Melatonin and Melatoninergic Drugs as Therapeutic Agents: Ramelteon and Agomelatine, the Two Most Promising Melatonin Receptor Agonists

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Abstract: Melatonin is a pineal hormone which basically acts through membrane receptors, but also as a free radical scavenger (requiring no receptors), and by binding to intracellular sites (calmodulin and nuclear receptors). Membrane receptors (MT_1, MT_2) are associated to G-proteins linked to inhibition of adenylyl cyclase and decrease of cAMP, and are expressed by almost all structures of the CNS (especially hypothalamic suprachiasmatic nucleus and pars tuberalis of the pituitary), as well as in peripheral tissues (gastrointestinal tract, thymus, smooth muscle of blood vessels, adipocytes, lymphocytes, etc). Among the actions attributed to melatonin are those of antioxidant, controller of circadian rhythms (especially sleep-wake and core body temperature), immunomodulation, antidepressant, etc. This wide spectrum of actions suggests many possible therapeutic applications for melatonin. However, its use as a drug presents some limitations (to optimise pharmacological responses of each subtype or receptors, its rapid metabolic inactivation, etc.) that have caused many laboratories to develop analogues without the above mentioned problems. Two are the patented melatoninergic drugs with more interesting properties: one is ramelteon (US6034239; Rozerem™), approved by the FDA for the long-term treatment of sleep disturbances characterized by difficulty with sleep onset; the second, agomelatine (US5318994; Valdoxan™), which is completing the phase III trial, was designed for the treatment of symptoms of major depressive disorders, particularly anxiety, sleep troubles and circadian disturbances.

Keywords: Melatonin, agomelatine, valdoxan, ramelteon, rozerem, sleep troubles, depression, MT1 melatonin receptors, MT2 melatonin receptors.

INTRODUCTION

Melatonin: Synthesis, Circulation, Metabolism and Regulation of Secretion

Melatonin is an indoleamine (N-acetyl-methoxy-tryptamine) widely distributed in nature, from plants, bacteria and unicellular algae to humans [1,2]. Although melatonin is synthesized in numerous organs (pineal, retina, lymphocytes, gastrointestinal tract, etc.), circulating melatonin in mammals is mainly of pineal origin [2,3]. Mammalian pinealocytes take tryptophan, the aminoacid precursor of melatonin, from blood, forming first 5-hydroxy-tryptophan and then serotonin. This last is acetylated to form N-acetylserotonin, a reaction catalyzed by the enzyme arylalkylamine N-acetyltransferase (AANAT), which, in most cases, represents the rate-limiting enzyme in melatonin synthesis. N-acetylserotonin is finally converted to melatonin by the enzyme hydroxyindole-O-methyltransferase [4]. Once synthesized, melatonin, because of its high liposolubility, diffuses out into the capillary blood and cerebrospinal fluid (CSF). In blood, melatonin circulates bound to proteins (70%) and free, rapidly reaching all tissues. Melatonin arrives at the CSF through the pineal recess, and its concentration in the third ventricle is up to 20 times higher than in blood [5]. The half-life of melatonin shows a bi-exponential pattern, with a first distribution half-life of 2 min, followed by a second of 20 min [6]. Circulating melatonin is metabolized in the liver by hydroxylation, dependent on cytochrome P450 mono-oxygenases, and then conjugated with sulphate (6-sulphatoxymelatonin) and, to a lesser extent, to glucuronide [6]. In tissues of neural origin, such as the retina and pineal gland, melatonin can be deacetylated to 5-metoxytriptamine [7]. In the brain, melatonin is metabolized to derivates of kynuramine [8].

The most striking feature of melatonin secretion is their rhythmicity. The pineal production of melatonin shows a circadian rhythm with low circulating levels during the day, and high plasmatic concentrations of hormone at night [4]. This rhythmicity is controlled by an endogenous clock, the suprachiasmatic nuclei of hypothalamus (SCN). The activity of the SCN is synchronized by the light/dark cycle. Light, detected by photoreceptors located at the retinal ganglion cells and containing the photopigment melanopsin, initiates the signals which, all along a neural pathway (the retino-hypothalamic tract) synchronizes the endogenous oscillator (SCN), to the day/night period [4]. Neurons from SCN project, through the paraventricular nucleus, medial forebrain bundle, reticular formation and intermediolateral horn of the spinal cord, to the superior cervical ganglia (SCG). Postganglionic noradrenergic fibres from SCG innervate pinealocytes (conarii nervi). In darkness, the norpinephrine released by the conarii nerves binds to β-adrenergic receptors on the pinealocytes, activates adenylate cyclase via the α-subunit of G_s protein, increases the cAMP, and promotes the synthesis and activation of AANAT, thus triggering the synthesis of melatonin [4]. During the light phase of the daily cycle, the release of norepinephrine is low and, consequently, melatonin synthesis decreases.

