Toxicology of Melatonin

Béatrice Guardiola-Lemaître
Servier International Research Institute, 6 place des Pléiades, 92415 Courbevoie Cedex, France

Abstract Despite the fact that melatonin has been released for public use in the United States by the Food and Drug Administration and is available over the counter nationwide, there currently is a total lack of information on the toxicology of melatonin. In Europe, melatonin has a completely different status in that it is considered a "neurohormone" and cannot be sold over the counter. Even though administration of melatonin in humans, as well as in animals (even at supraphysiological doses), has not shown evidence of toxicological effects (i.e., no deaths), a drug toxicological file still would need to be prepared and approved by the regulatory authorities. Several features that are specific to this neurohormone need to be taken into consideration. Whatever the species concerned, melatonin is secreted during the night; it is the "hormone of darkness." It presents a circadian rhythm and a circannual rhythm (in photoperiodic species). The duration of these secretions could have an impact on the reproductive system, for example, showing the importance of the pharmacodynamics of melatonin. An inappropriate time schedule of melatonin administration could induce supraphysiological concentrations of the neurohormone and a desensitization of melatonin receptors. A long duration of exposure to melatonin also could mimic an "artificial darkness" condition when a circadian rhythm with a basal zero level during the day needs to be conserved for a physiological function. Furthermore, administration of large doses of melatonin could induce high concentrations of melatonin and of different metabolites that could have deleterious effects per se. Numerous books, magazines, and articles have praised melatonin as a "miraculous cure-all" for ailments ranging from sleeplessness, to aging, without any clinical evidence of efficacy (with the exception of its chronobiotic and resynchronizing effect). Very little attention has been paid to the possible side effects of melatonin. Nightmares, hypotension, sleep disorders, abdominal pain, etcetera, have been reported. In fact, analysis of the known pharmacological profile of melatonin and/or of its metabolites, based on scientific preclinical studies, constitutes a basis for prediction of adverse drug reactions or side effects. These include (1) the central nervous system, (2) the cardiovascular system and platelet aggregation, (3) glucose metabolism, (4) immunology, and (5) cancer. The knowledge of the fundamental mechanism of action of melatonin, including molecular biology, also needs to be taken into account for evaluation of possible side effects. Two types of melatonin receptors have been cloned (related to cyclic AMP), and the possibility of intracellular action of melatonin cannot be excluded. Melatonin receptors are present in the periphery and also at the level of the central nervous system, particularly on the suprachiasmatic nucleus that "drives" a circadian rhythm to many other areas on which it projects. Among those, the hypothalamus (which has melatonin

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receptors) plays a fundamental role in the hormonal homeostasis and modulation control of the organism. Special preclinical and pharmacological studies that take into account all these parameters need to be designed for safety evaluation and risk assessment of this specific neurohormone.

Key words melatonin, toxicology, adverse drug reactions, side effects, pharmacology, pharmacodynamics, metabolism, time of administration, dosage

> What is there that is not poison? All things are poison and nothing [is] without poison. Solely the dose determines that a thing is not poison.
>
> —Paracelsus (1493-1541)

According to the definition given by Gallo (1976, p. 1) in his chapter on the history and scope of toxicology,

> Modern toxicology goes beyond the study of the adverse effects of exogenous agents to the study of molecular biology, using toxicants as tools. Historically, toxicology formed the basis of therapeutics and experimental medicine. A recent addition to toxicology is the application of the discipline of *safety evaluation and risk assessment*. Toxicology, like medicine, is both a science and an art.

