribute to preterm labor. In this context, an application is claimed for MT_2 receptor antagonists (e.g., 4-P-PDOT, Figure 6) for the treatment of pre-term labor [100].This patent application also

reports a combination of melatonin and oxytocin to induce labor in a pregnant woman, allowing the reduction of oxytocin dose and limiting its side effects.

11. Urinary incontinence

A patent application reports a composition for treating or preventing urinary incontinence which contains a melatonin receptor agonist, such as melatonin, agomelatine, ramelteon, tasimelteon or TIK-301 [101]. Indeed, in aged guinea pigs, melatonin was able to restore the contractile response of bladder smooth muscle. This effect resulted from an increase in Calcium sensitivity, normalization of oxidative stress parameters and polarity of mitochondria [102].

12. Ethanol acute intoxication

A patent claims the application of melatonin to overcome the symptoms of acute alcohol intoxication. The patent describes an experiment in which melatonin is administered before going to bed. The morning after, no hangover symptoms were reported [103].

13. Conclusions

In the 3-years period covered by the review, many patents and patent applications related to melatonin have appeared, indicating a persistent interest in the therapeutic exploitation of its beneficial effects. Melatonin has been claimed for the treatment or prevention of a variety of pathologies, comprising CNS diseases and pathologies affecting peripheral sites as diverse as the bladder and the skin. Some patents also report melatonin as one ingredient of health functional foods.

Depending on the pathology, the rationale for the application of melatonin is based either on its antioxidant activity or on the stimulation of its G protein-coupled receptors. In some cases the two mechanisms have been shown to exert synergistic effects.

Melatonin has been claimed of therapeutic utility both as a single agent or in combination with other therapeutically relevant drugs, acting as an adjuvant to potentiate their pharmacological effects. In fewer cases melatonin is proposed as the principal active component combined with other substances able to promote its effects.

The number of new melatonin analogs described and proposed for some therapeutic application is limited, comprising essentially two classes of bitopic melatonin derivatives and some indole derivatives with antioxidant effect.

14. Expert opinion

Patents and patent applications published in the period 2012-2014 reveal an opposite trend compared to previous years [104]. In fact, the greatest number of recent patents deals with the therapeutic applications for melatonin or its known analogs, with only a minor number of patents reporting new chemical classes of melatonin derivatives. A similar decrease in the number of new melatonin analogs can be perceived also in the scientific literature. This may reflect the hard walk to get a marketing authorization for new chemical entities compared to melatonin which is categorized by FDA as a dietary supplement. The appeal of new synthetic analogs of melatonin for therapeutic exploitation depends on better characterization of subtype-selective effects [105] and significant improvements in pharmacokinetic properties (bioavailability, duration of action).

In 2012-2014 patent applications, melatonin has been proposed for the treatment of a surprisingly diverse set of medical conditions, not only related to its effects on the central nervous system, but also involving many diverse peripheral districts. This is probably a consequence of its multiple mechanisms of action and its diverse pharmacological activities at cellular level. In many cases

melatonin is proposed as an adjuvant substance that can be combined with consolidated therapies either to enhance their efficacy or to reduce the amount of administered drugs and dose-related toxicities. A number of current clinical trials are evaluating the efficacy and toxicity of drug combinations comprising melatonin. If the use of melatonin in combination therapies allows to exploit its beneficial effects, on the other side it highlights that these effects are probably not sufficient to treat or cure pathologies by monotherapy. In this context, the safe profile of melatonin is an important requisite for combination. On the other hand, the molecular and cellular mechanisms underlying additive or synergistic effects of melatonin with other drugs need to be further explored, to rationally devise new therapeutic strategies.

The use of melatonin in combination with other drugs seems to be the newest trend, not only as a physical mixture of active ingredients, but also in the form of dual-acting ligands, i.e. compounds characterized by a chemical structure able to exert two different effects. A nice example of such compounds is reported in the patent by Witt-Enderby, in which a class of compounds is described as able to interact with both melatonin and estrogen receptors [53]. Other hybrid derivatives combining melatonin and a known neuroprotective agent have been reported as therapeutic options for neurodegenerative disorders. These compounds inhibit cholinesterases and exert an antioxidant effect that could be exploited in the treatment of Alzheimer's disease [34,106,107].

