

Melatonin and Sleep

Nava Zisapel*

Department of Neurobiology, Tel Aviv University, Tel Aviv 69978, and Neurim Pharmaceuticals, Israel

Abstract: Melatonin is produced over the night in a process driven by the circadian clock that resides in the suprachiasmatic nuclei (SCN), and by ambient light. It is a time cue to tune the sleep-wake and other circadian rhythms with the day-night cycles and thus useful in circadian rhythm sleep disorders. In addition melatonin induces fatigue and concurrent sleep-like changes in brain activation patterns in humans. With age SCN activity and melatonin production capacity decline thus depriving the brain of an important regulator of the body's circadian rhythms. Melatonin substitution therapy with a prolonged-release formulation that mimics the physiological release profile of the hormone (PRM) may effectively treat insomnia in older patients. Large-scale, randomized, placebo-controlled clinical trials in insomnia patients aged 55 years and older indicated that PRM given for 3 weeks or more improved sleep quality, facilitated sleep onset and improved morning alertness, daytime functioning and quality of life and reduced hypnotic drug usage. Preliminary results of a 6-months long term placebo controlled study announced at the 2009 meeting of the Associated Professional Sleep Societies indicated that PRM efficacy was sustained. There were no main safety concerns and in particular, none of the risks associated with hypnotic drugs use (e.g. memory and cognitive impairments, falls and accidents, residual daytime or 'hangover' effects, rebound insomnia and withdrawal symptoms). There is clinical data showing efficacy of PRM in circadian rhythm sleep disorders in totally blind individuals and children with neurodevelopmental disabilities. PRM thus represents a new therapeutic principle for treating sleep disorders.

Keywords: Melatonin, sleep, memory, insomnia, circadian, disorders.

INTRODUCTION

The biological clock is essential for our daily well-being. It controls the timing of our sleep and prepares us for the upcoming period of activity by an anticipatory rise in heart rate, glucose and cortisol. At the same time the 'hormone of the darkness', melatonin, decreases. Thus, the time-of-day message penetrates into all tissues and influences the organs of the body [1]. The 24-hour rhythm is regulated by an intrinsic body clock residing in the suprachiasmatic nucleus (SCN) within the brain [2]. While the master clock resides in the brain SCN, a functional clock appears to reside in most cells of the body. In all these tissues, at least some output genes are controlled at the transcriptional level directly by clock proteins; others appear to be regulated by cascades of circadian transcription factors or neuronal stimuli [3]. The individual period of the endogenous clock is usually 24 hours and is normally entrained by the ambient light to match the environmental light/dark cycle. When humans are isolated from all time cues, their circadian rhythms tend to free-run, or cycle at the endogenous rhythm, which in individual and population studies appears to be between 24 and 25 hours [4,5] Light is perceived by the retina through a special retino-hypothalamic tract, which is distinct from the optic nerve. In the case of no light perception to the SCN, mostly seen in totally blind individuals with no eyes present,

this entrainment does not occur, resulting in their period being unmatched to the daily 24-hour rhythm.

The sleep-wake cycle is the most overt circadian rhythm. Sleep, an essential state marked by lessened consciousness, lessened movement of the skeletal muscles, and slowed-down metabolism, is an orchestrated neurochemical process involving sleep-promoting and arousal centers in the brain [6]. From animal studies it is known that wakefulness is promoted by brainstem and hypothalamic neurons; each of these arousal networks is capable of increasing wakefulness, but coordinated activity in all these pathways is required for complete alertness and cortical activation[7]. Among the major sleep functions are the restorative effect on body and mind, resulting in a sense of well being and daytime vigilance, and the facilitation of the plastic cerebral changes that underlie learning and consolidation of memory[8].

Sleep propensity depends on the amount of sleep deprivation (homeostatic component) and on the circadian clock phase. The interaction between these processes forms the basis of consolidate bouts of sleep at night and wakefulness throughout the day [9]. The timing of sleep and wakefulness as well as other physiological and behavioral rhythms is tuned by the SCN[3]. A close interaction exists between circadian rhythms such as body temperature, blood pressure, immune and hormonal rhythms and the sleep-wake cycle leading to optimization of the internal temporal order. Indeed 66% of totally blind persons with no light perception had complained of mild to severe sleep disorders [10] due to this circadian rhythm disorder. Among other free running rhythms in totally blind individuals are the rhythms of production of melatonin, a hormone of the pineal gland that

*Address correspondence to this author at the Department of Neurobiochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel; Tel: +972-3-6409611; Fax: +972-3-6407643; E-mail: navazis@post.tau.ac.il

mediates the timing of sleep and of cortisol, a hormone that promotes arousal state [11,12]. When the free-running sleep propensity rhythm passes out of phase with the desired time for sleep, night-time insomnia and associated occupational and social difficulties result [12,13].

Melatonin (N-acetyl-5-methoxytryptamine) is the primary hormone produced by the pineal gland. Endogenous synthesis and secretion of melatonin are controlled by the SCN, and occur during the dark (quiescent) phase of the SCN electrical activity [2,14]. Melatonin secretion is halted during the light phase and increases in the evening to reach a peak in the middle of the night [15]. Recent data obtained from sheep and humans indicate that melatonin is released directly into the cerebrospinal fluid (CSF), particularly in the third ventricle. As a consequence, melatonin is present in much higher concentrations (100 times) in the CSF than in blood [16-18]. Light has two effects on pineal melatonin production: light-dark cycles synchronize the rhythm (due to entrainment of the circadian pacemaker) and acute light exposure at night rapidly reduces serotonin N-acetyltransferase (SNAT) activity and thus melatonin production is stopped [19] and melatonin levels in blood and CSF rapidly decrease [20]. The dim light melatonin onset (DLMO), which is the initial surge in melatonin release in the early part of the night under low light conditions, is a consistent and reliable measure of the intrinsic circadian phase [20]. Melatonin undergoes first pass hepatic metabolism (half-life in the human serum is less than 1 hr.) [21] and over 80% is excreted exclusively in the urine as 6-sulfatoxymelatonin (6-SMT) [22]. Therefore, the mean urinary excretion profile for 6-SMT reflects blood levels; the amount of melatonin produced endogenously may be reliably based upon cumulative urinary excretion of 6-SMT [22]. This review will focus on recent evidence on the role of melatonin in sleep regulation, its potential use in the treatment of circadian rhythm sleep disorders (particularly non-24 hour sleep wake cycles in the blind, delayed sleep phase syndrome and sleep disorders in neurodevelopmentally challenged children) and the recent developments in the clinical use of melatonin and other melatonin receptors agonists in insomnia.

MELATONIN AS A TIME CUE AND A SLEEP REGULATOR

Melatonin in Circadian Clock Regulation

Melatonin can relay time-of-day information (signal of darkness) to various organs, including the SCN itself. The phase-shifting effects of melatonin are essentially opposite to those of light [23]. Consistent with a synchronizing effect on the biological clock the MT1 and MT2 melatonin receptors are present in the SCN [24]. Thus melatonin is able to phase shift the endogenous circadian clock and, in the absence of light, to entrain the sleep-wake and neuroendocrine rhythms [14,22]. Exogenous melatonin can entrain or phase shift the endogenous melatonin cycle *in vivo* [22,25]. A phase response curve for this effect has been reported [25] which appears to be 180° out-of-phase with that effected by light exposure. Contemporaneous melatonin administration modifies the capability of light to induce circadian phase shifts [26]. The synchronization by melatonin of circadian rhythms may be mediated by the SCN [27]. Melatonin can

acutely suppresses electrical activity of the SCN during daytime [24], affect gene expression in the SCN and phase delay or advance the SCN phase on the forthcoming days (depending on whether it is given at the beginning or end of the light phase, respectively) [25]. It is yet unknown whether the acute suppression of SCN electrical activity by melatonin is involved in phase resetting because application of melatonin to SCN from knockout mice *in vitro* did cause phase shift of the electrical activity without acutely suppressing the firing rate of SCN neurons [24].

Melatonin in Sleep Regulation

There is a considerable body of evidence that melatonin regulates sleep in humans. The circadian rhythm in synthesis and secretion of melatonin is closely associated with the sleep rhythm in both normal and blind subjects. Thus, melatonin onset would typically start 14 hours after spontaneous wakeup (i.e. CT14 which corresponds to 21:00 in a sighted person who wakes up at 07:00 hours). Electrophysiological recordings demonstrated that the timing of the steepest increase in nocturnal sleepiness ("sleep gate") was significantly correlated with the rise of urinary 6-sulfatoxymelatonin excretion [28]. Endogenous melatonin levels are correlated with daytime napping in the blind [29,30]. In delayed sleep phase syndrome patients, the melatonin rhythm is delayed and there is a significant positive correlation between sleep and melatonin phase markers [31]. Aging, presence of certain diseases (e.g. primary degeneration of the autonomic nervous system and diabetic neuropathy, some types of neoplasms, Alzheimer's disease), and certain drugs (e.g. b-blockers, Clonidine, naloxone and non-steroidal anti-inflammatory drugs), abolish the nocturnal production of melatonin and are associated with impaired sleep [32]. Administration of melatonin during daytime (when its endogenous levels are minimal) results in induction of fatigue and sleepiness in humans [33-35]. The sleep-promoting effects of melatonin become significant about 2 hours after intake similar to the physiological sequence at night [36]. The size of the sleep promoting effect of melatonin is similar to that of a potent hypnotic, zopiclone [35]. When given at daytime the architecture of sleep induced by melatonin resembles to some extent the contribution of the endogenous circadian pacemaker to the spectral composition of the sleep EEG at night [37]. In preparation for sleep, melatonin acts to induce heat loss, reduce arousal and related brain activation and delay cortisol production [38,39].