From this definition, toxicology is described not as a static or fixed science but rather as a fully evolving science concerned with finding its place within pharmacology and pharmacokinetics through the voice of researchers who are experts in these particular fields as well as in the physiopathology field. Even if the pharmacology of melatonin and its mechanism of action have made great progress, we still are far from understanding the "whole picture." Certain therapeutic indications today have been defined in double-blind controlled studies in humans in which even the choice of the dose remains somewhat arbitrary. Other potential therapeutic indications of melatonin can be glimpsed through a better knowledge of its pharmacological and physiological roles. There currently is a total lack of information on the toxicology of melatonin despite the fact that melatonin has been released for public use in the United States by the Food and Drug Administration (FDA) and is available over the ounter nationwide (Lamberg, 1996). Numerous books, magazines, and articles have been published about the "miracles" of melatonin, and melatonin is used by millions of Americans as a "cure-all" for ailments ranging from sleeplessness to aging without any clinical evidence of efficacity in humans. In Europe, melatonin has a completely different status in that it is considered a "neurohormone" and cannot be sold over the counter. In this article, the known physiological and pharmacological effects of melatonin and/or of its metabolites will be described to see in what way studies could be designed to fit in with the targeted pathology and to approach safety and risk assessment. The fact that, physiologically, melatonin is the "hormone of darkness" secreted with a circadian rhythm during the night, whatever the species concerned, and that inappropriate timing of the hormone could have deleterious effects will be taken into account. Like a drug, its long-term use in humans would require a definition of its therapeutic indications, a specification of the dose and a related pharmacodynamic and drug toxicological file.

1. What Is Melatonin?

Melatonin is a neurohormone that, in humans as well as in animals, is synthesized mainly by the pineal gland, although other sources of synthesis have been described. A major and very fundamental aspect that needs to be taken into consideration is that melatonin is secreted *only during the night, whatever the species concerned*. Thus, in humans, melatonin is secreted during the night, when human activity is weak and dedicated mainly to rest and sleep, whereas their social activities are diurnal. Conversely, in nocturnal animals (e.g., rats, hamsters), melatonin also is secreted during the night when these animals are very active and dedicate their night to foraging for food and motor activity. In this way, melatonin could be considered as the hormone of darkness or hormone of time. It also is of interest to consider that in both diurnal and nocturnal rodents or mammals, it is during the light period that the peak of neuronal activity of the "clock" (i.e., the suprachiasmatic nucleus [SCN]) appears. The SCN is the main target for the activity of melatonin at the level of melatonin receptors. Melatonin exhibits a circadian rhythm with a high level during the night and a low (or zero) level during the day. Melatonin also exhibits a circannual rhythm: the shorter the day, the longer the night and, therefore, the larger the duration

of melatonin secretion. On the contrary, the longer the day, the shorter the night, the shorter the duration of melatonin secretion demonstrating the adaptive role of melatonin between the individual (or animal) and his or her (or its) environment (light-dark cycle).

2. *What Are the Well-Defined Activities of Melatonin in Humans?*

Up to now, studies on the chronobiotic effects of melatonin have focused mainly on the reentrainment of circadian rhythm sleep disorders. A chronobiotic has been defined as a "chemical substance able to therapeutically reentrain short-term dissociated or long-term desynchronised circadian rhythms or prophylactically preventing their disruption following environmental insult" (Armstrong, 1991, p. 265). The only well-defined activities of melatonin in humans that we consider here are those that have been performed in carefully controlled clinical studies. However, the choice of doses used has not always been preceded by a "dose effect" study.

Delayed sleep phase syndrome. In a randomized, double-blind, placebo-controlled trial in 8 subjects with delayed sleep phase syndrome, 5 mg of melatonin given daily for 4 weeks 5 h before the mean time of sleep onset significantly advanced both sleep and wake onset. After stopping melatonin administration, all subjects reverted to their previous sleep-wake times within 2 or 3 days. In this study, the melatonin treatment had a comparable effect with a fixed bed wake, dark-light cycle timing. These results were confirmed with a similar protocol (Dahlitz et al., 1991).

Jet lag syndrome. Melatonin is effective in resynchronizing circadian rhythms at doses from 1 to 5 mg in uncontrolled or double-blind placebo-controlled studies on the condition that it is taken at the appropriate time (Arendt et al., 1987).