Melatonin Receptors

By using as a probe the agonist radioligand 2-[^{125}I]-iodomelatonin [9] and through experiments of binding and autoradiography, binding sites for melatonin were characterized in different tissues. Initially, two kinds of membrane receptors: ML_1 (high affinity, pM range) and ML_2 (low affinity, nM range), were described. Three subtypes of ML_1 high affinity receptors were then characterized and named Mel_{1a}, Mel_{1b} and Mel_{1c}, the last not being present in mammals [10]. Now, in accordance with the criteria of the International Union of Pharmacology (IUPHAR) the following melatonin membrane receptors are under consideration: MT_1 (the former Mel_{1a}), MT_2 (the previously identified as Mel_{1b}), and MT_3 (the former ML_2). Both the MT_1 and MT_2 receptors belong to the superfamily of G-protein-coupled membrane receptors linked to the inhibition of the adenylyl cyclase and the subsequent decrease of cAMP [11]. The third subtype of melatonin membrane receptors, MT_3, with an affinity range 10 nM, has been described in hamsters as the human homologue of the cytoplasmic enzyme quinone reductase 2 [12], which participates in the protection against

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oxidative stress by preventing the electron transferring reaction of quinones [12]. They are expressed with the highest amounts in liver and kidney, modest amounts in brain, heart, brown adipose tissue, and low amounts in skeletal muscle and lung [12].

The human MT_1 and MT_2 melatonin receptor subtypes have a 55% overall identity in their aminoacid sequence and about 70% within the transmembrane domains [11]. These receptors have been described in almost all structures of the CNS, although the highest density is found in the hypothalamic SCN and the pars tuberalis of the pituitary [13]. This distribution explains the melatonin actions as a chronobiotic agent as well as its modulatory effects on the neuroendocrine reproductive axis. MT_1 and MT_2 receptors are also present in the gastrointestinal tract, lungs, thymus, smooth muscle of blood vessels, adipocytes, lymphocytes and neutrophiles. The peripheral receptors could explain some of the melatonin effects on cardiovascular, gastrointestinal or immune systems [13]. cDNAs and DNAs encoding high affinity melatonin receptors have been recently patented [14]. Nuclear receptors of the retinoic acid receptors superfamily (RZR/ROR) have also been proposed as melatonin binding sites, and would be especially related with the immunomodulatory role of melatonin [15]. Melatonin also binds to calmodulin [16] and this binding is involved in the antiestrogenic actions of melatonin [17].

MELATONIN ACTIONS AND APPLICATIONS

The adjective "pleiotropic", frequently used to design the actions of melatonin, defines the great number of functions attributed to this indoleamine. Let us briefly review some of the best known actions ascribed to melatonin:

Antioxidant Properties

Melatonin has been shown to be a free radical scavenger more efficient than most of the naturally occurring antioxidants [7]. Melatonin exerts its antioxidative effects by three different mechanisms:

I) Direct interaction with reactive oxygen species. A melatonin metabolite of non-hepatic tissues, the N^1-acetyl-N^2-formyl-5-methoxykinuramine, is the key to melatonin's ability to neutralize free radicals. In the formation process of this metabolite from melatonin, up to four free radicals are consumed. Furthermore, another metabolite of the same family, the N^1-acetyl-5-methoxy-kinuramine, is a potent radical scavenger not only for reactive oxygen but also with reactive nitrogen species [18].

II) Indirectly, by up-regulating the expression of antioxidant enzymes such as superoxide dismutases, peroxidases and gluthathione, and down-regulating pro-oxidant ones such as nitric oxide synthase and 5- and 12-lipo-oxigenases [19].

III) By preventing the formation of oxidative radicals. This antioxidant effect takes place at mitochondrial level. Mitochondria are the main source of free radicals because of the reactions of the respiratory chain. Melatonin is capable of supporting the electron flux through the respiratory chain, of preventing the breakdown of the mitochondrial membrane potential, and of decreasing electron leakage, thus reducing the formation of superoxide anions [19]. The melatonin metabolite N^1-acetyl-5-methoxykynuramine may also reduce the mitochondrial formation of oxidative radicals [20].

Control of the Circadian System (Chronotropic Agent)

The robust circadian rhythm of melatonin secretion, which is not disturbed by practically any situation or agent except light exposure, suggests a role as an endogenous synchronizer [21]. Melatonin is secreted under the control of the SCN and, at the same time, SCN itself has MT_2 melatonin receptors [22]. Thus, the SCN and the pineal gland integrate a feedback loop for the synchronization of many biological functions with the daily light/darkness cycles. Melatonin administration changes the timing of sleep, core body temperature, cortisol or melatonin rhythms [23]. The sense of these changes depends on the time of administration of melatonin. When given during the evening or the first half period of night, melatonin induces a phase-advance, whereas during the second half of the night or in the morning it induces a phase-delay of the rhythms [23].

Melatonin could be involved in the control of the circadian rhythm of core body temperature. Both, the circadian rhythms of melatonin and core body temperature are inversely coupled. It has been demonstrated that the hypothermic properties of melatonin are responsible for at least 40% of the amplitude of the circadian rhythm of body temperature [24].