It has been suggested that melatonin participates in the regulation of the sleep-wake cycle by inhibiting the wakefulness generating system in the hypothalamus [28]. The brain networks affected by melatonin are now starting to unravel, mostly due to molecular and functional brain imaging studies [40,41]. Melatonin, but not placebo, reduced task-related activity in the rostro-medial aspect of the occipital cortex during a visual-search task and in the auditory cortex during a music task. These effects correlated with subjective measurements of fatigue. In addition, melatonin enhanced the activation in the left parahippocampus in an autobiographic memory task. Results demonstrate that melatonin modulates brain activity in a manner resembling actual sleep although subjects are fully awake. Furthermore, the fatigue inducing effect of melatonin

on brain activity is essentially different from that of sleep deprivation thus revealing differences between fatigues related to the circadian sleep regulation as opposed to increased homeostatic sleep need [40]. These studies indicated that melatonin modulates brain activity patterns in awake subjects in a manner resembling actual sleep and are essentially different from those seen after sleep deprivation [40]. Furthermore, some of these patterns resembled those in subjects tested in the late evening, when melatonin is produced endogenously [41,42]. Thus, activation in the rostro-medial and lateral aspects of the occipital cortex and the thalamus diminished at 22:00 h. Activation in the right parietal cortex increased at night and correlated with individual fatigue levels, whereas exogenous melatonin given at 22:00 h reduced activation in this area. The right dorsolateral prefrontal cortex, an area considered to reflect homeostatic sleep debt, demonstrates increased activation at 22:00 h which is surprisingly correlated with endogenous melatonin. These results demonstrate and partially differentiate circadian effects (whether mediated by melatonin or not) and homeostatic sleep debt modulation of human brain activity associated with everyday fatigue at night [43]. Hence, melatonin may play an important role in priming sleep-associated brain activation patterns in anticipation of sleep.

Brain imaging studies also demonstrate that memory related activation in the hippocampus and parahippocampus are affected by time of day and melatonin in a differential manner and may implicate the circadian clock and melatonin in human memory processing during the sleep [41]. activation in the left hippocampus at 22:00 hr is significantly reduced compared with afternoon hours compatible with diurnal variation in hippocampal activity. Exogenous melatonin further reduced activation in this region, only in subjects who already crossed the melatonin onset phase at this hour and in correlation with endogenous melatonin levels. As such an effect was not demonstrated with afternoon administration of melatonin, a time depended effect is suggested. Contrary, activation in the left parahippocampus at 22:00 hr was higher in subjects that crossed the melatonin onset phase. Parahippocampal activation correlated with individual endogenous melatonin levels and was not further affected by exogenous melatonin. These results demonstrate that memory related activation in the hippocampus and para-hippocampus are affected by time of day and melatonin in a differential manner and may implicate the circadian clock and melatonin in human memory processing during the night [41]. Further studies demonstrate that nap and melatonin given in the afternoon act in a similar manner on verbal memory processing in the hippocampus [42]. The effect of a 2-hr mid-day nap versus an equal amount of wakefulness on a verbal memory task (unrelated word pair association) was compared to the effect of melatonin versus placebo (both conditions without nap) on a similar task. Following a nap relative to wakefulness, successful retrieval-related activation in the parahippocampus is decreased. A similar but smaller decrease is seen in wakefulness with melatonin but not placebo. In parallel, an improvement in verbal memory recall was found after a nap compared with wakefulness but not with melatonin during wakefulness compared with placebo. These findings demonstrate effects of melatonin that

resemble those of sleep on verbal memory processing in the hippocampus thus suggesting that melatonin, like sleep, can initiate offline plastic changes underlying memory consolidation; they also suggest that concomitant rest without interferences is necessary for enhanced performance [42].

Melatonin acts via its own receptors (MT1, MT2), which are members of the G protein-linked receptor family [44]. In addition, lower affinity melatonin binding sites have been described [45]. A melatonin binding site termed MT3 has recently been identified as quinone reductase 2 [44] but its physiological role has not been elucidated.

Immunohistochemical evidence indicates the presence of melatonin receptors (both MT1 and MT2) in the human hippocampus [46]. Melatonin exerts opposite effects on GABA-A receptors in specific brain areas, potentiating the GABA-A receptors in the SCN via MT1 receptors, while inhibiting it in the hippocampus via MT2 receptors [47]. The brain imaging data agree well with the presence of melatonin receptors of both MT1 and MT2 subtypes and the low affinity melatonin binding sites in the SCN and hippocampus. The GABA-A effects of melatonin at the SCN may explain some of its sleep promoting effects, whereas the anti GABA-ergic effect explains why melatonin would not cause amnesia. Importantly, melatonin is not sedating: in nocturnally active animals melatonin production is associated with wake, not sleep, periods [48] and in humans its sleep-promoting effects become significant about 2 hours after intake similar to the physiological sequence at night [36]. Because melatonin does not increase the amount of slow-wave sleep [37], which is considered a marker of the homeostatic sleep drive [49], it is reasonable to assume that the sleep promoting effects of melatonin are mostly ascribed to the circadian component of sleep regulation.

CLINICAL USE OF MELATONIN

Treatment of Circadian Rhythms Sleep Disorders

A circadian rhythm sleep disorder is a chronic condition in which an individual's circadian rhythm of sleep and wakefulness is out of phase with the conventional environmental patterns. Several circadian sleep disorders have been classified: delayed sleep phase syndrome (DSPS), advanced sleep phase syndrome (ASPS), irregular sleep-wake patterns and non 24-h sleep-wake syndrome in blind and sighted persons. In all of these the misalignment with the environmental day-night cycle is persistent, or periodic, and adjustment never seems to occur, or at best is most difficult. The pathophysiological process of chronic and periodic circadian rhythm sleep disorders is presumed to be associated with abnormalities occurring in the pacemakers, their coupling to the external cues, or their downstream synchronizing mechanisms. Two additional circadian rhythm sleep problems, i.e. those associated with jet lag and shift work, are due to temporary (circumstantial) misalignment of the circadian sleep-wake rhythm with environmental patterns.

Presently melatonin appears the only effective drug for treating circadian rhythms sleep-wake disorders [14,33]. The ability of melatonin to increase sleep propensity and synchronize the internal clock make it a reasonable

therapeutic candidate for facilitation of sleep and treatment of circadian rhythm sleep disorders. Apart from its sleep-anticipating effect, exogenous melatonin may affect sleep through its phase-resetting action on the biological clock. In delayed sleep phase syndrome (DSPS) the endogenous melatonin rhythms are delayed compared with those in normal individuals [31]. There is compelling evidence indicating that melatonin effectively adjusts sleep time in subjects with DSPS [22]. In these studies melatonin was given orally, at a 5 mg dose once daily for 28-30 days. In one study melatonin was administered to all patients in the evening (22:00 h) [50] whereas in the other studies in each patient melatonin was administered 5 hours before his own melatonin onset time (measured in dim light) [51,52]. In both protocols melatonin significantly advanced sleep onset and wake times of subjects with DSPS to earlier hours compared to placebo [50-52] and in the latter it was proven to improve vigilance and cognitive functions in DSPS patients [52]. It is yet to be found whether the effects of melatonin in DSPS reflect a phase advance of other rhythms besides the sleep-wake cycle.

When human subjects are isolated from all time cues, their circadian rhythms tend to "free run", namely cycle at the endogenous rhythm which in different individuals is close to 24 h and in population studies was on average between 24-25 h [4]. Subjects with no conscious light perception have a higher occurrence and more severe sleep disorders than those with some degree of light perception because they tend to free-run [13,53]. When they free-run, the sleep/wake cycle is pushed toward 24 h cycle due to social cues and the relationship between sleep and other bodily rhythms which keep free-running (e.g. melatonin, cortisol, body temperature) is constantly changing [13,53]. Non-24-hour (or free running) sleep-wake disorder is characterized by periods of good and bad sleep at which the circadian system periodically attains a normal phase position with the behaviorally imposed 24 h sleep wake cycle. Melatonin at a daily dose of 3-5 mg has been successfully utilized for adjusting the sleep-wake cycle in the blind, where the light-dark cycles are ineffective [11,54]. Presently melatonin appears the only effective drug for blind people with Non-24-hour sleep-wake disorder [11,23,54]. Furthermore, exogenous melatonin administration synchronized neuroendocrine rhythms to the day-night cycles in totally blind subjects with non-24h sleep wake disorder [11,54]. Melatonin administration appears to be the most promising approach, with long-term benefit, for circadian rhythm sleep disorders because it simultaneously treats the sleep and the wake state problems and in addition, synchronizes other bodily rhythms so as to maintain the body's internal temporal order and prevent internal desynchronization. The effective doses reported are 0.5-10 mg without a clear dose-response relationship [11,23,54-57]. This mainly reflects the large inter-individual variability in melatonin bioavailability which is well known in the published literature and are attributed to differences in first-pass metabolism [58-60]. This is also the case with endogenous melatonin levels which may vary 20 fold among healthy adults [61]. Further well controlled clinical trials are needed to establish the efficacy and safety of well characterized pharmaceutical preparations of melatonin to

gain regulatory approvals for the treatment of these disorders.