Shiftwork syndrome. A preliminary study involving a small group of workers on night shift taking melatonin (5 mg orally) at bedtime has shown an improvement in both sleep and alertness at work (Folkard et al., 1993).

Non-24-h sleep-wake disorder. Several studies have been done mainly in blind subjects who have sleep disorders. Noncontrolled and double-blind placebo-controlled trials with melatonin from 0.5 to 6 mg orally in the evening demonstrated that melatonin is able to entrain the sleep-wake cycle with (Sack et al., 1991) or without (Sarrafzadeh et al., 1991) entrainment of other rhythms (temperature cortisol).

In physiological conditions, plasma concentration levels vary during the night from 30 to 200 pg/ml^{-1} (Waldhauser et al., 1984). The plasma concentration reached after 5 mg administered orally in the evening is about 10 to 30 times more than in normal conditions.

3. *What Is Melatonin's Underlying Mechanism of Action?*

So far as the chronobiotic or resynchronizing effect of melatonin is concerned, the underlying mechanism of action is a centrally mediated effect with an activity of melatonin on receptors located on the SCN. The SCN could modulate the activity of the hypothalamus and then the secretion of several hormones including glucocorticods. The *chronobiotic* effect of melatonin is observed only if melatonin is given on an appropriate time schedule. Melatonin could phase advance, phase delay, or have no effect on circadian parameters, depending on the time of its administration. A phase response curve has been determined in humans by Lewy et al. (1992) using the dim light melatonin onset as a marker. Melatonin administration causes phase delays in the morning and phase advances in the afternoon.

4. *What Are the Other Pharmacological or Fundamental Properties of Melatonin?*

The first property of melatonin that has been described and studied extensively in animals is the activity of melatonin on reproduction (Reiter, 1980). This property certainly has contributed in naming melatonin as a neurohormone. More recently, one of the major roles of melatonin that has been studied and focused on more extensively is its "resynchroniszing/resetting" effect on the biological clock (i.e., the SCN) at the level of the central nervous system (McArthur et al., 1991). The synthesis of melatonin derives from *N*-acetylation of serotonin via *N*-acetyl-serotonin-transferase and then from methoxylation of *N*-acetyl-serotonin (Klein, 1974). Thus, melatonin also could be considered as a "neurotransmitter" even though there has been very little evidence up to now to uphold this hypothesis from a physiological point of view.

A great analogy also exists between melatonin and serotonin, from which it is derived, so far as the targets

of their activity are concerned. As for serotonin, studies have shown the role of melatonin at the level of the periphery on different systems in animals: (1) the cardiovascular system, (2) glucose and lipid metabolism, (3) the gastrointestinal system, (4) immunology, and (5) cancer. Although many animal studies concerning the preceding activities have been performed, the exact role of melatonin in these different systems has not yet been fully defined. One of the major problems in assessing the role of melatonin in animals is to properly define the experimental pharmacological conditions to be able to reproduce these activities.

So far as the melatonin receptors are concerned, two major subtypes have been cloned and identified in humans: Mel_{1a} and Mel_{1b} (Reppert et al., 1994, 1995). Both are related to the activity of cyclic AMP, but their exact role, as well as their distribution at the central or peripheral level, has not yet been elucidated. Another type of receptor that has not yet been cloned (Mel_2) also could play a role in the functionality of melatonin (Dubocovich, 1988). Finally, a nuclear receptor, RORα, also has been described for melatonin, although the difficulty of reproducibility of experimentation has made it impossible to confirm this receptor. It also is difficult, at the moment, to rule out a possible role of melatonin at the cellular level (cytosol). Antioxidant-like activity of melatonin also has been described (Reiter, 1997). However, the concentrations at which this antioxidant property occurs (10^{-7} M) in vitro does not fit in very well with the picomolar concentration of melatonin in blood in either humans or animals. This activity will need a high intracellular concentration of melatonin (10^{-7} M). It also must be remembered that melatonin has a very high lipophilic character.

