Clinical aspects of melatonin

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ABSTRACT

يتم إنتاج الميلاتونين من الغدة الصنوبرية لدى الإنسان، خاصة في الليل، مع كون النظم اليومي لمستويات الميلاتونين في الدم قريبة ومتوازية، ويتم إنتاجها من الغدة الصنوبرية. يؤثر التعرض للضوء في الليل أو عبر الظهيرة بشدة على الإنتاج اليومي للميلاتونين. يساهم النظام اليومي المضطرب في عدم كفاءة النوم، ويتحسن ذلك بواسطة التحكم في الميلاتونين. يعتبر الميلاتونين أيضاً، مضاد للأكسدة وكانس للبقايا عالي المفعول. في هذه الكمية، يقوم الميلاتونين بتقليص التكوين المتكدس للتجارب والإصابات الكدمية للحبل الشوكي والدماغ ويحمي ضد تلف التأكسد للعصبات والدبق في نماذج الجلطات الدماغية، وأمراض باركنسون والزهامير، إضافة إلى أن الميلاتونين والاستقلاب فعالين بصورة عالية في الحماية ضد الإشعاع المؤيني. أخيراً، قد يكون الميلاتونين علاجاً لارتفاع ضغط الدم. الميلاتونين ذو فعالية عالية، وأمان عالي، وقلة السمية تجعله ذو أهمية في العلاج السريري.

Melatonin is produced in the human pineal gland, particularly at night, with the circadian rhythm of blood melatonin levels closely paralleling its production within the pineal gland. Light exposure at night, or rapid transmeridian travel severely compromises the circadian production of melatonin. The disturbed melatonin rhythm contributes to jet lag and sleep inefficiency, both of which are improved by melatonin administration. Melatonin is also a highly effective direct free radical scavenger and antioxidant. In this capacity, melatonin reduces experimental cataractogenesis, traumatic injury to the spinal cord and brain, and protects against oxidative damage to neurons and glia in models of stroke, Parkinsonism, and Alzheimer's disease. Additionally, melatonin and its metabolites are highly effective in protecting against ionizing radiation. Finally, melatonin may be a treatment for hypertension. Melatonin's high efficacy, its high safety profile, and its virtual lack of toxicity make it of interest in clinical medicine.

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Enormous advances have been made in the last 2 decades regarding the potential utility of melatonin (N-acetyl-5-methoxytryptamine), a secretory product of the human pineal gland,[1] in disease management. The results of several ongoing clinical studies will likely provide additional valuable information and further impetus for the use of melatonin in the clinical setting. Even today however, the results of numerous experimental investigations as well as descriptions of its successful use in humans, certainly support its consideration in pathophysiological situations. Melatonin was initially isolated from the bovine pineal gland, and subsequently structurally identified roughly 50 years ago.[2] This was a remarkable discovery considering that, at that time, the pineal gland was considered by most scientists and certainly by clinicians to be a vestigial non-functional outgrowth of the diencephalon. Due to the discovery of the multiple actions of its secretory product, melatonin, in the intervening years, it is now known that the gland is not only not a morphological remnant, but is an important organ whose function is relevant to a number of pathophysiological situations.[3,4] The current survey will summarize some of the known functions of melatonin, and describe how this indoleamine has been used at the clinical level.

Melatonin production and the light:dark cycle. Within the pineal gland melatonin is a product of the metabolism of tryptophan, an amino acid consumed in the diet. The molecular aspects of its biosynthesis have been worked out in significant detail, this information is available in a number of reviews dedicated to this subject.[5,6] One of the most interesting aspects of its synthesis is that, within the pineal of all vertebrates including man, melatonin's production is relegated almost exclusively to the daily period of darkness, for example, night. During the day, the gland is essentially dormant in terms of the generation of melatonin. This changes however, with darkness onset, when