For sure there are different aspects related to melatonin effects that still need to be better elucidated. One such aspect is the involvement of melatonin's different mechanisms of action on the observed pharmacological activities. In particular the role exerted by MT_1 and MT_2 membrane receptors should be clarified and their effects distinguished from non-receptor mediated ones. The better understanding of their role in the pathogenesis of diseases and of their potential in the treatment of such pathologies may provide a therapeutic opportunity to receptor subtype-specific compounds and to melatonin receptor antagonists, which at present do not find any application, nor are currently evaluated in clinical trials. Also the antioxidant activity of melatonin, which is often invoked to explain different kinds of cytoprotective effects, should be characterized in a more clear

and thoroughly way. Another important point is the comprehension of the role of timing and pattern of melatonin administration to ensure the maximum therapeutic efficacy. Melatonin is able to influence the expression of genes in a number of physiological districts or cell types characterized by a circadian activity. The correlation between melatonin levels, gene expression and protein production should be further investigated in these districts, as it could influence the way of melatonin administration, either in a fast- or a controlled-release form, and the most suitable moment of day for administration to better achieve the aims of the therapy.

Bibliograpy

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[1] Lerner AB, Case JD, Takahashi Y, et al. Isolation of melatonin: the pineal gland factor that lightens melanocytes. J Am Chem Soc 1958;80:2587

[2] Acuña-Castroviejo D, Escames G, Venegas C, et al. Extrapineal melatonin: sources, regulation, and potential functions. Cell Mol Life Sci 2014;71:2997-3025

[3] Pandi-Perumal SR, Trakht I, Srinivasan V, et al. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol 2008;85:335-53

[4] Carpentieri A, Díaz de Barboza G, Areco V, et al. New perspectives in melatonin uses. Pharmacol Res 2012;65:437-44

[5] Sanchez-Barcelo E, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: evaluation of human trials. Curr Med Chem 2010;17:2070-95

[6] Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of basic and clinical pharmacology. LXXV. Nomenclature, classification and pharmacology of G protein-coupled melatonin receptors. Pharmacol Rev 2010;62:343-80 **Comprehensive review on MT1 and MT2 distribution, signaling and function.

[7] Slominski RM, Reiter RJ, Schlabritz-Loutsevich N, et al. Melatonin membrane receptors in peripheral tissues: distribution and functions. Mol Cell Endocrinol 2012;351:152-66 *Review on MT1 and MT2 distribution and functions on peripheral tissues

[8] Hardeland R. Melatonin: signaling mechanisms of a pleiotropic agent. Biofactors 2009;35:183-92

[9] Reiter RJ, Tan DX, Rosales-Corral S, Manchester LC. The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. Mini-Rev Med Chem 2013;13:373-384

[10] Lemoine P, Zisapel N. Prolonged-release formulation of melatonin (Circadin) for the treatment of insomnia. Expert Opin Pharmacother 2012;13:895-905

[11] Zlotos R, Jockers R, Cecon E, Rivara S, Witt-Enderby PA. MT1 and MT2 melatonin receptors: ligands, oligomers and therapeutic potential. J Med Chem 2014;57:3161-85

[12] Leclerc V, Ettaoussi M, Rami M, et al. Design and synthesis of naphthalenic derivatives as new ligands at the melatonin binding site MT3. Eur J Med Chem 2011;46:1622-9

[13] Spadoni G, Bedini A, Rivara S, Mor M. Melatonin Receptor Agonists: New Options for Insomnia and Depression Treatment. CNS Neurosci Ther 2011;17:733–41

[17] Requis Pharmaceutical Inc. Antihistamines combined with dietary supplements for improved health. US20140199417; 2014

[18] Casal Y Garzov RE. Drug composition for the treatment of insomnia. WO2012109695; 2012

[19] Nestec SA. Methods and compositions useful for promoting sleep in animals. US20130273176; 2013

[20] Nestec SA. Compositions and methods for enhancing cognitive and related functions in animals. US20120171303; 2012

[21] Tianjin Tiens Biolog Dev CO, Tianjin Tiens Life Sources CO LTD; Tiens Group CO LTD. Medlar and tuckahoe composition and preparation method thereof. CN102552536; 2012

[22] Guangzhou Gonghe Medicine Tecnology CO LTD; Guangzhou Xiangkun Medicine Tecnology Co LTD. Anti-insomnia composition, application of composition, multi-phase pulse immediate-

release preparation prepared by use of composition and preparation method of preparation. CN103550775; 2014

[23] Dongguan Zhaoyan Biotechnology CO LTD. Nutritional health-care product for improving agrypnia. CN102688416; 2012

[24] Keller RM. Formulation and method to induce a deep state of relaxation. WO2013166246; 2013