5. *Is Time of Administration Important?*

The "window of time sensitivity" of melatonin receptors, as well as the quick up-and-down regulation of melatonin receptors after melatonin exposure, shows the important role of *time* in this regulation (Masson-Pévet et al., 1993). This window of time sensitivity of the melatonin receptor is critical for the activity of melatonin and is found in both the rat and human between 1700 and 2000 h. Because melatonin exhibits a physiological circadian rhythm, this rhythm needs to be conserved with high concentrations during the night in humans (peaking at the beginning of the night from 1900 to 2000 h) and low concentrations during the day (falling in the early morning between 0500 and 0800 h). This is particularly important for long-term treatment with melatonin.

6. *Pharmacokinetics and Metabolism of Exogenous Melatonin*

In physiological conditions, melatonin is secreted only during the night, and concentration levels vary in humans and during the night from 30 to 200 pg/ml^{-1} (Waldhauser and Dietzel, 1985). Following oral administration of 80 mg of melatonin in gelatin capsules, the absorption half-life has been reported as 0.4 h, the elimination half-life has been reported as 0.8 h, and the melatonin levels range from 350 to 10,000 times those occurring physiologically (Waldhauser et al., 1984). Other pharmacokinetic studies have shown that depending on the dose, the time of administration, and the oral preparation (e.g., gelatin capsule), the half-life of melatonin and the plasma melatonin concentrations can vary from one to another. To have an idea of what the plasma concentrations in humans would be after different doses of melatonin and hypothesizing what the consequences would be of an inappropriate timing of dosing, particularly in the case of high doses, a simulation has been done (Bouzom and Merdjan, unpublished data) in the following conditions: (1) physiological circadian rhythm; (2) exogenous administration of melatonin at night (5 and 80 mg orally); (3) exogenous administration in the morning, at noon, and in the evening (5 and 80 mg orally); and (4) administration of huge doses of melatonin.

Methodology

The curve showing the physiological plasma level of melatonin was obtained from a representative subject receiving placebo in a study including 33 young healthy male volunteers (Fig. 1).

The simulated curves showing plasma levels of melatonin after oral administration of 5 and 80 mg were calculated from data published by Matthews et al. (1981). This article presents data observed in 2 subjects after single oral administration of 2.5 mg of melatonin. From average concentrations, the elimination half-life of melatonin has been evaluated at 1.2 h. Then it has been assumed that the kinetics of melatonin is linear, which means that plasma concentrations of melatonin increase proportionally to the administered dose. By using this hypothesis and the elimination half-life, plasma concentrations of melatonin have been calculated after single oral administration of 5 and 80 mg of melatonin. Plasma concentrations calculated (Fig. 1) were in the range of those observed after single oral administration of 80 mg of melatonin by Waldhauser et al. (1984) and Dollins

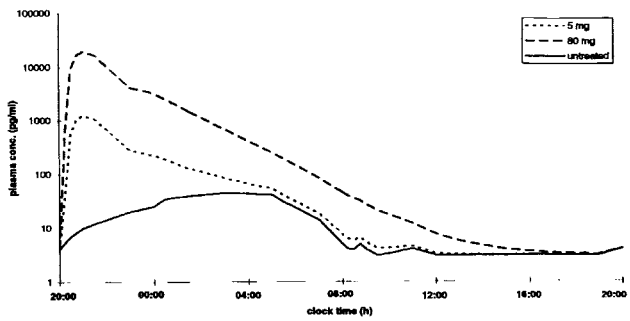

Figure 1. Plasma concentrations of melatonin (picograms/milliliter) simulated after single oral administration of 5 mg (dotted line) and 80 mg (dashed line) of melatonin in humans. The solid line represents typical spontaneous circadian fluctuations.