During the first few days after traveling across several time zones, most travelers complain that they are suffering from "jet lag". Although the term "jet lag" refers to disturbances in a variety of symptoms, jet-lagged travelers mostly complain of loss of sleep and of its consequences (e.g., diurnal sleepiness, depressed mood, decreased efficiency, premature awakening, headaches, reduced cognitive skills, poor psychomotor co-ordination, moodiness or general malaise). The jet lag syndrome is largely due to the inability of the circadian system to resynchronize rapidly after sudden shifts in the timing of the environmental light-dark cycles. A spontaneous phase shift toward the new timing of the light-dark cycle gradually resolves the problem. There is evidence for improvement of sleep and self-reported jet lag symptoms by melatonin (3-5 mg) in subjects with jet lag [14,22]. Both melatonin (1.5 mg in a surge sustained formulation) and the investigational melatonin receptors agonist tasimelteon (10-100 mg), given to healthy volunteers in a 5-h advance of sleep-wake schedule reduced sleep latency and increased sleep efficiency compared with placebo and shifted the endogenous plasma melatonin rhythm to an earlier hour [62,63]. It seems, therefore, that melatonin receptor agonists may have some beneficial effects on the symptoms of jet lag, and circadian phase position, although the optimal dose, timing of ingestion and safety of such treatments have yet to be determined.

Treatment of Sleep Disorders in Children With Neurodevelopmental Disabilities

Several published studies have looked at the use of melatonin to treat serious sleep disorders among children who are blind, have autism spectrum conditions, attention-deficit/hyperactivity disorder (ADHD), cerebral palsy, or foetal alcohol syndrome. In most of these disorders, endogenous production of melatonin is impaired or its circadian rhythmicity is abnormal. In a review article on the therapeutic uses of melatonin, 24 paediatric studies from 1991-1998 were summarized [64]. Additional studies were published later on [65-72]. There is strong consensus among these researchers that exogenous melatonin is beneficial for treating chronic sleep-wake cycle disorders of children who have neurodevelopmental and neuropsychiatric difficulties. None of the authors noted significant adverse side-effects. In most instances, fast-release (FR) melatonin preparations that are available as food supplements in the USA were used rather than prolonged release melatonin or controlled-release (CR) forms of melatonin, possibly because the PRM and CR melatonin became available only later.

Several published studies performed in Canada [64,73] have studied PRM (Circadin[®] Neurim Pharmaceuticals; authorized in the EU and other countries for the treatment of insomnia in patients aged 55 years and older) for sleep wake cycle disturbances in children with neurodevelopmental disabilities. The average final PRM dose in the children was 5.7 mg (2-12 mg). The studies showed that the FR melatonin was most effective when there was only delayed sleep onset, but PRM was more useful for sleep maintenance. Other studies [67-69] reported on the use of PRM in the treatment

of children with Smith-Magenis syndrome (SMS). SMS, which is a clinically recognizable contiguous gene syndrome, caused by interstitial deletion of chromosome 17p11.2 is associated with major sleep disturbances ascribed to a phase shift of their circadian rhythm of melatonin with a paradoxical diurnal secretion of the hormone. Morning beta-blockers (in a single morning dose to suppress the endogenous melatonin production during the day) and evening PRM (6mg/day) reinstated a normally timed melatonin circadian rhythm, improved daytime behavior and restored normal sleep habits, resulting in a greatly improved quality of life for both SMS patients and their families. This pattern was maintained for 6 months to 3 years with no signs of tolerance or treatment related adverse events.

Additional studies reporting use of an experimental controlled release melatonin formulations (CR-melatonin) have been published recently [70,71] reporting on long term effectiveness of CR-melatonin therapy at a maximal dose of 4 mg in children under 4 year and 6 mg over this age in improving sleep in children with neurodevelopmental disabilities and treatment-resistant circadian rhythm sleep disorders (CRSD) and in autistic children with chronic sleep disorders. Adverse reaction to melatonin therapy and development of tolerance were not evident. At the end of the study, the parental comments regarding the effectiveness of long-term melatonin therapy were highly positive. It was concluded that controlled-release melatonin may provide an effective and well-tolerated treatment for autistic children with chronic sleep disorders. Further well controlled clinical trials, are therefore needed to establish the efficacy and safety and gain regulatory approvals of well characterized melatonin formulations for the treatment of sleep disorders in children with neurodevelopmental and neuropsychiatric difficulties or blindness.

Treatment of Insomnia

Insomnia is defined in the fourth revision of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV) as well as in the tenth revision of the International Classification of Diseases (ICD-10). Key elements for the definition of Primary Insomnia that are described in DSM-IV as well as in ICD-10 include insufficient sleep quantity and/or non-restorative sleep (inadequate sleep quality) over a certain period as well as negative effects on subsequent day-time functioning. The sleep disorder persists over a month and is associated with significant daytime distress. No satisfactory correspondence has ever been established between the common complaint of sleep quality and sleep laboratory measurements. Recent findings suggest that patients suffering from sleep quality problems have a lighter sleep. Hence complaints on the quality of sleep remain essentially subjective. Similarly, estimates of impaired daytime functioning remain essentially subjective. Epidemiological studies throughout the world (USA, Canada, Europe, Australia, Asia, Russia) have found that at least 10% of the total population in these countries rates their sleep problem as serious. Women were 1.5 times more likely than men to have a sleep problem.

The elderly are particularly liable to suffer from insomnia. Whereas the prevalence of sleep problems in 18-35 year olds is about 10% -15%, it rises to 30% in the over-

65 year olds [74,75]. This is reflected in the usage of hypnotic medication with roughly 10-15% of the elderly population in the UK, France and Germany. Furthermore, such usage tends to be long term and to give rise to complications such as increased risk for falls, amnesia, driving accidents, dependency and tolerance and serious withdrawal syndrome when they attempt to discontinue the hypnotic drugs. Hypnotics address insomnia related to quantitative sleep problems (increased sleep latency, decreased total sleep time) but none of these treat sleep quality or improve the restorative value of sleep. The targets of the therapeutic process are now changing from improving symptoms such as difficulties in initiating or maintaining sleep to re-establishing restorative sleep and improving daytime functioning.

The production of melatonin decreases with age and is low in elderly insomnia patients. Older subjects show an increased lag from sunset to the onset of the melatonin pulse and to the melatonin pulse peak so that the pulse starts later, peaks lower and later and ends sooner [76]. 6-SMT excretion in insomniacs aged > 55 years is about one third of that in healthy adults aged 20-35 years and about half of that in healthy adults > 55 years without sleep complaints [77,78]. A low melatonin amplitude was found to be associated with insomnia complaints in adult and elderly subjects [77,79-81] and intensive care patients [82]. Endogenous melatonin levels decrease with age presumably due to age related calcification of the pineal gland [77-79,83-87]. These age-related changes are associated with sleep disturbances and most probably reflect age-related diminution in output of the circadian pacemaker [88]. Actually, functional disturbances of SCN circadian activity may start already around the age of 50, reflected in a disruption in the circadian rhythmicity of the AVP-expressing neurons in the SCN [18,89] while the number of AVP-expressing neurons is still intact [90].

In addition, the sleep of the elderly is characterized by increased fragmentation and maintenance problems which may not be linked to disturbances in the circadian pacemaker. No systematic relationship between melatonin production and objectively measured sleep laboratory parameters has been demonstrated so far [91-93]. However, individual melatonin levels, as well documented in the published literature, may vary 20 fold among healthy adults [61] and this variability may mask such relationship. Melatonin deficiency should thus be viewed as relative (e.g. a decline from when young to when old) rather than as absolute [94]. Indeed, a recent report shows that the degree of pineal calcification, which better reflects the individual loss in melatonin production capacity, is better correlated to poor sleep in insomnia patients than the actual melatonin levels [87]. Recent finding also indicate that the degree of pineal calcification rather than actual melatonin levels corresponds to with poor quality of sleep in insomnia patients [79,87]. The decline in melatonin may contribute to the common complaint of poor sleep quality seen amongst elderly people [77-79].

Taking into consideration the preeminence of the circadian clock and melatonin in timing sleep, it is likely that insomnia is also linked to melatonin abnormalities. The "melatonin replacement" hypothesis states that well-evidenced age-related decline in melatonin production

contributes to poor sleep quality and thus, melatonin therapy may replenish the deficiency in the endogenous sleep-regulating hormone and improve sleep.