norepinephrine (NE) is released from the postganglionic sympathetic nerve terminals that innervate the gland, and end in the vicinity of pinealocytes, the functional units of the gland. After its interactions with ß and α-adrenergic receptors in the pinealocyte membrane, NE promotes a cascade of events that culminate in the rapid conversion of tryptophan, via the intermediary serotonin, to melatonin (**Figure 1**). The synthesis of melatonin is followed by its rapid discharge from the pinealocyte into the rich capillary bed that pervades the gland, and possibly directly or indirectly into the cerebrospinal fluid (CSF) of the third ventricle. As a result of its nocturnal synthesis and rapid release, blood and CSF levels of the indoleamine are significantly higher at night than during the day (**Figure 1**).[1,7] Typical daytime levels of melatonin in the circulation of humans are 10 pg/ml serum or less, while at night they typically range from 50-150 pg/ml. This circadian melatonin rhythm exists in all vertebrates and is unrelated to the behavioral characteristics of the species; thus, whether animals are diurnally active, nocturnally active, or whether they have a crepuscular activity pattern, pineal melatonin synthesis and release, uniquely occur at night. Because of this, melatonin is referred to as the "chemical expression of darkness".[6] Given that pineal melatonin synthesis requires darkness, the world-wide use of artificial light is eroding the ability of humans and animals (in the vicinity of humans) to produce this important indoleamine. Extension of the daily light period into the night, which virtually all cultures currently do, truncates melatonin production, and decreases the total amount of melatonin generated on a daily basis.[8] As a result during the course of a lifetime, modern day humans are producing much less melatonin than they would be, if they were under natural photoperiods with light and darkness, being determined by the rising and the setting of the sun. To make matters worse, it is not uncommon for humans to turn on a light if awakened at night. Depending on the brightness (intensity) and wavelength (color) of that light, blood melatonin levels may drop precipitously. The reduction in total melatonin produced due to misuse of light and/or light pollution, which is essentially ubiquitous in urban areas particularly as societies become economically advanced,