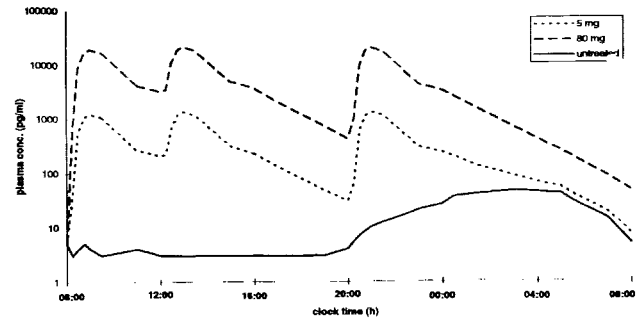

Figure 2. Plasma concentrations of melatonin (picograms/milliliter) simulated after repeated oral administrations of 5 mg (dotted line) and 80 mg (dashed line) of melatonin in humans. The solid line represents typical spontaneous circadian fluctuations.

et al. (1993). These data were used for the determination of the whole concentration time profile after repeated administration (Fig. 2) by summing the concentrations corresponding to each administration using the superposition principle.

Results

Figure 1 shows the physiological plasma level of melatonin in 1 patient. The levels are very low during the day (between 1 and 10 pg/ml), peaking at 2000 h and progressively reaching levels ranging from 10 to 100 pg/ml during the night (maximum obtained between 0000 and 0400 h). The levels then progressively decrease from 0400 h to return to basal value at 0800 h. After one dose of 5 mg given orally, the levels reached 1000 pg/ml within the hour following administration (10-fold times the noctural level), decreasing progressively to return to the basal physiological line at 0800 h. For 80 mg given orally, the levels reached more than 10,000 pg/ml within 1 h after administration, only returning to basal level at 1600 h.

Figure 2 shows the simulated plasma concentration of melatonin after repeated oral administration of 5 mg at 0800, 1200, and 2000 h. The concentrations show three peaks reaching levels of 1000 pg/ml in the hour after dosing. Between 0800 and 1600 h, the concentrations stay high at levels of the same magnitude or higher than those obtained during the night in physiological conditions. In any case, the circadian rhythm disappears and the concentrations always are higher than the nocturnal physiological one or reach 10-fold this physiological one. After administration of 80 mg in the same conditions (i.e., 0800, 0000, and 2000 h, orally), the same profile is obtained with concentrations that always are higher than 1000 pg/ml, that is, 10-fold the nocturnal physiological one and with three peaks reaching 10,000 pg/ml (i.e., 100-fold the nocturnal physiological concentrations).

From these data, several comments can be made: First, unique oral dosing of 80 mg of melatonin induces levels of melatonin that are 100-fold higher than the nocturnal physiological one, and basal level returns only at 1600 h. These results mimic what is observed in photoperiodic animals during "short days exposure."

Second, the inappropriate timing of dosing of 5 mg of melatonin given orally (three times per day) induces a high level of melatonin that remains higher over a 24-h period than the nocturnal physiological one. The consequences of such a high level over a 24-h period never have been investigated, at least in animals. The question can be asked about the desensitization of melatonin receptors, particularly at the level of the SCN, and of the hypothalamus (status of the secretions of the hormone, particularly after long-term treatment).

Third, after dosing of 80 mg, the situation is more dramatic, and in this particular case another question can be asked about the quantity of metabolites synthesized and their possible pharmacological or deleterious activities.

Disruption of such a rhythm could be deleterious from a physiopathological point of view (1) if the time schedule of dosing is not appropriate (e.g., morning or noon instead of evening) or (2) if large doses of melatonin are absorbed conducting particularly after chronic treatment to a steady state of melatonin with-

out returning to the basal level when circadian rhythm is required and needs to be respected to reactivate the functionality of the receptor.

The reproductive system and the visual tract, for which circadian melatonin rhythms play a fundamental role, could be taken as examples to illustrate this point.