Sleep/Wake Rhythm

Circulating levels of melatonin are highest during the night and the timing of highest urinary excretion of 6-sulphatoxymelatonin overlaps with the greatest nocturnal sleepiness [25]. This fact suggested a possible role of melatonin in the mechanism of sleeping. Melatonin, at physiologic doses (0.1-0.3 mg) promotes sleep onset and maintenance, decreases sleep latency, increases sleep efficiency and increases total sleep time [26,27]. However, not all the results are similar, and the efficacy of melatonin as a hypnotic agent is still controversial. Discrepancies could be explained by the different dosage and time of administration of the indoleamine or the different way of assessing sleep quality [28]. It has been suggested that melatonin hypnotic effects are secondary to its ability to induce a decrease in core body temperature [29]. A good example of the role of melatonin in the sleep/wake rhythm can be obtained from patients of Smith-Magenis syndrome, originated by a partial deletion of chromosome 17. These patients show a characteristic inversion of the circadian rhythm of melatonin, with a phase advance shift of 9.6 ± 0.9 h with respect to control subjects. Behavioural phenotype includes sleep disorders with early sleep onset, difficulty falling asleep, difficulty staying asleep, frequent awakening, early waking, reduced REM sleep and decreased sleep time [30]. The administration to these patients of a β1-adrenergic antagonist in the morning (to abolish the diurnal secretion of melatonin) associated with the administration of melatonin in the evening (to generate a nocturnal peak, restoring the normal day/night rhythm of melatonin), induced a dramatic improvement in sleep quality [30,31].

Melatonin synthesis declines with age and sleep troubles of elderly people have been attributed to this low melatonin secretion. Consequently, melatonin, rather than the therapies based on benzodiazepines [32], has been proposed as an elective treatment for insomnia in elderly patients, with positive results.

It is in those sleep disorders originated by a desynchronization of activity rhythms with the dark-light cycle, such as shift-work, jet-lag or absolute blindness, where melatonin is more effective [33]. However, since in these situations circadian rhythms can also be entrained by light, the effects of melatonin can be obscured [28].

Immunomodulation

The relationship between melatonin and the immune system was demonstrated several years ago [34]. Immunomodulatory properties of melatonin seem to be mediated via membrane and nuclear receptors [35]. Both melatonin MT_1 membrane receptors and the putative nuclear RZR/RORα melatonin receptors have been described in various immune cells of humans [36]. Melatonin enhances the production of interleukins by cultured mononuclear cells (IL-2 and IL-6) and macrophages (IL-2 and IL-12)[37]. Furthermore, human lymphocytes synthesize melatonin, which could stimulate the production of IL-2 as an autocrine or paracrine substance [38].

Mood Disorders

The cyclic nature of depressive illness, together with the diurnal changes in its symptoms as well as the troubles in the circadian rhythms of core body temperature and sleep/wake, were the basis to consider that a dysfunction of the circadian system could be among the ethiological factors of depression [39]. Because of its role as regulator of circadian rhythms, measurement of melatonin secretion in depression has gained attention [39]. Serum melatonin concentrations are low in patients with major depressive disorders and, after treatment with antidepressants, melatonin secretion (measured by the urinary excretion of its metabolite 6-sulphatoxy-melatonin) increases coinciding with the improvement of the symptoms [40,41]. Not only the serum concentration of melatonin is altered in patients with bipolar affective disorder or seasonal affective disorder (winter depression) but a phase delay of melatonin rhythm is characteristic of these mood disorders [39,40]. Because of the circadian component of depressive illness, treatment with phase-resetting agents (bright light or melatonin) at critical timings was proposed several years ago [42]. Recently, the description of depression-like behaviour in melatonin MT_1 receptor knockout mice [43] has caused interest to be regained in the role of melatonin in depression as well as in the development of melatonin receptor agonists as antidepressants.

Cardiovascular System

It has been suggested that melatonin regulates blood pressure through its control of the autonomic nervous system [44]. A nocturnal serum concentration of melatonin lower than normal has been described in subjects with coronary diseases [45]. Furthermore, melatonin increases the vagal tone, decreases plasmatic levels of NE, and reduces blood pressure in hypertensive patients [46]. Melatonin receptors have been identified in the cardiac ventricular wall, coronary arteries, aorta and systemic arteries [13] although their physiological meaning is not completely understood.

Oncostatic Properties of Melatonin

The possible influence of melatonin on the growth and spread of different kinds of tumors as well as the mechanisms involved in such oncostatic properties, have been widely studied in animal models [47,48] and also explored in human clinical trials [49]. However, it is on hormone-dependent breast tumors where the oncostatic actions of melatonin have been most studied [50]. Melatonin inhibits the promotion, growth and invasiveness of estrogen-receptor positive mammary tumors by interacting with the estrogen-signaling pathway [17,51,52], and enhances the antiestrogenic action of tamoxifen by acting on a site other than this antiesterogen [52]. Furthermore, melatonin exerts antiaromatase actions *in vitro* (MCF-7 cells) [53] as well as *in vivo* (murine chemically induced mammary tumors) [54]. That is to say, melatonin shares properties of selective estrogen receptor modulators (SERMs) such as tamoxifen and selective estrogen enzyme modulators (SEEMs) such as letrozole or formestane, thus being a drug with potential interest in prevention and treatment of human breast cancer. This property of melatonin reinforces the idea of the possible role of this hormone as an adjuvant therapy for prevention and treatment of cancer [52]. Since oxidative stress has been implicated in the initiation, promotion and progression of carcinogenesis [55], the anticarcinogenic actions of melatonin can be also attributed to its antioxidative properties [56].

So far, the highest clinical experience in the use of melatonin in treatment of cancer belongs to the group of Lissoni in Italy. They have used melatonin not only as an anticancer drug for different kinds of neoplasias but also to reduce the adverse effects of other chemotherapeutic agents. The conclusion is that treatment with melatonin or an association of different pineal indoles, improves the efficacy of chemotherapy in terms of both survival and quality of life [57,58].