Early studies have shown Improvement of sleep in elderly insomnia patients by very high doses of melatonin. [95] whereas the first attempts to study the efficacy of low doses of melatonin (acute administration; 1 and 5 mg regular release at night) failed to show significant effects on the polysomnography-recorded sleep in younger insomnia patients [96]. Later on, the majority of studies showing efficacy of lower doses (0.3-5 mg per os.) of melatonin in insomnia were performed with elderly insomniacs or patients in whom melatonin production was suppressed. In most of these studies melatonin therapy resulted in shortening of sleep latency compared to placebo. [92,97-99]. A meta-analysis of published studies on the efficacy of exogenous melatonin in the management of primary insomnia indicated a decrease in sleep onset latency (weighted mean difference from placebo) by -7.2 minutes (95% confidence interval [CI] -12.0, -2.4; n=12) [100]. Another meta analysis of 15 studies that enrolled healthy subjects or people with no relevant medical condition other than insomnia, indicated that melatonin reduced sleep onset latency by 3.9 min (95% CI -2.5, -5.4); and increased sleep efficiency by 3.1% (95% CI 0.7, 5.5) and sleep duration by 13.7 min (95% CI 3.1, 24.3). [101]. Further studies indicated that melatonin is beneficial for improving the quality of life in the elderly [102] which agrees well with the importance of good sleep to quality of well being [103].

Melatonin is short-lived in humans and its half-life in plasma is only 40-50 min. Following oral administration, melatonin is rapidly absorbed with peak plasma levels occurring between 20 minutes and 2 hours depending on dose [104]. To cover the whole night period, high doses or repeated administration of FR melatonin would thus be needed. Down regulation and desensitization of melatonin receptors by high melatonin concentrations have been demonstrated experimentally [105-107]. Although there are no studies showing that the risk of down regulation exists in humans, a therapeutic formulation of the hormone that would reproduce the normal nocturnal increase in melatonin concentrations is deemed more suitable for melatonin substitution therapy than high dose FR-melatonin.

PRM (Circadin[®]) was designed to provide following ingestion in the evening (ca. 22-23 pm) a melatonin profile in the blood more closely related to the normal physiological release pattern (Fig. 1). A pharmacokinetic study looking at melatonin blood levels in healthy males after ingestion of the 2 mg dose at 9am shows peak concentration of melatonin in the blood occurring 2.6 hours and persisting over 3.5 hours after ingestion and declining towards the morning. Ingestion of a 2 mg PRM yields about 6-8 fold higher blood levels than the endogenous levels at night [108] which would correspond to brain melatonin levels comparable to those normally present in the brain at night.

A number of exploratory double blind randomized, placebo-controlled studies were performed early in the clinical development of PRM using objective measurement tools (actigraphy) in insomnia patients aged 55 years and older [97,98]. In these subjects the peak excretion of the main melatonin metabolite 6-SMT during the night was

lower and/or delayed in comparison with non-insomniac elderly people. One study (two-way crossover) reported that sleep efficiency was significantly greater after PRM than after placebo and wake time after sleep onset was significantly shorter. Sleep latency showed a trend to benefit and total sleep time was not affected. The only adverse effects reported were two cases of pruritus, one during melatonin and one during placebo treatment; both resolved spontaneously. Another, three-way crossover double blind randomized study, reported on the effects of 2 mg FR-melatonin, PRM and placebo on sleep. Treatment for 1 week with 2 mg PRM improved sleep efficiency while the FR-melatonin treatment improved sleep initiation compared to placebo. Sleep maintenance and initiation were further improved following two-months treatment with nightly PRM 1 mg dose, and declined after treatment was stopped, suggesting that tolerance had not developed. The results of these proof-of-concept studies encouraged the development of PRM to seek regulatory pharmaceutical drug approval.

The efficacy and safety of PRM 2 mg tablets in inducing and maintaining sleep were investigated in a sleep laboratory trial. This was a double blind, placebo controlled study in 24 male and 16 female insomniacs aged 55 years and above [109]. Patients were treated nightly single-blind with placebo (2 weeks), randomized double-blind to PRM or placebo (3 weeks) followed by withdrawal period (3 weeks). Sleep was assessed by polysomnography, all-night sleep electroencephalography spectral analysis, and questionnaires. Psychomotor performance was assessed by the Leeds psychomotor test battery. By the end of the double blind treatment, the PRM group had significantly shorter sleep onset latency compared with placebo (-9 minutes; $p=0.02$) and scored significantly better in psychomotor tests without negative effects on sleep structure and architecture. Half of the patients reported substantial improvement in sleep quality at home with PRM compared to 15% with placebo ($P=0.018$). No rebound effects on sleep or daytime vigilance were observed during withdrawal [109].

Other objective assessments of the sleep inducing effects of PRM as compared to those of conventional hypnotics were performed in randomized controlled studies at Defence Research and Development Canada. In a repeated measures, placebo-controlled protocol, 30 aircrew flew 3 transatlantic missions over which they took each of the 3 medications (placebo, PR- melatonin 2 mg, or zopiclone 5 mg) at an early body clock time (17:00) and completed a sleep questionnaire on arising from their medicated sleep. The results of the actigraphic data showed that relative to placebo, aircrew on PRM and zopiclone fell asleep more quickly, slept more, had fewer awakenings after sleep onset, and spent less time awake after sleep onset. The results of the questionnaire data show that relative to placebo, aircrew on PRM and zopiclone experienced less difficulty getting to sleep, had fewer awakenings, less difficulty returning to sleep after awakening, and reported a better sleep quality. There were no statistically significant differences between PRM and zopiclone in any of the actigraphic or questionnaire sleep parameters [110].

As no hypnographic correlates of the perceived quality of sleep exit, further studies on the efficacy of PRM in the improvement of quality of sleep (or non-restorative sleep) were based on patient reported assessments using the Leeds

Sleep Evaluation Questionnaire (LSEQ), the Pittsburgh Sleep Quality Index (PSQI) and patients' self reported diaries. The LSEQ includes 10 visual analogue scales (VAS) that measure four domains of sleep and morning behaviour: ease of getting to sleep (GTS- mean of questions 1, 2, and 3); quality of sleep (QOS-mean of questions 4 and 5); hangover on awakening from sleep (AFS- mean of questions 6 and 7) and alertness and behavioural integrity the following morning (BFW- mean of questions 8, 9, and 10). It has been validated in a number of studies involving healthy volunteers and the target population of insomnia patients aged 55 years and older [111,112]. Clinical response was defined as an improvement of 10 mm or more on the visual analogue scales which is considered to be of clinical importance and relevance [113]. The PSQI comprises nine questions relating to the patient's usual sleep habits during the previous four [114] or two [115] weeks. It addresses possible reasons for trouble in sleeping as well as daytime behaviour. The patient is asked to give the most accurate reply for the majority of days and nights during this period. An algorithm is used to calculate seven component scores and these are added to give a global PSQI score. The PSQI has been recommended as an essential measure for global sleep and insomnia symptoms in recent expert consensus recommendations for a standard set of research assessments in insomnia [116].

Two phase III double-blind placebo controlled studies of the efficacy and safety of PRM on the quality of sleep and behavior during the day were carried out in adult males and females (170 and 354 patients, respectively), aged 55 years or more suffering from primary insomnia according to DSM-IV criteria and for whom this was the consultation complaint [117,118]. The studies started with a run-in of 2 weeks (single-blind with placebo treatment), followed by a randomized treatment period of 3 weeks (double-blind, placebo-controlled, parallel group design). A 2 weeks withdrawal period with placebo was also included in one of these studies [117].

In these clinical trials benefits were shown in treated patients compared to placebo in quality of sleep and morning alertness. The apparent association between the quality of sleep at night and morning alertness was further investigated by comparing clinical response rate (the rate of patients improving concomitantly by 10 mm or more in QOS and BFW) in the two groups. This analysis revealed that 47% in the PRM group compared to 27% in the placebo group improved concomitantly in quality of sleep and morning alertness; the difference between treatment groups was significant ($P=0.009$) [117]. The second phase III trial also demonstrated significant differences in favour of PRM *vs.* placebo treatment in concomitant and clinically relevant improvements in quality of sleep and morning alertness, demonstrated by responder analysis (26% *vs.* 15%; $p=0.014$; Odds Ratio 1.97) as well as on each of these parameters separately and in quality of sleep assessed by the PSQI component 1 [118]. In the very severe insomnia population (baseline PSQI>13) the difference in responder rate between the PRM and placebo groups was somewhat greater (18.5%; odds ratio of 3.6) suggesting that the therapeutic benefit of PRM is higher in the severely ill patients. A better outcome for the PRM group on the WHO-5 well being index was also demonstrated and 70% of patients who responded to PR melatonin (*i.e.* demonstrated concomitant improvements in

QOS and BFW), also experienced a clinically relevant improvement in quality of life (equivalent to 3 units or more on the WHO-5 scale) compared to only 24% in non-responders [118] suggesting beneficial treatment effect on the restorative value of sleep.