The reproductive system. As seen in point 4, the first property of melatonin that has been described is the activity of melatonin in reproduction. In hibernating animals, there is a gonadal regression after melatonin treatment in Syrian hamsters or induced by exposure to a decreasing natural photoperiod (garden dormouse) or exposure to short days (mimicking longer amplitude of melatonin exposure). This effect is mainly a central effect through the activation of the hypothalamus, which regulates luteinizing hormone (LH)-releasing hormone (RH) neurons and then follicle-stimulating hormone and LH release, stimulating or inhibiting the gonads. Such activity has been taken advantage of for the development of B-Oval (Applied Medical Research), a contraceptive pill (combination of melatonin and a synthetic progesterone) that is now in Phase 3. Obviously, contraceptive pills need a toxicological file before being put on the market. Strict timing schedule compliance (i.e., taken regularly and without skipping a pill) is necessary for the success of contraception.

Consequently, any observations, predictions, or hypotheses that the inappropriate timing of melatonin administration could cause serious and detrimental side effects on the reproductive system in humans (even humans are not yet considered as a "photoperiod species") should be further verified before its administration to humans.

Deleterious effect of melatonin on the eye. In nonmammalian retinas, light and dark adaptational reactions in photoreceptor cells and retinal pigment are driven by an intrinsic oscillator that controls a circadian rhythm. During the day, cones contract, rods elongate, and pigment granules migrate into the optical villi of retinal pigment epithelium cells. The inverse retinomotor occurs at night. These daily rhythms are controlled by dopamine during the daytime and melatonin during the nighttime. Such a mechanism has not been demonstrated in mammals, but the rhythm of photoreceptor renewal has been shown to be modulated by both dopamine and melatonin. From these pharmacokinetic data, we can again formulate the hypothesis that inappropriate melatonin administration or high doses of melatonin could induce form deprivation myopia, intraocular pressure glaucoma, or an increase of the phagocytosis of retinal pigment epithelium inducing age-related maculopathy (Nguyen-Legros, 1996; Nguyen-Legros et al., 1996). Such a hypothesis should be verified before administration to humans.

Metabolites of melatonin are mainly (1) 6-sulfatoxy-melatonin, (2) kynurenamines, (3) *N*-acetyl-5-methoxy-kynurenamine (AMK), and (4) *N*-acetyl-2-formyl-5-methoxy-kynurenamines (AFMK). AMK has been shown to accelerate the reentrainment of melatonin rhythm following a phase advance of the light-dark cycle in male rats (Kennaway et al., 1989) and also is a potent inhibitor of prostaglandin synthesis. AMK also is able to inhibit diazepam binding from brain synaptosomes. AMK and AFMK have been shown to inhibit sexual development in a protein-restricted prepubertal rat model (Kennaway et al., 1988).

Because the melatonin synthesis pathway comes from L-tryptophan, it is of interest to consider its metabolization. Among several metabolites, L-kynurenine has been reported to have a convulsant effect and quinolinic acid to be a neurotoxin with neuroexcitatory activity at the level of the *N*-methyl-*D*-aspartate receptor (Huether et al., 1992; Heyes et al., 1994). Once more, the pharmacological and pharmacodynamic effects of melatonin and/or of its metabolites need to be taken into account for assessment of the safety of melatonin, particularly when huge doses of melatonin have been absorbed.

7. What Could Be the Toxicological Studies for Melatonin?

So far as we know, to date no toxicology in the regulatory sense of the term has been undertaken on melatonin. It is sold over the counter in the United States even though no security studies have been performed. In France, melatonin is considered a neurohormone and, as such, is not freely sold. In subjects taking melatonin, no evidence of toxicity in a strictly conventional sense of the term has been reported (i.e., no deaths or serious accidents). However, secondary effects have been reported such as gastrointestinal disorders, hypotension, headaches, and nightmares. As we saw in point 6, these secondary effects could be linked to pharmacological activity or pharmacodynamics and metabolism of melatonin. Theoretically,

Table 1. Timing of toxicological studies versus clinical trials.