Bone Metabolism

Bone marrow cells express N-acetyltransferase and hydroxy-indole-O-methyltransferase, the two basic enzymes for melatonin synthesis, and melatonin is present in these cells at concentrations higher than in plasma [59,60]. Bone marrow melatonin could play a role as antioxidant or be involved in osteoblast differentiation [60, 61]. Melatonin promotes osteogenesis and prevents bone deterioration [60,62]. These effects could be mediated by either: a) the suppression of osteoclasts activity, because of its free-radicals' scavenging properties or actions on the receptor activator of NF-κB ligand (RANKL), b) by increasing the osteoblast's activity through specific receptors for melatonin [60-61,63].

Energy Expenditure and Body Mass Regulation

Melatonin concentration in the gastrointestinal tract of vertebrates is 10-100 times higher than in plasma [64]. The release of gastrointestinal melatonin seems to be related with the periodicity of food intake [64]. Thus, melatonin could play a role in the regulation of energy balance and body weight. The decline of melatonin secretion with aging has been related with the age-dependent increase of visceral fat [65] although, in human adults, obesity is not accompanied by significant changes in melatonin secretion [66]. However, obese pubertal boys (10-15 year-old) show an increased excretion of 6-sulphatoxymelatonin [67]. Human pathologies associated with desynchronization of circadian rhythms and melatonin secretory patterns could lead to carbohydrate craving and overall weight gain [68].

In rats rendered obese by being fed a hypercaloric diet, melatonin reduces body weight gain [69] and daily administration of melatonin to middle age male rats feeding normocaloric food reverses visceral fat and circulating levels of glucose, leptin, insulin and triglycerides to the youthful values [70]. The presence of a melatonin receptor in human adipocytes [71] could explain, at least in part, these effects. Recently, studies carried out on rabbits, demonstrated that melatonin improves metabolic and morphological pathologies associated with obesity [72].

POTENTIAL INTEREST IN THE UTILIZATION OF MELATONIN AS A THERAPEUTIC DRUG

From the reading of the above exposed wide catalogue of melatonin properties, it is reasonable to consider many possible therapeutic applications of this molecule. Table 1 summarizes some of the hundreds of patented applications of melatonin. However, for different reasons, some of which will be described in the next section, there is little experience in therapeutic uses of melatonin, at least in controlled trials with criteria for any other drugs. This paucity of "controlled uses" contrasts with the extensive use of melatonin without medical control. What we are going now to analyze are the pathologies for which melatonin could represent a therapeutic agent:

- Deficits of the Immune System

The role of melatonin as an immunoenhancer [34] opened up interest towards this substance in at least two fields. One is the treatment of the HIV infection. Serum melatonin concentration seems to be decreased in HIV-infected patients and this fact could be related with the impairment of the T-helper response [73]. Melatonin implants have recently demonstrated that they are able to enhance the T-Helper immune response [74]. On the other hand, some autoimmune diseases, such as rheumatoid arthritis, develop with elevated serum melatonin. In these cases the use of a melatonin receptor antagonist would be recommended.

- Breast Cancer

The properties of melatonin as an antiestrogenic and antiaromatase drug [17, 52] with action mechanisms different to those of the drugs currently being used, open up interest for the

Table 1. Some Patents of Melatonin Uses or Melatoninergic Synthetic Drugs

Uses for Melatonin or Melatoninergic Synthetic Drugs.	US Patents Numbers
Alzheimer disease treatment	65958964, 274615
Androgenic alopecia	6281241
Anesthesia	6552064, 6638966
Anticancer	5196435
Benign prostatic hyperplasia	5750557, 5770610, 6048888
Cardiac Antiarhythmic	6620836
Cerebral infarction treatment	5700828, 6075045
Contraception and control of menstrual cycle	4855305, 5272141
Circadian rhythms troubles	4600723, 4665086, 5242941, 5420152, 5591768, 5707652 5716978, 6069164, 6180657, 6423738, 6638963, 6794407
Dermocosmetics	4746674, 5891903, 5932608, 5939084, 5985293, 6093409
Drug dependencies treatment	6469044, 6833383
Intraocular pressure reduction (Treatment of Glaucoma)	4654361, 6730707
Immunomodulation	5519047
Migraine prevention	5688520
Premenstrual syndrome	4945103
Rheumatoid arthritis	5609877
Sleep troubles	5449683, 5641801, 5654325, 6703412, 6998112
Melatoninergic drugs	5071875, 5151446, 5449689, 5464872, 5498423, 5530012, 5541228, 5596019, 5677328, 5703239, 5763471, 5780470, 5856529, 5808151, 5889031, 5922771, 5948817, 5981571, 6028112, 6060506, 6090854, 6114373, 6140372, 6211225, 6214869, 6436984, 6495543, 6569894, 6620809, 6737431, 6844445, 6908931

study of the possible utility of melatonin in the prevention and treatment of hormone-dependent mammary cancer, at least as an adjuvant of the consolidated therapies.

- Other Neoplasias

Not only mammary tumors but other neoplasias expressing estrogen receptors (glioma, melanoma, etc.) are susceptible to treatment with melatonin and some experimental results support this possibility [75,76]. Furthermore, oncostatic properties of melatonin are possibly not exclusively dependent on its antiestrogenic properties. Immunoenhancing [34,37,38] and antioxidant [7,18-20] actions of melatonin are also useful in antineoplasic treatments, as has been assessed in experimental studies [4, 47,48].