Sleep promoting effects of PRM resulting in mean net reduction in sleep latency with PRM of 9 minutes over that with placebo were also demonstrated in these studies [118]. The magnitude of the effect resembled that observed by objective assessments with PRM [109] and is similar to those of currently used hypnotic drugs of the benzodiazepine and non-benzodiazepine class, *i.e.* zaleplon [119], zopiclone [35,110], zolpidem [120] and other melatonergic agents, *i.e.* Ramelteon [121].

At the same time PRM does not impair vigilance [122] driving performance and memory and has no discernible withdrawal symptoms which are associated with the GABA-A receptor modulators (*i.e.* benzodiazepine and non-benzodiazepine hypnotics) [123]. Importantly, melatonin potentiates the effects of GABA-A receptor modulators [36,124], and co-administration of PRM during the withdrawal period is thus useful to facilitate discontinuation of hypnotic drugs [125]. A unique feature of the PRM is the improvement in morning alertness which has hitherto not been demonstrated with any insomnia drug, neither with the MT1/MT2 melatonin receptor agonist ramelteon [126].

The secretion of the arousal hormone cortisol occurs earlier during sleep in older than young adults [127]. Middle-aged individuals not only have higher cortisol levels in the early portion of the night compared to younger adults, but also an increased vulnerability of sleep to stress hormones, possibly resulting in impairments in the quality of sleep [128,129]. PRM treatment delayed the nocturnal cortisol production in elderly insomnia patients towards the morning hours [38]; this delay may explain in part some of the beneficial effects of PRM on sleep and daytime alertness in elderly patients with insomnia [117,118]. No such effect was observed with FR-melatonin (unpublished data). This suggests that the effects of PRM on the circadian clock contribute to its efficacy in insomnia. This is further supported by the gradual development of the hypnotic effects of PRM, which in many patients, as is the case with blind individuals, may take days to weeks to respond [109].

Pre-clinical data on melatonin has not revealed any effects suggesting concern over the long-term use of melatonin in humans at the 2 mg dose proposed for PRM.

The total number patients exposure to PRM was 1926, of which 1361 patients received PRM for 3 weeks, 46 received PRM for 4-6 weeks, 373 patients received the compound for 6 months and 146 patients received PRM for one year or longer [108]. The most common adverse events (>2%) were headache, pharyngitis, back pain, and asthenias, which were common, by MedDRA definition (>1/100, <1/10), both in the PRM and placebo treated groups and were not necessarily related to treatment. When normalized for exposure period (per 100 patients' weeks) the rate of adverse events with PRM was less than with placebo for each as well as the total adverse events (3.17 with PR melatonin *vs.* 8.21 with placebo). No significant findings were observed from the Physical Examination or from the Vital Signs recording and

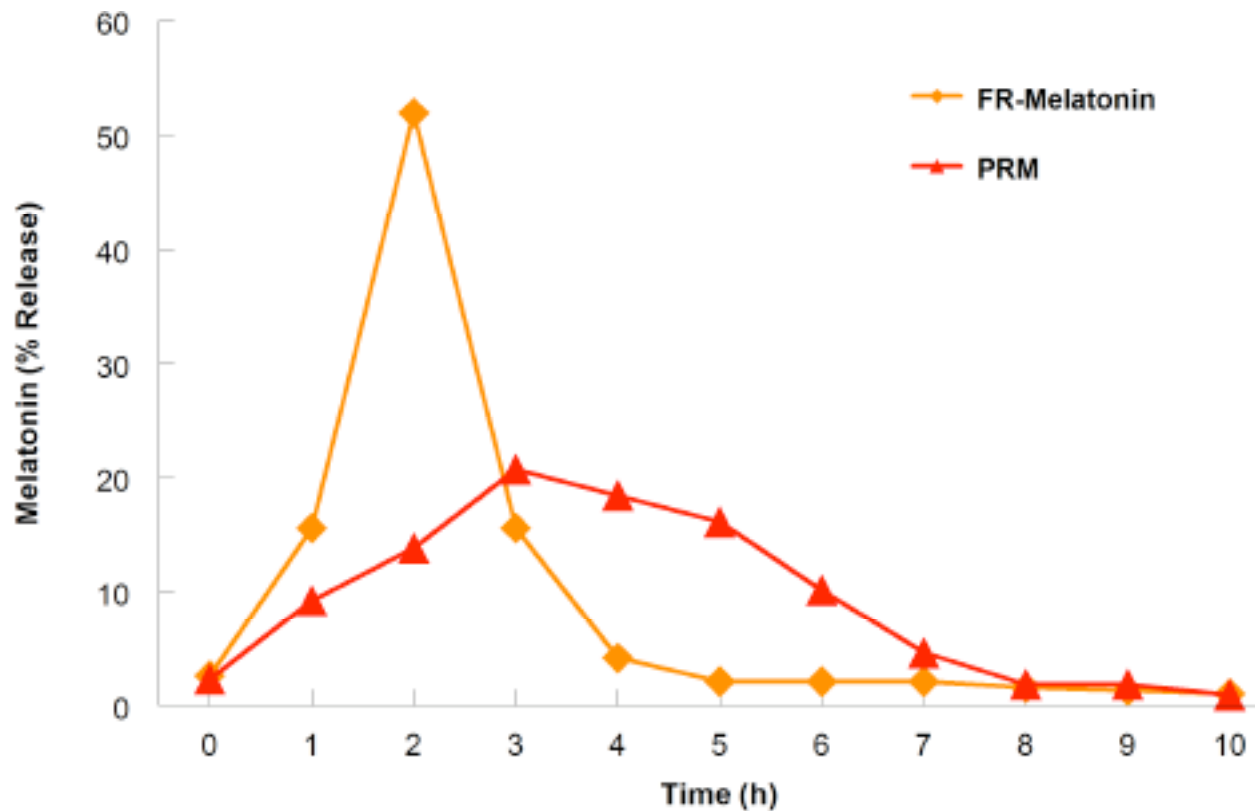


Fig. (1). Pharmacokinetic profile of PRM (Circadin[®]) compared to FR-melatonin 2 mg formulations (after [58] and [133]).

no safety concerns were raised. Discontinuation of therapy, whether after short term or long term period did not cause rebound or withdrawal symptoms [130]. Results of a six-month double blind placebo controlled study with PRM (Circadin[®]) were announced at the 2009 meeting of the Associated Professional Sleep Societies, indicating that efficacy is sustained over long-term periods without signs of tolerance development and confirming the safety of this drug.

Importantly, melatonin (as also PRM) potentiates the effects of GABA-A receptor modulators (i.e. benzodiazepine and non-benzodiazepine hypnotics) [36,123,124,131], and co-administration of PRM during the withdrawal period is thus useful to facilitate discontinuation of hypnotic drugs [125]. There were no discernible differences in efficacy and safety of PRM between patients who were previously treated with hypnotics and naïve patients [130].

PRM (Circadin[®], Neurim Pharmaceuticals) was authorised by the European Commission on 26 July 2007 for the short-term treatment of primary insomnia characterized by poor quality of sleep in patients who are aged 55 or over. This was the first melatonin receptor agonist approved for clinical use in the European Union. Since then, a second melatonin receptor agonist, agomelatine, which is also an antagonist of serotonin 5HT-2C receptors (Valdoxane[®], Servier) was authorised by the European Commission in 2009 for its use in the treatment of major depressive episodes in adults. Data from the clinical development programme show that agomelatine improves subjective sleep and daytime fatigue in these patients [132]. Ramelteon (Rozerem[®], Takeda), was approved by the USA FDA for the

treatment of insomnia characterized by difficulty falling asleep. Based on lack of withdrawal effects, it is not a controlled substance, and of all the prescription products approved by the FDA for insomnia its use is not limited to short term periods. Ramelteon is the only melatonergic drug approved by the FDA so far. Uncontrolled melatonin products are however available in the USA as food supplements for which no data on quality, pharmacokinetics pharmacodynamics or safety exist.

CONCLUSIONS

Melatonin is produced endogenously at night and its production overlaps the dark period. It is a signal of darkness thus affecting the circadian clock phase and melatonergic drugs may thus be useful in the treatment of circadian rhythm-related sleep disorders (i.e. non 24h sleep wake disorders in the blind, delayed sleep phase syndrome, sleep disorders in children with neurodevelopmental disabilities) and jet lag.

Melatonin also has sleep promoting effects demonstrated in changes in brain activation patterns and fatigue induction. The SCN function and melatonin production capacity decline with age thus depriving the brain from an important time cue and sleep regulator. PRM, which circumvents the fast clearance of the hormone and essentially mimics the physiological release pattern, significantly improves sleep quality and latency in insomnia patients aged 55 years and older and at the same time improves morning alertness and quality of life (evidence of restorative sleep) compared to placebo. PRM efficacy, being demonstrated on the basis of

responder analysis, is considered clinically significant and relevant [118].