[25] Takeda Pharmaceutical Company Limited. Tricyclic compound and pharmaceutical use thereof. US8552037; 2013

[27] Vanda Pharmaceuticals Inc. Use of melatonin agonist for the treatment of sleep disorders including primary insomnia. US20120136050, 2012

[28] Vanda Pharmaceuticals Inc. Treatment of circadian rhythm disorders. US8785492; 2014

[29] Obshchestvo S Ogranichennoj Otvetstvennost'ju "Ehr Ehnd Di Farma". Pharmaceutical composition for prevention and treatment of depressions. RU2011109649; 2012

[30] Parshin AN, Gilod VM. Method of treating adaptation disorders in patients with risk of suicidality. RU2506073; 2014

[31] Joshi N, Biswas J, Nath C, Singh S. Promising role of melatonin as neuroprotectant in neurodegenerative pathology. Mol Neurobiol 2014, in press DOI 10.1007/s12035-014-8865-8

[32] Polimeni G, Esposito E, Bevelacqua V, et al. Role of melatonin supplementation in neurodegenerative disorders. Front Biosci (Landmark ed) 2014;19:429-46

[33] Bennett M, Peller G. Compositions and methods for treating traumatic brain injury. CA2799127; 2014

[34] Centrum Medyczne Ksztalcenia Podyplomowego - Uniwersytet Warszawski. Novel hybrid cholinesterase inhibitors. US8841453; 2014

[35] Regents of University of Minnesota. Ischemia/reperfusion protection compositions and methods of using. US8728532; 2014

[38] Morozova MA, Beniashvili AG, Zapolskii ME. Combination for the prophylaxis treatment of behavioural, mental, and cognitive disorders. WO2014054965; 2014

[39] Jeong JK, Moon MH, Lee YJ, et al. Melatonin-induced autophagy protects against human prion protein-mediated neurotoxicity. J Pineal Res 2012;53:138-46

[40] Industrial Cooperation Foundation Chonbuk National University. Novel uses of melatonin. WO2013109016; 2013

[41] Industry-Academic Cooperation Foundation, Yonsei University. Pharmaceutical composition for preventing or treating protein aggregate-inducing diseases. KR20140014801; 2014

[42] Kanno H, Ozawa H, Sekiguchi A, et al. The role of mTOR signaling pathway in spinal cord injury. Cell Cycle 2012;11:3175-9

[43] Nestec SA. Methods for reducing neurodegeneration. US20140113949; 2014

[44] Wilhelmsen M, Amirian I, Reiter RJ, et al. Analgesic effects of melatonin: a review of current evidence from experimental and clinical studies. J Pineal Res 2011,51:270-7

[45] Cecype Services S. DE R.L. DE C.V. Pharmaceutical composition comprising a combination of a non-steroidal anti-inflammatory agent, an adjuvant and an antineuritic analgesic, having an antinociceptive effect. WO2014098552; 2014

[46] Bizzarri M, Proietti S, Cucina A, Reiter RJ. Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. Expert Opin Ther Targets. 2013;17:1483-96

[47] Clinical trial identifier NCT01965522. Anti-proliferative Effects of Vitamin D and Melatonin in Breast Cancer.

[48] Clinical trial identifier NCT00668707. Adjuvant Melatonin for Prevention of Lung Cancer Recurrence and Mortality.

[49] Ekmekcioglu C. Expression and putative functions of melatonin receptors in malignant cells and tissues. Wien Med Wochenschr 2014; in press DOI 10.1007/s10354-014-0289-6

[50] Grant SG, Melan MA, Latimer JJ, Witt-Enderby PA. Melatonin and breast cancer: cellular mechanisms, clinical studies and future perspectives. Expert Rev Mol Med 2009;11:e5

[51] Sanchez-Barcelo EJ, Mediavilla MD, Alonso-Gonzales C, Reiter RJ. Melatonin uses in oncology: breast cancer prevention and reduction of the side effects of chemotherapy and radiation. Exp Opin Invest Drugs 2012;21:819-831. *Review on melatonin potential in prevention, treatment and protection from chemotherapy side effects

[52] Duquesne University of the Holy Spirit. Combination hormone replacement therapy (HRT) and melatonin to prevent and treat mammary cancer. US8618083; 2013

[53] Duquesne University of the Holy Spirit. Anti-cancer tamoxifen-melatonin hybrid ligand. US8785501; 2014

[54] Scott & White Healthcare. Melatonin-based treatment and diagnosis of bile duct disease. US20140079680; 2014

[55] Han Y, Demorrow S, Invernizzi P et al. Melatonin exerts by an autocrine loop antiproliferative effects in cholangiocarcinoma: its synthesis is reduced favoring cholangiocarcinoma growth. Am J Physiol Gastrointest Liver Physiol 2011;301:G623-33

[56] Clinical trial identifier NCT01557478. Melatonin as adjuvant therapy in breast cancer patients.