- Circadian Rhythm Disturbances.

This is, without doubt, the field of highest interest up to date. The chronobiotic effects of melatonin [21] make its use viable in different kinds of alterations of the circadian system such as: a) Desynchronizations because of shift-work or transmeridian flights (jet-lag). b) Sleep troubles of circadian origin (delayed or advanced sleep onset syndromes), c) General desynchronization in elderly people in which endogenous melatonin secretion is very low. The treatment of sleep troubles of circadian origin is probably the most successful application of melatonin [25-29] and one of the reasons for the interest in the development of melatonin receptor agonists for this purpose. One example is ramelteon, the drug we will comment on in greater detail in the next section.

- Treatment of Mood Disorders

Depression is one of the most common mental health problems. One in five women and one in ten men will suffer from depression at some point in their lives, these data explaining the interest in development of antidepressant drugs. Essential features of a major depression include sleep disturbances [77], which could be related with changes in melatonin secretory patterns. Furthermore, some forms of depression show seasonal components (SAD), and light, the principal *zeitgeber* of melatonin circadian rhythms of secretion, is being used as a tool for its treatment. All these data reinforce the

role of the circadian system in the pathophysiology of depression [42] and, consequently, the interest in the development of antidepressant drugs, such as melatonin receptor agonists, with incidence in the circadian rhythmicity.

- Treatment of Neurodegenerative Diseases

The antioxidant effects of melatonin protect neurons against amyloid-β-induced effects in Alzheimer's disease [78]. Similar positive effects of melatonin have been described for Parkinson's disease, Huntington's Corea and other neurodegenerative diseases [79-81]. This is a field of melatonin therapeutic uses with great future prospects.

- Control of Food Ingestion and Body Mass

Although the role of melatonin in the control of food intake and body mass is still controversial [64-72] there is evidence that melatoninergic drugs could, in the future, be used in the prevention of obesity. Some laboratories such as Servier, interested in development of melatonin analogues, have been studying, in animal models, the effects of a melatonin agonist (S20304) and an antagonist (S30982) in body weight control [82].

PROBLEMS REGARDS THE THERAPEUTIC USE OF MELATONIN

As stated in the previous section, the description of the multiple action of melatonin suggests a possible usefulness in the treatment of pathological situations such as depression, jet-lag, work-shift syndrome, sleep disorders, some disorders associated with reproduction, some kinds of hormone-dependent cancer, immune disorders, aging, etc. However, the use of the natural molecule as a drug presents some limitations. The first is the difficulty to obtain selective pharmacological responses mediated by each kind or subtype of receptors. The second is its rapid metabolic inactivation (short half-life) which signifies a low oral bioavailability as well as a great interindividual variation. Other reasons which limit the pharmacological (medically controlled) use of native melatonin are not of scientific nature. The use of melatonin for human consumption is differently regulated in many countries. Thus, whereas in USA melatonin is considered as a "dietary supplement" which can be obtained without prescription, in some countries of the EU melatonin is a medicine available only under prescription but in others its use is illegal. The consequence of this lack of an international regulation is that people interested in using melatonin can obtained it by internet, in preparations with doses which gives plasmatic concentrations hundreds of times higher than the physiological ones, with potential side effects. Furthermore, no pharmaceutical company has requested regulatory approval to market it as a drug, perhaps because it is a non patentable molecule.

These reasons have led many laboratories to look for melatonin analogues able to mimic the effects of the native hormone without the above mentioned problems.

BASIS FOR THE DEVELOPMENT OF MELATONINERGIC SYNTHETIC DRUGS

The relatively simple structure of melatonin (Fig. **1**) and the well known relationships between structure and function offer many possibilities for development of synthetic analogues [82]. Both, the ethyl amide side chain and the methoxy group of melatonin, are critical for the affinity to receptors. Thus, whereas the removal of the methoxy group reduces the affinity, the increase in the length of the alkyl substituent attached to the carbonyl group from CH_3 to C_3H_7 has the contrary effect [83]. The indole ring is not necessary for melatonin binding to the receptor and even its replacement by a naphthalene ring increases the affinity. The development of receptor ligands is based on modifications of different regions or the melatonin molecule, according to different strategies. The authors recommend an excellent review from Zlotos [83], which describes the main synthetic melatonin analogues grouped by their chemical structure: indoles, tetralines, napftalenes, indanes, benzoxazoles, benzofuranes, and others. From the melatonin derivates patented as melatoninergic drugs (see Table **1**) we have selected the two most successful patents.

THE TWO MOST PROMISING MELATONIN AGONISTS

Among the hundreds of patents for melatoninergic drugs, there are two that show the most interesting properties. One of them, ramelteon (US6034239) [84] has already been approved by the FDA and commercialized by Takeda Pharmaceuticals North America under the name of Rozerem™. The second, agomelatine (US5318994) [85] is completing the phase III trial and will be soon available from Servier and Novartis, under the commercial name of Valdoxan™.