Phase I: single dose	Single dose studies in two rodent species, one or two routes of administration
	Genotoxicity: two in vitro tests (bacterial test and chromosome aberration test)
	Repeated dose studies: 2 weeks in two species (rodent and nonrodent)
Phase I: repeated dose up to 7 days	Repeated dose studies: 4 weeks in two species (rodent and nonrodent)

In consequence, 2-week studies are of little interest, and generally 4-week studies are directly performed, including a careful examination of reproductive organs (first stage of reprotoxicology). *Furthermore*, given that the day-for-day strategy is now accepted (animals vs. humans), 4-week studies allow starting Phase II studies up to 4 weeks.

Phase II: for studies beyond 4 weeks	Repeated dose studies of 6 months in rodents and of 6 or 12 (9 in the future?) months in nonrodents
	In most cases, in practice, fertility and embryo-fetal development studies
	One or more tests for genotoxicity (one in vivo test)
	Starting of carcinogenic studies for drugs administered beyond 3 months, according to International Conference on Harmonisation (6 months in the European Community [EC] guideline)
Phase III	Peri- and postnatal development
	Result of the carcinogenic studies
	Other studies (mechanistic?)

there could be any number of adverse reactions when a natural product is administered at highly supraphysiological doses. Therefore, these reactions could be much worse when a neurohormone is administered.

Taking into consideration this neurohormonal action, the strict regulatory studies listed in Table 1 should be performed to evaluate the toxicity and security of melatonin (or its synthetic analogs) before using it over a long period in humans. Analytical data concerning the purity of the neurohormone also must be taken into consideration because impurities could affect the results of genotoxicity tests.

Preclinical studies play an important role in the FDA's evaluation of human drugs. The current law requires premarket approval for both safety and efficacy of all "new" drugs (Merrill, 1996). Investigation of therapeutic agents in humans has long been accepted; accordingly, the primary evidence of safety comes from clinical and not laboratory studies. However, animal studies are the sole source of information about a substance's biological effects before human trials are begun, and their results influence not only the decision whether to expose human subjects but also the design of clinical protocols.

In the particular case of melatonin, an adjustment to, or the setting up of, appropriate studies according to different therapeutic indications should be envisaged. As seen earlier on, these studies also should take into account the pharmacokinetics of the product: (1) duration and amplitude of secretion, (2) respect of nycthemeral and circadian rhythms, (3) transposition of the effects of administration of melatonin (or its analogs) in nocturnal animals to humans (who are a diurnal species), (4) respect of timing and consequences of inappropriate timing of administration, (5) chronic and long-term administration, and (6) metabolism studies.

8. Side Effects Directly Linked to the Pharmacological Profile of Melatonin

The side effects (mild or severe) that can occur after administration of a drug are more often directly linked to the pharmacological profile of this drug or its metabolites. As seen in point 4, there is a great analogy between melatonin and serotonin (its precursor) so far as their pharmacological targets are concerned.

Cardiovascular system. Depending on the concentrations of melatonin or the preparation used, melatonin can exert either a vasoconstrictory effect at physiological concentrations (nanomolar) or a vasodilatory effect at higher concentrations (micromolar or millimolar), suggesting a biphasic pharmacology of melatonin (Mahle et al., 1995). The subcellular mechanism of such an activity is as yet unknown despite the fact that melatonin receptors have been identified in different structures (arteries). Melatonin and its main target, the SCN, are able to modify cardiovascular rhythms (e.g., blood pressure, heart rate). Kawachi et al. (1995) showed a higher risk of coronary heart disease in shift workers. Taken together, these data, among others, show that melatonin could modulate the rhythmicity of the cardiovascular system. Again, alterations of the circadian rhythmicity of melatonin could be deleterious from a long-term effect point of view.

Gastrointestinal system. Recent studies have shown the existence of melatonin binding sites at the level of the gastrointestinal tract and that melatonin could modulate intestinal motricity. Several studies performed in humans have reported that nonsteroid antiinflammatory drugs produce a greater number of undesirable side effects including gastric ulcerations when delivered in the morning rather than in the evening, which shows a time-dependent effect (Moore and Goo, 1987). Melatonin has a worsening effect incidence on these ulcerations. These scientific facts give support to keeping a constant watch over the apparition of ulcerations after chronic treatment with melatonin, particularly when combined with antiinflammatory treatment.