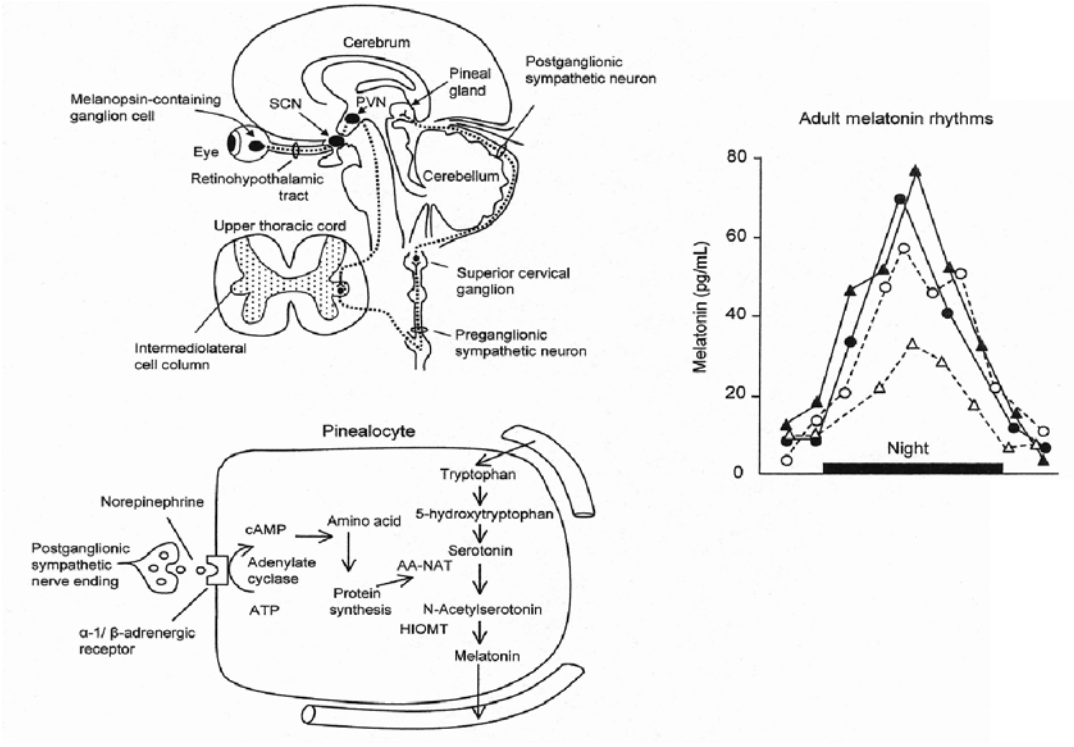

Figure 1 - Neural connections between the eyes and the pineal gland (upper left), melatonin synthesis from tryptophan in the pinealocyte (lower left) and nocturnal rises in melatonin in the blood of 4 adult humans (right). Retinohypothalmic fibers from the retina project to the biological clock (the suprachiasmatic nuclei or SCN); from the SCN, axons travel to the paraventricular nuclei (PVN) which sends descending fibers to the intermediolateral cell column (preganglionic sympathetic neurons) in the upper thoracic cord; the final synapse (on postganglionic sympathetic neurons) occurs in the superior cervical ganglia. Norepinephrine (which is released at night) induces the conversion of serotonin to melatonin via 2 enzymes, N-acetyltransferase (AA-NAT) and hydroxyindole-O-methyltransferase (HIOMT). Once produced, melatonin is quickly released, which causes the nighttime rise in blood melatonin levels.

may be a greater problem in terms of human health than originally realized. Given the widely beneficial actions of melatonin, its inadvertent inhibition by artificial light-at-night may have consequences that are now only becoming apparent. As an example, epidemiological studies have revealed that individuals who are deprived of nocturnal darkness due to chronically working at night, exhibit an increased risk of cancer.[9] In essentially all of these reports, it was proposed that the reduction in melatonin, a known anti-cancer agent, may have been responsible for the higher cancer frequency. It is also conceivable that other diseases may be either precipitated, or aggravated by what is being referred to as a relative hypomelatonemia resulting from excessive light exposure.

Jet lag. Melatonin has the capability of synchronizing the biological clock (the suprachiasmatic nuclei [SCN] in the brain), and thereby stabilizing 24 hour or circadian rhythms. These rhythms, which most functions of the body exhibit, are important for optimal health and well being.[10] In most species, the SCN contains an abundance of melatonin receptors, which respond to fluctuating levels of the indoleamine in the blood.[11,12] A major disturbance of circadian rhythms is experienced during the phenomenon referred to as jet lag. Long haul transmeridian flights over multiple time zones are frequently associated with poor sleep quality upon arrival, fatigue, increased headache frequency, inability to optimally perform mental or physical tasks, and gastrointestinal disturbances, among others. These symptoms are a result of the slow adjustment of the SCN to the new time zone, so the bodily functions are not in synchrony with the new environment.[13] Recovering from jet lag typically requires several days to a week, depending on the number of time zones transversed.[14] Commonly, easterly flights cause greater jet lag in most individuals, while flights in the westerly direction are less consequential.[15] To illustrate how potentially detrimental jet lag may be, the experimental findings of Filipski et al[16] and Davidson et al[17] are noted. In their studies with rodents, simulated long haul travel in an easterly direction caused more rapid growth of transplanted tumors and premature death of the animals, relative to those maintained under stable environmental conditions. Given these findings, it is not surprising that one of the first proposed uses of melatonin was in the treatment of jet lag. This suggested use was understandable considering melatonin's ability to act on, and synchronize the activity of the SCN, and thereby other bodily rhythms as well.[18] The best way to alleviate the symptoms of jet lag would be to more rapidly adjust the biological clock to the new environment.[19] The 24 hour melatonin cycle,[5] which is a reflection of the activity of the SCN, is markedly disturbed during jet lag. Re-synchronizing the circadian timing system, including the melatonin cycle, facilitates adaptation of the individual to the environmental changes associated with rapid transmeridian travel and improves physiological performance. A large number of investigations have confirmed the effectiveness of melatonin ingestion in reducing the symptoms of jet lag.[20-23] The usual dose taken orally in these studies, is 3-5 mg ingested before bedtime (3 hours to 30 minutes before the desired sleep). The majority of the studies employed subjective means, usually questionnaires, in evaluating the severity of jet lag symptoms after melatonin ingestion; most often the questions related to the degree of fatigue and sleep efficiency. As an example, in their study Suhner et al[24] concluded that melatonin usage improved sleep (assessed in the morning), and reduced sleepiness, lethargy, and fatigue (assessed in the evening). This latter finding importantly indicates that the sleep promoting activity of melatonin is not carried over to the following day. The measured outcomes of the melatonin/jet lag reports have been subjected to several meta-analyses.[25-27] These examinations of the published data indicate that melatonin is generally beneficial in improving the symptoms of jet lag particularly in reducing sleep latency, improving the quality of sleep by reducing awakenings, and curbing daytime fatigue. Whether the success of melatonin as a jet lag treatment relates to its natural ability to promote sleep, or to its ability to adjust the functioning of the biological clock is yet unresolved. While the collective data almost overwhelmingly document the benefits of melatonin in jet lag treatment, in a few cases the outcomes have been less convincing. In addition to the use of melatonin, there may be other strategies to assist in coping with jet lag. Some of these appropriately include time light exposure, behavioral adjustments; in other words, exercise, the use of hypnotics, and the use of drugs that enhance daytime alertness.[28]