RAMELTEON

Structure and Nomenclature

Ramelteon (TAK-375); systematic (IUPAC) name: [(*S*)-*N*-[2-(1,6,7,8-tetrahydro-2*H*-indeno-[5,4-*b*]furan-8-yl)ethyl]propionamide]; MW 259.34; formula: ($C_{16}H_{21}NO_2$); CAS number: (196597-26-9); PubChem: 208902; marketed as Rozerem™ by Takeda Pharmaceuticals North America.

Stage of Development of the Patent

Ramelteon (US6034239) was approved (July, 2005) by the FDA (Food and Drug Administration) for long-term use for treatment of sleep disturbances characterized by difficulty with sleep onset.

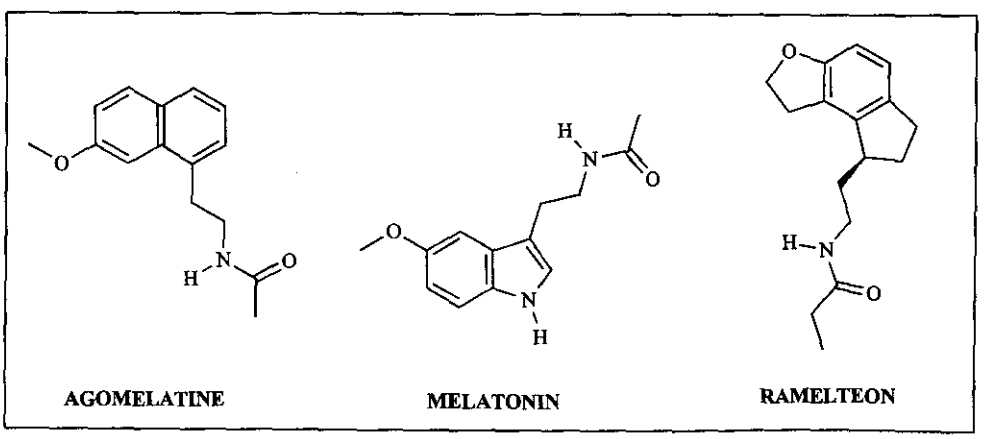

Fig. (1). Structure of melatonin and two melatonin receptor agonists.

Properties

Ramelteon is an indenofuran derivative of melatonin, the first in a new class of sleep agents that selectively binds to the MT_1 and MT_2 melatonin receptors [86] in the hypothalamic suprachiasmatic nucleus . Ramelteon inhibits forskolin-stimulated cAMP production in neonatal pituitary rats, with an IC_{50} value of 20.8 pM, indicating a strong MT_1 agonistic activity [83,86]. Its binding activity to the MT_2 receptors in Chinese hamster ovarian cells (Ki = 45 pM) is 3-fold lower than for the MT_1 receptors (Ki=14 pM) and 4-fold higher than the MT_2 affinity of melatonin (Ki=195 pM) [83]. The affinity of ramelteon for hamster brain MT_3 receptor binding sites is extremely weak (Ki=14 pM) compared to that of melatonin (Ki= 24nM) [83,87]. Ramelteon affinity for other ligand binding sites such as receptors for benzodiazepines, dopamine, opiates, ion channels and transporters, is not measurable [83,87,88]. Ramelteon also does not interfere with the activity of a number of selected enzymes in a standard panel [83].

Among the pharmacokinetic properties of ramelteon it is remarkable that its half life in humans is longer than that of melatonin [89,90] and is more potent than the indoleamine as a sleep promoter. Its chronobiotic properties have been demonstrated in rats. Like melatonin, ramelteon accelerates the re-entrainment of running wheel capabilities [91].

The major metabolite of ramelteon, designed M-II, is also active and has approximately one tenth and one fifth the binding affinity of the parent molecule for the human MT_1 and MT_2 receptors, respectively, and is 17 to 25-fold less potent than ramelteon on *in vitro* functional assays. Although the potency of M-II at MT_1 and MT_2 receptors is lower than the parent drug, after oral administration of ramelteon, M-II circulates at concentrations higher than those of ramelteon, resulting in a systemic exposure that is 20 to 100-fold greater than the parent compound [92]. M-II has weak affinity for the serotonin $5\text{-}HT_{2B}$ receptor, but no appreciable affinity for other receptors or enzymes and, similar to ramelteon, does not interfere with the activity of a number of endogenous enzymes [89].

Specific Applications

Insomnia is one of the most common causes of medical consulting. The pharmacological options available for the treatment of insomnia included benzodiazepines and non-benzodiazepine hypnotics, which have the potential to induce addiction, cause withdrawal symptoms, or trigger rebound insomnia [93]. Since melatonin had been demonstrated to have soporific effects [26,27], the design of a melatonin receptor agonist for treatment of insomnia was a reasonable hypothesis. Thus, ramelteon, a MT_1 and MT_2 melatonin receptor agonist, was conceived for the treatment of patients with insomnia and circadian rhythm troubles; especially for long-term treatment of sleep disturbances characterized by difficulty with sleep onset [88,92,93]. Compared with melatonin, ramelteon selectivity for MT_1 receptors is more than 1000-fold higher than for MT_2 ones, this data suggesting that ramelteon may target sleep onset more specifically than melatonin, and may be more suitable for insomnia characterized by difficulty in falling asleep [88,92]. Ramelteon promotes sleep by regulating the normal sleep-wake cycle rather than having generalized depressant effects on the central nervous system [88,92-95].