[57] Universidad de Granada. Use of melatonin for treating and/or preventing mucositis. US20140243384; 2014

[58] Ruiqiong R. Vulcanized aspartic acid modified melatonin derivative and application thereof. CN102816103; 2014

[59] Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res 2011; 51:1-16

[60] Luchetti F, Canonico B, Betti M. Melatonin signaling and cell protection function. FASEB J 2010;24:3603-24.

[61] Carrillo-Vico A, Lardone PJ, Alvarez-Sánchez N, et al. Melatonin: buffering the immune system. Int J Mol Sci 2013;14: 8638-83

[62] University of Sunderland. Melatonin derivatives and their use as antioxidants. US8258173; 2012

[63] Iasomai AB. New combination comprising N-acetyl-L-cysteine and its use. WO2012130609; 2012

[64] Nutri-Fit GMBH. Melatonin and its use in preventing postoperative complications. US20140249198; 2014

[65] Yang Y, Sun Y, Yi W, et al. A review of melatonin as a suitable antioxidant against myocardial ischemia-reperfusion injury and clinical heart diseases. J Pineal Res 2014;57:357-66.

[66] Tianjin Pacific Pharmaceutical. Traditional Chinese medicine composition used for treating low immunity in middle-aged and elderly people. CN102872294; 2013

[67] Slominski AT, Kleszczynski K, Semak I, et al. Local melatoninergic system as the protector of skin integrity. Int J Mol Sci 2014;15:17705-32

[68] Universidad de Granada. Composition comprising melatonin or its derivatives with coenzyme Q10 and use thereof against ageing of the skin. WO2014083227; 2014

[69] Mavi Sud s.r.l. Melatonin and immunostimulating substance-based compositions. US8802117; 2014

[70] Novaera s.r.l. Melatonin and an antimicrobial or antibacterial agent for the treatment of acne. US20140199413; 2014

[71] Suzhou Guli Biotechnology Co. Skin whitening cream. CN102860978; 2013

[72] Luchetti F, Canonico B, Bartolini D, et al. Melatonin regulates mesenchymal stem cell differentiation: a review. J Pineal Res 2014;56:382-397

[73] Maria S, Witt-Enderby PA. Melatonin effects on bone: potential use for the prevention and treatment for osteopenia, osteoporosis, and periodontal disease and for use in bone-grafting

procedures. J Pineal Res 2014; 56:115-25 *Review on the mechanisms sustaining melatonin effects on bone

[74] Duquesne University of the Holy Spirit. Enhanced bone healing. US20130029960; 2013.

[75] Riukowski JL. Formulations and methods for recovery from dental surgery. EP2711012; 2014

[76] Sun Yat-Sen University. Application of melatonin synergistic extracellular matrix biomaterial to preparation of medicament for promoting osteoblast differentiation of mesenchymal stem cells. CN103736150; 2014

[77] Sun Yat-Sen University. Application of melatonin in preparation of drugs for promoting mesenchymal stem cell chondrogenic differentiation. CN103494808; 2014

[78] Medical College of Georgia, University of South Florida. Method of disease-induced and receptor-mediated stem cell neuroprotection. US20120009271; 2012

[79] Brzozowska I, Strzalka M, Drozdowicz D, et al. Mechanisms of esophageal protection, gastroprotection and ulcer healing by melatonin. implications for the therapeutic use of melatonin in gastroesophageal reflux disease (GERD) and peptic ulcer disease. Curr Pharm Des 2014;20:4807-15

[80] De Souza Pereira R. Method using a dietary supplement for healing or regressing symptoms of gastroesophageal reflux disease, gastritis and ulcers. US20130090305; 2013

[81] De Souza Pereira R. Regression of gastroesophageal reflux disease symptoms using dietary supplementation with melatonin, vitamins and aminoacids: comparison with omeprazole. J Pineal Res 2006;41:195-200

[82] Obshchestvo S Ogranichennoj Otvetstvennost'ju "Ehr Ehnd Di Farma", Pharmaceutical composition for gastric and/or duodenal ulcer. RU2010129474, 2012

[83] Celinski K, Konturek SJ, Konturek PC, et al. Melatonin or L-tryptophan accelerates healing of gastroduodenal ulcers in patients treated with omeprazole. J Pineal Res 2011;50:389-94

[84] Clinical trial identifier NCT01566474. Melatonin Associated to Acid Inhibition for Chemoprevention in Barret Esophagus: a Pilot Study.