Sleep. Sleep induction has also been a major interest for melatonin researchers.[29,30] Sleep problems are remarkably widespread, and the causes are highly diverse. Sleep, a lessened state of consciousness, has a variety of identifiable characteristics including changes in the central nervous system typically evaluated by means of polysomnography. There are 2 distinct stages of sleep known as rapid eye movement, or REM sleep, and non-rapid eye movement (NREM) sleep.[31] Unlike many other mammals, humans have a propensity to consolidate sleep to an uninterrupted 7-8 hour period at night thus, when this pattern is disturbed, the period of wakefulness is made more difficult. In humans, the period of sleep at night is associated with elevated melatonin levels, but sleep per se does not induce pineal melatonin synthesis, rather, the nighttime rise

in elevated pineal activity is a function exclusively of darkness. While in humans the association between high circulating melatonin levels and sleep is typically in phase,[8] in many animals, for example nocturnally active species, the rhythms are 180 degrees out of phase with each other. In humans, sleep patterns change over the course of a lifetime. In newborns, sleep occurs any time throughout the 24 hour period, and becomes more-or-less confined to the night several months after birth at about the same age the low day time/high nighttime melatonin cycle matures.[32] In the elderly, sleep efficiency diminishes, sleep architecture deteriorates and it becomes fragmented and this correlates with a reduction in the nighttime rise in melatonin.[33] In patients suffering with insomnia who are older than 55 years of age, the mean level of the nighttime blood melatonin rise is much less than that of age-matched individuals without insomnia.[34] Because of these relationships, interest in the use of melatonin to correct sleep problems has become an active area of clinical investigation. Melatonin therapy to improve sleep in the elderly has been the subject of numerous investigations. Certainly, the ability of melatonin to improve sleep in the older population has proven successful for the most part.[35] Simultaneously, the use of melatonin by these individuals also commonly improves the quality of life, which would be expected considering the relationship between high quality of sleep, and an improved feeling of well being. For sleep in the elderly, commonly suggested doses of melatonin range from 250 μg to 10 mg nightly before bedtime. Alzheimer's disease (AD) is a late life-onset condition that is often accompanied by sleep disturbances. Patients with AD also have a more greatly attenuated melatonin rhythm than do elderly individuals who do not have this condition.[36] Melatonin replacement therapy has been shown to prolong nocturnal sleep time in AD patients.[37,38] Additionally, their scores on standardized cognitive and non-cognitive tests improved during melatonin treatment.[37] Melatonin therapy for improving sleep has been tested in a very wide variety of divergent sleep patterns and poor sleep quality in adult humans, and whereas it has frequently been beneficial, it is not always so.[27] The failure to improve sleep may relate to the widely different causes of poor sleep quality, thus, some respond to melatonin therapy while some individuals do not. Given the positive correlation between good sleep hygiene and reduced cardiovascular risk and slowed neurodegenerative decline, any pharmacotherapy that enhances sleep may well have benefits beyond improved nighttime rest. Melatonin therapy has also been successfully used to correct pediatric sleep problems. Childhood sleep disturbances are a major reason parents approach pediatricians. Disturbed sleep in children predisposes them to poor health as well as memory, and learning difficulties. Because of the role that melatonin has in improving sleep, the indoleamine is among the 8 most common medications prescribed by child psychiatrists in Britain.[39] A leading group in the area of the use of melatonin for aiding sleep in children is that of Jan et al.[40] Based on the published literature, it appears that 70-90% of children with circadian rhythm sleep disorders, respond with improved sleep quantity and quality when melatonin therapy is instituted.[41,42] However, not all children respond with the same degree of sleep improvement. Children who have degenerative brain diseases, massive brain damage, or neural tumors may benefit less than other children.[43] Also, persistent early morning awakening, which is common in children with neurodevelopmental disorders, is more difficult to successfully treat. In general, melatonin supplementation for the purpose of improving sleep in children is most effective when endogenous melatonin secretion is low, and the nighttime melatonin rise is attenuated. Exogenous melatonin treatment for sleep disorders is less effective when a normal light:dark melatonin cycle exists. Based on these observations, it is apparent that melatonin works differently than hypnotic drugs used to promote sleep. According to Jan et al,[44] melatonin is an effective therapy in many children with sleep disturbances, and the indoleamine has a benign safety profile; the development of tolerance to melatonin has never been observed. The therapeutic dose of melatonin to improve sleep in children is based on the clinical response.