An excellent review of the randomized controlled trials published up to July 2006 examining the efficacy and tolerability of ramelteon is that of Borja and Daniel [92]. Ramelteon is currently the only non-scheduled prescription drug for the treatment of insomnia available in USA. It has not exhibited potential for abuse or dependency in laboratory tests [96], and the withdrawal and rebound insomnia typical with other GABA modulators is not present in ramelteon, which has few side effects [96]. In a double-blind crossover study on fourteen adults with histories of sedative abuse, ramelteon demonstrated no significant effects indicative of potential for abuse or motor and cognitive impairment at up to 20 times the recommended therapeutic dose [97]. In individuals with mild to moderate obstructive sleep apnea (OSA), ramelteon was well-tolerated and, as expected, because of its lack of depressant effects on the central nervous system, did not worsen OSA when administered to subjects with mild to moderate symptoms [98].

The clinical efficacy of ramelteon was evaluated in clinical trials which have been analyzed in the above mentioned review [92]. The trial involving a highest number of cases has been a multicentric randomized, double-blind, placebo-controlled 35-night outpatient trial carried out on 829 older people ($\geq$ 65 years) with chronic insomnia. The conclusion was that ramelteon, at doses of 4 or 8 mg, taken nightly for five weeks, significantly reduced sleep latency with no significant rebound insomnia or withdrawal effects [94]. In a previous trial, the efficacy of ramelteon was checked not in chronic insomniacs but in patients with transient insomnia (375 volunteer aged 35-60 years). Ramelteon at doses 16 or 24 mg administered a half hour before scheduled bedtime significantly decreased sleep latency compared with placebo [95]. Results from other trials [99-101] show similar results of decrease of sleep latency, although the effect of ramelteon in sleep efficiency and total sleep at night is less pronounced [92].

Doses

Although doses used in clinical trials ranged from 4 to 64 g, the recommended dosage for treatment of insomnia in adults is 8 mg orally administered within 30 minutes of bed time [92].

Side effects

Ramelteon is generally well tolerated in clinical trials, with most adverse events classified as mild or moderate [99]. These are: headache (7%), somnolence (5%), dizziness (5%), fatigue (4%) and nausea (3%) [99]. Following the administration of ramelteon, patients did not report rebound insomnia or withdrawal effects [99]. Compared with triazolan, ramelteon was found not to have significant effects on behavioural and cognitive performance or abuse potential [102]. Since CYP1A2 is the mayor isoenzyme responsible for hepatic metabolism of ramelteon, the inhibitors of this enzyme, such as amiodarone, ciprofloxacin and fluvoxamine, increase the effects of this drug [92].

Shortcomings

The absence, up to date, of published trials comparing ramelteon with other hypnotic agents (including native melatonin) make it difficult to assess its efficacy relative to other treatments of insomnia. The usual dosage of ramelteon approved for treatment of insomnia is 8 mg. However, except a report on a two-day treatment in insomniac subjects [99], there are no comparative studies of the efficiency and toxicity of this new melatoninergic drug with melatonin, active at a much lower dose (0.3 mg).

AGOMELATINE

Structure and Nomenclature

Agomelatine (S20098); systematic (IAUPAC) name: (N[2-(7-methoxy-1-naphthyl)ethyl]-acetamide); MW 243.3; formula $C_{15}H_{17}NO_2$, CAS number 138112-76-2; PubChem 82148; marketed as Valdoxan™ by Servier and Novartis.

Stage of Development of the Patent

Agomelatine (US5318994) is being developed by Servier and Novartis under the name of Valdoxan™. In March 2006, Novartis acquired the exclusive rights to further develop and market agomelatine in the United States. Servier retains the rights to develop and market the product in the rest of the world. Currently, agomelatine has completed the phase III clinical trials for treatment of symptoms of major depressive disorders, particularly anxiety,

sleep troubles and circadian disturbances, and a registration dossier for an indication in major depressive disorder has been recently submitted to the European Regulatory Agency [103].

Properties

The replacement of the indole scaffold of melatonin by the naphthalene ring system yields agomelatine [83], which is a potent agonist at melatonin receptors (MT_1 and MT_2) [104] and antagonist at serotonin-2C ($5\text{-}HT_{2C}$) and serotonin-2B ($5\text{-}HT_{2B}$) receptors [104]. The affinity of agomelatine for cloned human receptors MT_1 and MT_2 subtypes (K_i=6.15 x 10^{-11} and 2.68 x 10^{-10} M, respectively) is comparable to that of melatonin (K_i=8.52 x 10^{-11} and 2.63 x 10^{-10} M, respectively). On the other hand, agomelatine behaves as a competitive antagonist for the $5\text{-}HT_{2C}$ receptor with an IC_{50}=2.7 x 10^{-7} M on human cloned $5\text{-}HT_{2C}$ receptors [83,104,105].

Specific Applications

This drug was developed basically as once-daily treatment for major depressive disorder and its symptoms, particularly anxiety and sleep and circadian disturbances [103]. Its efficacy was assessed on experiments carried out on different animal (rats or mice) models of depression (forced swimming test, olfactory bulbectomy, etc) as well as in transgenic murine models [82]. In these different animal tests, agomelatine, at an average dose of 10 mg/Kg, was as effective as imipramine or fluoxetine (the two antidepressant molecules of reference) at identical doses.