The efficacy of PRM in insomnia patients ≥ 55 years is probably derived from its physiological functions as a sleep regulator and circadian clock synchronizer. Improvement in morning alertness has hitherto not been documented with any conventional hypnotic drug, or the MT1/MT2 melatonin receptor subtype specific drug ramelteon. Some of PRM's effects may thus be possibly mediated by non-MT1/MT2 pathways. Further studies on PRM mechanism of action are thus warranted. There is no main safety concern. In particular, PRM treatment is not associated with memory impairments, residual daytime or 'hangover' effects, and there is no rebound insomnia or withdrawal symptoms upon discontinuation. Melatonergic drugs, particularly PRM thus represent a new therapeutic principle in sleep medicine.

REFERENCES

- [1] Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. *J Endocrinol* 2003; 177 (1): 17-26.
- [2] Van Someren EJ. More than a marker: interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. *Chronobiol Int* 2000; 17 (3): 313-54.
- [3] Levi F, Schibler U. Circadian rhythms: mechanisms and therapeutic implications. *Annu Rev Pharmacol Toxicol* 2007; 47: 593-628.
- [4] Czeisler CA, Duffy JF, Shanahan TL, *et al.* Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 1999; 284 (5423): 2177-81.
- [5] Campbell S. Is there an intrinsic period of the circadian clock? *Science* 2000; 288 (5469): 1174-5.
- [6] Saper CB, Lu J, Chou TC, Buttery R. The hypothalamic integrator for circadian rhythms. *Trends Neurosci* 2005; 28 (3): 152-7.
- [7] Espana RA, Scammell TE. Sleep neurobiology for the clinician. *Sleep* 2004; 27 (4): 811-20.
- [8] Gais S, Born J. Declarative memory consolidation: mechanisms acting during human sleep. *Learn Mem* 2004; 11 (6): 679-85.
- [9] Dijk DJ, Lockley SW. Integration of human sleep-wake regulation and circadian rhythmicity. *J Appl Physiol* 2002; 92 (2): 852-62.
- [10] Tabandeh H, Lockley SW, Buttery R, *et al.* Disturbance of sleep in blindness. *Am J Ophthalmol* 1998; 126 (5): 707-12.
- [11] Hack LM, Lockley SW, Arendt J, Skene DJ. The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *J Biol Rhythms* 2003; 18 (5): 420-9.
- [12] Skene DJ, Lockley SW, Arendt J. Melatonin in circadian sleep disorders in the blind. *Biol Signals Recept* 1999; 8: 90-5.
- [13] Leger D, Guilleminault C, Defrance R, Domont A, Paillard M. Prevalence of sleep/wake disorders in persons with blindness. *Clinical Science* 1999; 97: 193-9.
- [14] Zisapel N. Circadian rhythm sleep disorders: pathophysiology and potential approaches to management. *CNS Drugs* 2001; 15 (4): 311-28.
- [15] Brzezinski A. Melatonin in humans. *N Engl J Med* 1997; 336 (3): 186-95.
- [16] Rousseau A, Petren S, Planthorn J, Eklundh T, Nordin C. Serum and cerebrospinal fluid concentrations of melatonin: a pilot study in healthy male volunteers. *J Neural Transm* 1999; 106: 883-8.
- [17] Hedlund L, Lischko M, Rollag M, Niswender G. Melatonin: daily cycle in plasma and cerebrospinal fluid of calves. *Science* 1977; 195: 686-7.
- [18] Liu RY, Zhou JN, van Heerikhuizen J, Hofman MA, Swaab DF. Decreased melatonin levels in postmortem cerebrospinal fluid in relation to aging, Alzheimer's disease, and apolipoprotein E-epsilon4 genotype. *J Clin Endocrinol Metab* 1999; 84 (1): 323-7.
- [19] Gastel JA, Roseboom PH, Rinaldi PA, Weller JL, Klein DC. Melatonin production: proteasomal proteolysis in serotonin N-acetyltransferase regulation. *Science* 1998; 279 (5355): 1358-60.
- [20] Lewy AJ. The dim light melatonin onset, melatonin assays and biological rhythm research in humans. *Biol Signals Recept* 1999; 8 (1-2): 79-83.
- [21] Waldhauser F, Waldhauser M, Lieberman HR, Deng MH, Lynch HJ, Wurtman RJ. Bioavailability of oral melatonin in humans. *Neuroendocrinology* 1984; 39 (4): 307-13.
- [22] Arendt J, Skene DJ. Melatonin as a chronobiotic. *Sleep Med Rev* 2005; 9 (1): 25-39.
- [23] Skene DJ. Optimization of light and melatonin to phase-shift human circadian rhythms. *J Neuroendocrinol* 2003; 15 (4): 438-41.
- [24] Liu C, Weaver DR, Jin X, *et al.* Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* 1997; 19 (1): 91-102.
- [25] Lewy AJ, Ahmed S, Sack RL. Phase-shifting the human circadian clock using melatonin. *Behav Brain Res* 1996; 73: 131-4.
- [26] Deacon S, Arendt J. Adapting to phase shifts II. Effects of melatonin and conflicting light treatment. *Physiol Behav* 1996; 59: 675-82.
- [27] McArthur AJ, Gillette MU, Prosser RA. Melatonin directly resets the rat suprachiasmatic circadian clock *in vitro*. *Brain Res* 1991; 565: 158-61.
- [28] Shochat T, Haimov I, Lavie P. Melatonin--the key to the gate of sleep. *Ann Med* 1998; 30 (1): 109-14.
- [29] Lockley SW, Skene DJ, Tabandeh H, Bird AC, Defrance R, Arendt J. Relationship between napping and melatonin in the blind. *J Biol Rhythms* 1997; 12 (1): 16-25.
- [30] Nakagawa H, Sack RL, Lewy AJ. Sleep propensity free-runs with the temperature, melatonin and cortisol rhythms in a totally blind person. *Sleep* 1992; 15 (4): 330-6.
- [31] Shibui K, Uchiyama M, Okawa M. Melatonin rhythms in delayed sleep phase syndrome. *J Biol Rhythms* 1999; 14 (1): 72-6.
- [32] Zisapel N. Development of a melatonin based formulation for the treatment of insomnia. *Drug Dev Res* 2000; 50: 226-34.
- [33] Cajochen C, Krauchi K, Wirz-Justice A. Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol* 2003; 15 (4): 432-7.
- [34] Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc Natl Acad Sci USA* 1994; 91 (5): 1824-8.
- [35] Paul MA, Gray G, Sardana TM, Pigeau RA. Melatonin and zopiclone as facilitators of early circadian sleep in operational air transport crews. *Aviation Space Environ Med* 2004; 75 (5): 439-43.
- [36] Wesensten NJ, Balkin TJ, Reichardt RM, Kautz MA, Saviolakis GA, Belenky G. Daytime sleep and performance following a zolpidem and melatonin cocktail. *Sleep* 2005; 28 (1): 93-103.
- [37] Dijk DJ, Roth C, Landolt HP, *et al.* Melatonin effect on daytime sleep in men: suppression of EEG low frequency activity and enhancement of spindle frequency activity. *Neurosci Lett* 1995; 201 (1): 13-6.
- [38] Zisapel N, Tarrasch R, Laudon M. The relationship between melatonin and cortisol rhythms: clinical implications of melatonin therapy. *Drug Dev Res* 2005; 65 (3): 119-25.
- [39] Zisapel N. Sleep and sleep disturbances: biological basis and clinical implications. *Cell Mol Life Sci* 2007; 64 (10): 1174-86.
- [40] Gorfine T, Assaf Y, Goshen-Gottstein Y, Yeshurun Y, Zisapel N. Sleep-anticipating effects of melatonin in the human brain. *Neuroimage* 2006; 31 (1): 410-8.
- [41] Gorfine T, Zisapel N. Melatonin and the human hippocampus, a time dependant interplay. *J Pineal Res* 2007; 43 (1): 80-6.
- [42] Gorfine T, Yeshurun Y, Zisapel N. Nap and melatonin-induced changes in hippocampal activation and their role in verbal memory consolidation. *J Pineal Res* 2007; 43 (4): 336-42.
- [43] Gorfine T, Zisapel N. Late evening brain activation patterns and their relation to the internal biological time, melatonin, and homeostatic sleep debt. *Hum Brain Mapp* 2007; 30 (2): 541-52.
- [44] Witt-Enderby PA, Bennett J, Jarzynka MJ, Firestine S, Melan MA. Melatonin receptors and their regulation: biochemical and structural mechanisms. *Life Sci* 2003; 72 (20): 2183-98.
- [45] Laudon M, Nir I, Zisapel N. Melatonin receptors in discrete brain areas of the male rat: impact of aging on density and on circadian rhythmicity. *Neuroendocrinology* 1988; 48 (6): 577-83.
- [46] Mazzucchelli C, Pannacci M, Nonno R, Lucini V, Fraschini F, Stankov BM. The melatonin receptor in the human brain: cloning experiments and distribution studies. *Mol Brain Res* 1996; 39: 117-26.