[85] Nestec S.A. Methods for increasing absorption of peptides, peptidomimetics and other gastrointestinal transport protein substrates. US8492331; 2013

[86] Navarro-Alarcón M, Ruiz-Ojeda FJ, Blanca-Herrera RM, et al. Melatonin and metabolic regulation: a review. Food Funct. 2014;5:2806-32

[87] Korea Food Research Institute. Composition of food components for appetite control comprising specific compounds as effective component. KR20120046967; 2012.

[88] Korea Food Research Institute. Composition for promoting leptin secretion comprising specific compounds as effective component. KR20130010192; 2013.

[89] Pechanova O, Paulis L, Simko F. Peripheral and Central Effects of Melatonin on Blood Pressure Regulation. Int J Mol Sci 2014; 15:17920-17937

[90] Grossman E, Laudon M, Zisapel N. Effect of melatonin on nocturnal blood pressure: meta-analysis of randomized controlled trials. Vasc Health Risk Manag 2011;7:577-84

[91] Neurim Pharma 1991. Method and formulation for treating resistance to antihypertensives and related conditions. US8075914; 2011

[92] Obshchestvo S Ogranichennoj Otvetstvennost'ju "Ehr Ehnd Di Farma". Pharmaceutical composition for treating and/or preventing arterial hypertension. RU2010129473; 2012

[93] Peschke E. Melatonin, endocrine pancreas and diabetes. J Pineal Res 2008;44:26-40

[94] Kemp DM, Ubeda M, Habener JF. Identification and functional characterization of melatonin Mel 1a receptors in pancreatic β cells: potential role in incretin-mediated cell function by sensitization of cAMP signaling. Mol Cell Endocrinol 2002;191:157-166

[95] Takahashi JS, Kay SA. Compositions and methods for diabetes treatment. US20120232003; 2012

[96] Wang F, Tian X, Lu Zhang, Tan D, Reiter RJ, Liu G. Melatonin promotes the in vitro development of pronuclear embryos and increases the efficiency of blastocyst implantation in murine. J Pineal Res 2013;55:267–274

[97] Tamura H, Takasaki A, Miwa I, et al. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. J Pineal Res. 2008;44:280-7

[98] Maxia N. Use of N-acetyl-5-methoxytriptamine or analogs thereof, for promoting the mechanism of implantation of the embryo and related compositions and culture media. WO2013178587; 2013

[99] Olcese J, Beesley S. Clinical significance of melatonin receptors in the human myometrium. Fertil Steril 2014;102:329-35

[100] The Florida State University Research Foundation, Inc. Methods of treating pre-term labor. US20140088057; 2014

[101] Astellas Pharma Inc. Pharmaceutical composition for treating or preventing stress urinary incontinence or mixed incontinence, and method for screening compounds to be included in said pharmaceutical composition. WO2014010603; 2014

[102] Gomez-Pinilla PJ, Gomez MF, Swärd K, et al. Melatonin restores impaired contractility in aged guinea pig urinary bladder. J Pineal Res 2008;44:416-25

[103] Rassoulian D. Use of melatonin to treat acute alcohol intoxication. EP2570126; 2014

[104] Mor M, Rivara S, Pala D, Bedini A, Spadoni G, Tarzia G. Recent advances in the development of melatonin MT1 and MT2 receptor agonists. Exp Opin Ther Patents 2010;20:1059-77

[105] Ochoa-Sanchez R, Comai S, Lacoste B, et al. Promotion of non-rapid eye movement sleep and activation of reticular thalamic neurons by a novel MT2 melatonin receptor ligand. J Neurosci 2011;31:18439-52

[106] Rodríguez-Franco MI, Fernández-Bachiller MI, Pérez C, et al. Novel tacrine-melatonin hybrids as dual-acting drugs for Alzheimer disease, with improved acetylcholinesterase inhibitory and antioxidant properties. J Med Chem 2006;49:459–62

[107] López-Iglesias B, Pérez C, Morales-García JA, et al. New melatonin-N,N-dibenzyl(N-methyl)amine hybrids: Potent neurogenic agents with antioxidant, cholinergic, and neuroprotective properties as innovative drugs for Alzheimer's disease. J Med Chem 2014;57:3773-85