The range of currently available antidepressant treatments includes selective serotonin reuptake inhibitors (SSRIs) and serotonin and noradrenaline reuptake inhibitors (SNRIs). Both are effective in the treatment of major depressive disorders and have a better safety and tolerability profile than the older tricyclic antidepressants and monoamine oxidase inhibitors. However, the SSRIs and the SNRI are associated with side-effects of their own, such as gastrointestinal problems, weight gain, sexual dysfunction, drowsiness and sedation, and discontinuation symptoms, all of which contribute to a reduction in compliance and may lead to premature cessation of treatment [106,107]. In addition, some concerns about increased suicide rates in patients receiving SSRIs have recently been described [108] and there have been also concerns about the toxicity of SNRIs in overdose [106]. The antidepressant action of agomelatine is not mediated through the same mechanisms as selective serotonin reuptake inhibitors or tricyclics [109].

The current therapeutic strategies for major depressive disorders are, therefore, lacking in agents that combine antidepressant efficacy with a favourable tolerability profile. Agomelatine is a new antidepressant with an innovative pharmacological profile: it is the first melatoninergic antidepressant [82]. Several large, multicenter, multinational, placebo controlled studies and several double-blind, placebo-controlled trials of agomelatine have demonstrated that it is a clinically effective and well tolerated antidepressant in acute trials [107,110,111]. The antidepressant efficacy of agomelatine has been demonstrated in comparison with placebo at various levels of severity of depression. Indeed, it seems that the treatment effect of agomelatine tends to increase with the severity of the depression [107]. The results also indicate an early improvement of depressive symptoms and good response rates. Agomelatine has also been shown to have a comparable efficacy profile to the SSRIs, such as paroxetine, and the SNRIs, such as venlafaxine [112], but lacks typical antidepressant side effects. In rats, agomelatine potentiates the anxiolytic effects of diazepam [113].

The novel mode of action of agomelatine, involving melatoniergic agonism and $5\text{-}HT_{2C}$ and $5\text{-}HT_{2B}$ antagonism, has interesting consequences in terms of clinical benefits. The low rate of adverse events observed with this new drug contrasts with the SSRIs and SNRIs, for which the release of serotonin and noradrenaline can cause gastrointestinal, central nervous system and cardiovascular side-effects [107,109,110]. Furthermore, agomelatine prevents treatment-related sexual dysfunction and has positive effects on the relief of sleep disturbances in depression [110]. Indeed, agomelatine is the only antidepressant to have a specific action on circadian rhythms, which are often imbalanced in depressed patients [82], being capable of phase-shifting circadian rhythms in older men [114]. Furthermore, sleep electroencephalographic changes consistent with desirable sleep architecture improvements, as well as improved subjective sleep quality within the first week of administration of agomelatine, are accompanied by an improvement in daytime alertness [115].

In addition, a placebo-controlled, double-blind study comparing agomelatine with paroxetine showed that, after one week of treatment discontinuation, no signs of discontinuation symptoms were seen in the agomelatine group compared to significant discontinuation symptoms in the paroxetine group [112].

Doses

The antidepressant efficacy of agomelatine has been shown at a standard dose of 25 mg, once daily in the evening, in a dose-ranging study performed in major depressive disorder [116].

Side-Effects

Agomelatine, whatever the dose, has been shown to have a remarkable tolerability and safety profile, showing good acceptability with a side-effects profile close to that of the placebo [107,116]. The most common adverse events are reported to be headache, nausea and fatigue. The number of events remains very low and generally resolved within two weeks. The rates of discontinuation due to adverse events are low (8%) and similar to those for the placebo [107,116].

Advantages

The clinical advantage of agomelatine derives from its mechanism of action which gives this molecule antidepressant properties together with the ability to regulate sleep-wake rhythm without affecting daytime vigilance.

CURRENT & FUTURE DEVELOPMENTS

Ramelteon and agomelatine are two melatoninergic drugs which share its affinity for MT_1 and MT_2 melatonin receptors and consequently have some hypnotic and chronobiotic actions in common. Only the antagonism at $5\text{-}HT_{2C}$ receptors seems to be specific to agomelatine [105]. Both drugs have been patented as "specialized" for sleep disorders (ramelteon) or as antidepressant (agomelatine). Sleep dysfunction and psychiatric disorders are commonly associated. When insomnia is comorbid with depression, agomelatine therapy could be preferred whereas chronic insomnia in nonpsychiatric patients could be treated with ramelteon. This drug has not yet been studied in psychiatric patients [117]. Another substance developed for treatment of sleep troubles is LY 156735 (developed by Eli Lilly; USA patent 20050164987A1) [118], a β-substituted analogue of melatonin with higher hypnotic potency and a better pharmacokinetic profile than the native indoleamine [119]. Like the other melatoninergic drugs, it has a chronobiotic effect: it improves the adaptation to a phase-advance in the light-dark cycle [120], yet not lowering the core body temperature when administered during daytime [28]. LY 156735 is in the early stages of clinical studies.

No comparative studies have been carried out on the efficacy and side effects of both drugs for its common actions. Probably they are likely to be made in the future, especially after the commercialization of agomelatine. From the knowledge of the distribution and properties of MT_1 and MT_2 melatonin receptors it seems logical to expect that the potential peripheral effects (cardiovascular effects, oncostatic actions, etc) of ramelteon and agomelatine will be investigated by further studies

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