- [47] Wan Q, Man HY, Liu F, *et al.* Differential modulation of GABAA receptor function by Mel1a and Mel1b receptors. *Nat Neurosci* 1999; 2 (5): 401-3.
- [48] Reiter RJ. At the cutting edge melatonin: the chemical expression of darkness. *Mol Cell Endocrinol* 1991; 79: C153-C8.
- [49] Knoblauch V, Krauchi K, Renz C, Wirz-Justice A, Cajochen C. Homeostatic control of slow-wave and spindle frequency activity during human sleep: effect of differential sleep pressure and brain topography. *Cereb Cortex* 2002; 12 (10): 1092-100.
- [50] Dahlitz M, Alvarez B, Vignau J, English J, Arendt J, Parkes JD. Delayed sleep phase syndrome response to melatonin. *Lancet* 1991; 337 (8750): 1121-4.
- [51] Oldani A, Ferini-Strambi L, Zucconi M, Stankov B, Fraschini F, Smirne S. Melatonin and delayed sleep phase syndrome: ambulatory polygraphic evaluation. *Neuroreport* 1994; 6: 132-4.
- [52] Nagtegaal JE, Kerkhof GA, Smits MG, Swarts AC, Van Der Meer YG. Delayed sleep phase syndrome: a placebo controlled crossover study on the effects of melatonin administered five hours before the individual dim light melatonin onset. *J Sleep Res* 1998; 7(2): 135-43.
- [53] Lewy AJ, Newsome DA. Different types of melatonin circadian secretory rhythms in some blind subjects. *J Clin Endocrinol Metab* 1983; 56: 1103-7.
- [54] Sack RL, Brandes RW, Kendall AR, Lewy AJ. Entrainment of free-running circadian rhythms by melatonin in blind people. *N Engl J Med* 2000; 343 (15): 1070-7.
- [55] Folkard S, Arendt J, Aldhous M, Kennett H. Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. *Neurosci Lett* 1990; 113 (2): 193-8.
- [56] Sack RL, Lewy AJ, Blood ML, Stevenson J, Keith LD. Melatonin administration to blind people: phase advances and entrainment. *J Biol Rhythms* 1991; 6 (3): 249-61.
- [57] Lewy AJ, Emens JS, Bernert RA, Lefler BJ. Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: clinical implications. *J Biol Rhythms* 2004; 19 (1): 68-75.
- [58] Aldhous M, Franey C, Wright J, Arendt J. Plasma concentrations of melatonin in man following oral absorption of different preparations. *Br J Clin Pharmacol* 1985; 19 (4): 517-21.
- [59] Cavallo A, Ritschel WA. Pharmacokinetics of melatonin in human sexual maturation. *J Clin Endocrinol Metab* 1996; 81 (5): 1882-6.
- [60] Benes L, Claustrat B, Horriere F, *et al.* Transmucosal, oral controlled-release, and transdermal drug administration in human subjects: a crossover study with melatonin. *J Pharmacol Sci* 1997; 86 (10): 1115-9.
- [61] Mahlberg R, Kunz D. Melatonin excretion levels and polysomnographic sleep parameters in healthy subjects and patients with sleep-related disturbances. *Sleep Med* 2007; 8 (5): 512-6.
- [62] Rajaratnam SM, Middleton B, Stone BM, Arendt J, Dijk DJ. Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. *J Physiol* 2004; 561 (Pt 1): 339-51.
- [63] Rajaratnam SM, Polymeropoulos MH, Fisher DM, *et al.* Melatonin agonist tasimelteon (VEC-162) for transient insomnia after sleep-time shift: two randomised controlled multicentre trials. *Lancet* 2009; 373 (9662): 482-91.
- [64] Jan JE, Freeman RD, Fast DK. Melatonin treatment of sleep-wake cycle disorders in children and adolescents. *Dev Med Child Neurol* 1999; 41 (7): 491-500.
- [65] O'Callaghan FJ, Clarke AA, Hancock F, Hunt A, Osborne JP. Use of melatonin to treat sleep disorders in tuberous sclerosis. *Dev Med Clin Neurol* 1999; 41: 123-6.
- [66] Miyamoto A, Oki J, Takahashi S, Okuno A. Serum melatonin kinetics and long-term melatonin treatment for sleep disorders in Rett syndrome. *Brain Dev* 1999; 21 (1): 59-62.
- [67] De Leersnyder H, de Blois M-C, Vekemans M, *et al.* Beta1-adrenergic antagonists improve sleep and behavioural disturbances in a circadian disorder, Smith-Magenis syndrome. *J. Med Genet* 2001; 1 (38): 586-90.
- [68] De Leersnyder HD, Bresson JL, de BM-C, *et al.* B1-adrenergic antagonists and melatonin reset the clock and restore sleep in a circadian disorder, Smith-Magenis syndrome. *J Med Genet* 2003; 40: 74-8.
- [69] De Leersnyder H. Inverted rhythm of melatonin secretion in Smith-Magenis syndrome: from symptoms to treatment. *Trends Endocrinol Metab* 2006.
- [70] Giannotti F, Cortesi F, Cerquiglioni A, Bernabei P. An open-label study of controlled-release melatonin in treatment of sleep disorders in children with autism. *J Autism Dev Disord* 2006; 36 (6): 741-52.
- [71] Carr R, Wasdell MB, Hamilton D, *et al.* Long-term effectiveness outcome of melatonin therapy in children with treatment-resistant circadian rhythm sleep disorders. *J Pineal Res* 2007; 43 (4): 351-9.
- [72] Hoebert M, van der Heijden KB, van Geijlswijk IM, Smits MG. Long-term follow-up of melatonin treatment in children with ADHD and chronic sleep onset insomnia. *J Pineal Res* 2009; 47 (1): 1-7.
- [73] Jan JE, Hamilton D, Seward N, Fast DK, Freeman RD, Laudon M. Clinical trials of controlled-release melatonin in children with sleep-wake cycle disorders. *J Pineal Res* 2000; 29 (1): 34-9.
- [74] Ohayon M. Prevalence of DSM-IV diagnostic criteria of insomnia: distinguishing insomnia related to mental disorders from sleep disorders. *J Psychiatr Res* 1997; 31 (3): 333-46.
- [75] Ustun T, Privett M, Lecrubier Y, *et al.* Form, frequency and burden of sleep problems in general health care: a report from the WHO Collaborative Study on Psychological Problems in General Health Care. *Eur Psychiatry* 1996; 11 (1): 1087-96.
- [76] Nair NPV, Hariharasubramanian N, Pilapil C, Issac I, Thavundayil JX. Plasma melatonin - an index of brain aging in humans ? *Biol Psychiatry* 1986; 21: 141-50.
- [77] Haimov I, Laudon M, Zisapel N, *et al.* Sleep disorders and melatonin rhythms in elderly people. *BMJ* 1994; 309 (6948): 167.
- [78] Leger D, Laudon M, Zisapel N. Nocturnal 6-sulfatoxymelatonin excretion in insomnia and its relation to the response to melatonin replacement therapy. *Am J Med* 2004; 116 (2): 91-5.
- [79] Hajak G, Rodenbeck A, Staedt J, Bandelow B, Huether G, Ruther E. Nocturnal plasma melatonin levels in patients suffering from chronic primary insomnia. *J Pineal Res* 1996; 19: 116-22.
- [80] Mishima K, Okawa M, Shimizu T, Hishikawa Y. Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. *J Clin Endocrinol Metab* 2001; 86 (1): 129-34.
- [81] Riemann D, Klein T, Rodenbeck A, *et al.* Nocturnal cortisol and melatonin secretion in primary insomnia. *Psychiatry Res* 2002; 113 (1-2): 17-27.
- [82] Shilo L, Dagan Y, Smorjick Y, *et al.* Patients in the intensive care unit suffer from severe lack of sleep associated with loss of normal melatonin secretion pattern. *Am J Med Sci* 1999; 317 (5): 278-81.
- [83] Lynch HJ, Ozaki Y, Shakal D, Wurtman RJ. Melatonin excretion of man and rats: effect of time of day, sleep, pinealectomy and food consumption. *Int J Biometeorol* 1975; 19 (4): 267-79.
- [84] Sharma M, Palacios-Bois J, Schwartz G, *et al.* Circadian rhythms of melatonin and cortisol in aging. *Biological Psychiatry* 1989; 25: 305-19.
- [85] Kunz D, Schmitz S, Mahlberg R, *et al.* A new concept for melatonin deficit: on pineal calcification and melatonin excretion. *Neuropsychopharmacology* 1999; 21 (6): 765-72.
- [86] Kennaway DJ, Lushington K, Dawson D, Lack L, van den Heuvel C, Rogers N. Urinary 6-sulfatoxymelatonin excretion and aging: new results and a critical review of the literature. *J Pineal Res* 1999; 27: 210-20.
- [87] Mahlberg R, Kienast T, Hadel S, Heidenreich JO, Schmitz S, Kunz D. Degree of pineal calcification (DOC) is associated with polysomnographic sleep measures in primary insomnia patients. *Sleep Med* 2009; 10 (4): 439-45.
- [88] Czeisler CA, Dumont M, Duffy JF, *et al.* Association of sleep-wake habits in older people with changes in output of circadian pacemaker. *Lancet* 1992; 340 (8825): 933-6.
- [89] Hofman MA, Swaab DF. Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. *Brain Res* 1994; 651 (1-2): 134-42.
- [90] Swaab DF, Fliers E, Partiman TS. The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res* 1985; 342 (1): 37-44.
- [91] Carrier J, Monk TH, Buysse D, Kupfer D. Sleep and morningness-eveningness in the 'middle' years of life (20-59y). *J Sleep Res* 1997; 6: 230-7.
- [92] Hughes RJ, Sack R, Lewy A. The role of melatonin and circadian phase in age-related sleep-maintenance insomnia: assessment in a clinical trial of melatonin replacement. *Sleep* 1998; 21 (1): 52-68.
- [93] Kripke D, Elliot J, Youngstedt S, Smith J. Melatonin: marvel or marker ? *Ann Med* 1998; 30: 81-7.