Glucose metabolism. In humans, circadian (or circannual) variations of glucose tolerance, glycemia, and insulinemia have been reported (Jarett and Keen, 1970; Karvonen et al., 1993). Modulation of glucose metabolism by melatonin in rats has been reported despite sometimes contradictory results. We recently have shown that melatonin was able to suppress hyperglycemia caused by intracerebroventricular injection of 2-deoxy-*D*-glucose (Shima et al., in press), confirming its antihyperglycemic effect in rats. Again, such data, when transposed to humans, give support to keeping a constant watch on the glucose (and lipid) metabolism in people receiving chronic treatment, by melatonin, particularly in diabetes Type I, in which patients receive insulin treatment, or diabetes Type II, which more often is treated by sulfonylureas. Again, an inappropriate time schedule of melatonin dosing could have deleterious effects because many counterregulatory hormones of glucose metabolism are synthesized by the hypothalamus, which is "driven" by the SCN.

Cancer and immunology. It has been shown in animals that tumor growth was accelerated by pinealectomy and that restoration of normal melatonin levels in pinealectomized animals was able to inhibit this tumor growth. This property has been taken advantage of by clinicians, particularly in the treatment of breast cancer, with encouraging results (Blask et al., 1991). These effects of melatonin probably implicate its immunoregulatory role. Activation of melatonin receptors enhances the release of Th cell cytokines, such as γ-interferon and interleukin-2, as well as opioid cytokines. These mediators may counteract secondary immunodeficiencies, synergize with interleukin-2 in cancer patients, and affect hematopoiesis. The latter apparently is influenced by the action of melatonin-induced opioids on *x*-opioid receptors present on stromal bone marrow cells. In combination with cancer chemotherapeutic drugs, the results obtained so far are disappointing. Melatonin seems to worsen the bone marrow toxicity of common cancer chemotherapeutic regimens (Maestroni and Conti, in press). Again, these data lead us to believe that a careful watch needs to be kept on hematopoiesis before administration in humans and particularly in people treated by chemotherapy.

Conclusion

The analysis of hypothetical side effects or adverse reactions linked to the pharmacological profile of melatonin is supported by results of a recent clinical study (Nagtegaal et al., 1997). In this open study, 97 patients with circadian rhythm disorders were treated with melatonin (5 mg administered every evening 5 h before endogenous melatonin production) for 2-12 months. The adverse drug reactions were spontaneously reported by the patients. According to this publication, 25 patients mentioned a total of 35 adverse drug reactions including fever, hyperkinesia, dizziness, gastrointestinal disorders, headaches, hemorrhages, pigmentation, ankle edema, flushing, diplopia, hepatic pain, thrombosis, and hyperglycemia (in patients with diabetes Type I on insulin treatment). For each adverse drug reaction, the authors tried to give an explanation based on the pharmacological mechanism of the hormone. In each case, their explanation fit with the preceding hypotheses, developed in point 8 and based on the up-to-date known pharmacology of melatonin and/or its metabolites.

As the toxicology of melatonin is concerned, nothing at all is known. Therefore, if melatonin is to be considered as a neurohormone, then a lot of work on toxicology needs to be undertaken. In addition to regulatory studies, it should be taken into account that melatonin displays both a circadian and circannual rhythm and is secreted only during the night. This physiological rhythm needs to be conserved or modulated (i.e., advanced, reversed, diminished, or amplified) according to the appropriate therapeutic indications (Lamberg, 1996). All this information shows that safety evaluation and risk assessment of melatonin need to be seriously undertaken, taking into account the originality and the specificity of this

neurohormone and after having properly defined its therapeutic interest as well as dosage and duration of treatment.

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