- [94] Zhdanova IV. Melatonin as a hypnotic: pro. *Sleep Med Rev* 2005; 9 (1): 51-65.
- [95] MacFarlane JG, Cleghorn JM, Brown GM, Streiner DL. The effects of exogenous melatonin on the total sleep time and daytime alertness of chronic insomniacs: a preliminary study. *Biol Psychiatry* 1991; 30 (4): 371-6.
- [96] James SP, Sack DA, Rosenthal NE, Mendelson WB. Melatonin administration in insomnia. *Neuropsychopharmacology* 1990; 3 (1): 19-23.
- [97] Haimov I, Lavie P, Laudon M, Herer P, Vigder C, Zisapel N. Melatonin replacement therapy of elderly insomniacs. *Sleep* 1995; 18 (7): 598-603.
- [98] Garfinkel D, Laudon M, Nof D, Zisapel N. Improvement of sleep quality in elderly people by controlled-release melatonin. *Lancet* 1995; 346 (8974): 541-4.
- [99] Wurtman RJ, Zhdanova I. Improvement of sleep quality by melatonin. *Lancet* 1995; 346 (8988): 1491.
- [100] Buscemi N, Vandermeer B, Hooton N, *et al.* The efficacy and safety of exogenous melatonin for primary sleep disorders: a meta-analysis. *J Gen Intern Med* 2005; 20 (12): 1151-8.
- [101] Brzezinski A, Vangel MG, Wurtman RJ, *et al.* Effects of exogenous melatonin on sleep: a meta-analysis. *Sleep Med Rev* 2005; 9 (1): 41-50.
- [102] Karasek M. Melatonin, human aging, and age-related diseases. *Exp Gerontol* 2004; 39 (11-12): 1723-9.
- [103] Jean-Louis G, Kripke DF, Ancoli-Israel S. Sleep and quality of well-being. *Sleep* 2000; 23 (8): 1115-21.
- [104] Arendt J. Melatonin and the mammalian pineal gland. London: Chapman & Hall 1995.
- [105] Gilad E, Matzkin H, Zisapel N. Inactivation of melatonin receptors by protein kinase C in human prostate epithelial cells. *Endocrinology* 1997; 138 (10): 4255-61.
- [106] Godson C, Reppert SM. The Mel1a melatonin receptor is coupled to parallel signal transduction pathways. *Endocrinology* 1997; 138 (1): 397-404.
- [107] Gerdin MJ, Masana MI, Rivera-Bermudez MA, *et al.* Melatonin desensitizes endogenous MT2 melatonin receptors in the rat suprachiasmatic nucleus: relevance for defining the periods of sensitivity of the mammalian circadian clock to melatonin. *FASEB J* 2004; 18 (14): 1646-56.
- [108] EMEA. Circadin EPA Report. 2007; Available at: <http://www.emea.europa.eu/humandocs/PDFs/EPAR/circadin/H-695-en6.pdf>
- [109] Luthringer R, Muzet M, Zisapel N, Staner L. The effect of prolonged-release melatonin on sleep measures and psychomotor performance in elderly patients with insomnia. *Int Clin Psychopharmacol* 2009.
- [110] Paul MA, Gray G, Maclellan M, Pigeau RA. Sleep-Inducing Pharmaceuticals: a comparison of melatonin, zaleplon, zopiclone, and temazepam. *Aviat Space Environ Med* 2004; 75 (6): 512-9.
- [111] Parrott AC, Hindmarch I. The Leeds sleep evaluation questionnaire in psychopharmacological investigations - a review. *Psychopharmacology (Berl)* 1980; 71 (2): 173-9.
- [112] Tarrasch R, Laudon M, Zisapel N. Cross-cultural validation of the Leeds sleep evaluation questionnaire (LSEQ) in insomnia patients. *Hum Psychopharmacol* 2003; 18 (8): 603-10.
- [113] Zisapel N, Nir T. Determination of the minimal clinically significant difference on a patient visual analog sleep quality scale. *J Sleep Res* 2003; 12 (4): 291-8.
- [114] Buysse D, Reynolds III C, Monk T, Berman S, Kupfer D. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; 28: 193-213.
- [115] Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. *Psychosom Res* 2002; 53: 737-40.
- [116] Buysse DJ, Ancoli-Israel S, Edinger JD, Lichstein KL, Morin CM. Recommendations for a standard research assessment of insomnia. *Sleep* 2006; 29 (9): 1155-73.
- [117] Lemoine P, Nir T, Laudon M, Zisapel N. Prolonged-release melatonin improves sleep quality and morning alertness in insomnia patients aged 55 years and older and has no withdrawal effects. *J Sleep Res* 2007; 16 (4): 372-80.
- [118] Wade A, Ford I, Crawford G, *et al.* Efficacy of prolonged release melatonin in insomnia patients aged 55-80 years. *Curr Med Res Opin* 2007; 23 (10): 2597-605.
- [119] Ancoli-Israel S, Walsh JK, Mangano RM, Fujimori M, Zaleplon, A Novel nonbenzodiazepine hypnotic, effectively treats insomnia in elderly patients without causing rebound effects. *Primary Care Companion J Clin Psychiatry* 1999; 1 (4): 114-20.
- [120] FDA. Zolpidem F. Clinical Review. 1992; http://www.fda.gov/cder/foi/nda/pre96/019908_S000_AmbienTOC.htm. 1992.
- [121] Borja NL, Daniel KL. Ramelteon for the treatment of insomnia. *Clin Ther* 2006; 28 (10): 1540-55.
- [122] Paul MA, Gray G, Kenny G, Pigeau RA. Impact of melatonin, zaleplon, zopiclone, and temazepam on psychomotor performance. *Aviat Space Environ Med* 2003; 74 (12): 1263-70.
- [123] Otmani S, Demazieres A, Staner C, *et al.* Effects of prolonged-release melatonin, zolpidem, and their combination on psychomotor functions, memory recall, and driving skills in healthy middle aged and elderly volunteers. *Hum Psychopharmacol* 2008; 23 (8): 693-705.
- [124] Garfinkel D, Laudon M, Zisapel N. Improvement of sleep quality by controlled-release melatonin in benzodiazepine-treated elderly insomniacs. *Arch Gerontol Geriatr* 1997; 24 (2): 223-31.
- [125] Garfinkel D, Zisapel N, Wainstein J, Laudon M. Facilitation of benzodiazepine discontinuation by melatonin: a new clinical approach. *Arch Intern Med* 1999; 159 (20): 2456-60.
- [126] Roth T, Seiden D, Sainati S, Wang-Weigand S, Zhang J, Zee P. Effects of ramelteon on patient-reported sleep latency in older adults with chronic insomnia. *Sleep Med* 2006; 7: 312-8.
- [127] Van Cauter E, Leproult R, Plat L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. *JAMA* 2000; 284 (7): 861-8.
- [128] Vgontzas AN, Bixler EO, Wittman AM, *et al.* Middle-aged men show higher sensitivity of sleep to the arousing effects of corticotropin-releasing hormone than young men: clinical implications. *J Clin Endocrinol Metab* 2001; 86 (4): 1489-95.
- [129] Vgontzas AN, Zoumakis M, Bixler EO, *et al.* Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: physiologic and therapeutic implications. *J Clin Endocrinol Metab* 2003; 88 (5): 2087-95.
- [130] Zisapel N. Controlled release melatonin (Circadin) in the treatment of insomnia in older patients: efficacy and safety in patients with history of use and non-use of hypnotic drugs. *Harefuah* 2009; 148 (5): 337-41, 48.
- [131] Ferini-Strambi L, Oldani A, Zucconi M, *et al.* Triazolam and melatonin effects on cardiac autonomic function during sleep. *Clin Neuropharmacol* 1995; 18 (5): 405-9.
- [132] Lemoine P, Guilleminault C, Alvarez E. Improvement in subjective sleep in major depressive disorder with a novel antidepressant, agomelatine: randomized, double-blind comparison with venlafaxine. *J Clin Psychiatry* 2007; 68 (11): 1723-32.
- [133] EMEA. European Public Assessment Report (EPAR) CIRCADIN. 2007; Available from: <http://www.emea.europa.eu/humandocs/PDFs/EPAR/circadin/H-695-en1.pdf>

Received: September 09, 2009

Revised: October 20, 2009

Accepted: October 24, 2009

© Nava Zisapel; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.