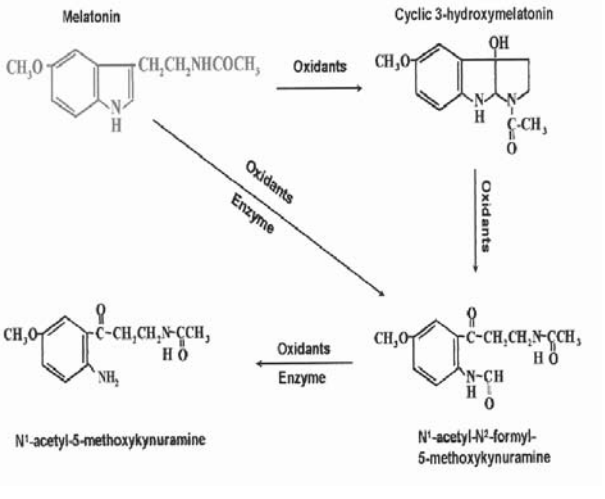

Figure 2 - Melatonin produces each of these metabolites when it functions as a free radical scavenger. Moreover, these products themselves are free radical scavengers. The successful neutralization of radicals in this process is referred to as the antioxidant cascade. Via this cascade, melatonin may scavenge as many as 10 radicals.

Reduction of oxidative stress. The discovery that melatonin is a direct free radical scavenger,[45,46] and indirect antioxidant[47,48] has been a major stimulus for investigations on the role of this indoleamine in a variety of diseases,[49,50] and processes that generate toxic oxygen derivatives.[51,52] While the initial report documented melatonin's ability to scavenge the highly toxic hydroxyl radical,[45] subsequent studies have shown melatonin to be capable of neutralizing a wide variety of reactive oxygen and nitrogen-based toxic molecules.[53-56] Importantly, not only is melatonin itself a radical scavenger, but so are several of its metabolites, which are formed when the indoleamine functions in the capacity of a direct antioxidant. These products include cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxylkynuramine (AFMK), N1-acetyl-5-methoxykynuramine (AMK), and possibly others (**Figure 2**). In this reaction cascade, a single melatonin molecule may scavenge as many as 10 free radicals.[57]

In addition to the direct actions of melatonin as a free radical scavenger, the indole stimulates a variety of enzymes that detoxify radicals and their products. These enzymes are critical for optimal antioxidative defense, and they include the superoxide dismutases, glutathione perioxidase, and catalase.[46,47,58,59] Since these seminal observations, melatonin has captured the interest of many researchers who have now tested melatonin as a therapy in a large variety of experimental and clinical conditions. The following sections consider some of these data where the findings have clear clinical implications. The literature cited for each of these conditions is in no way exhaustive, but the discussion directs the reader to some of the most germane literature.

Cataracts. Cataract is a common cause of blindness in many countries, and lenticular surgery is the most frequently performed operation in patients older than 65 years of age. Non-congenital cataracts develop when the lens of the eye becomes opaque due to molecular degradation, caused most frequently by the hydroxyl radical.[60] The immediate precursor of the hydroxyl radical, in other words, hydrogen peroxide is elevated above normal in the aqueous and vitrous humors of human cataractous eyes,[61] and likewise nitric oxide is higher in the aqueous humor of humans when traumatic cataracts are present.[62] Given that nitric oxide couples with the superoxide anion radical to form the peroxynitrite anion, the latter highly toxic reactant also is believed to contribute to lenticular damage, and cataract formation. After the information was uncovered that melatonin is a free radical scavenger, there was a reasonable expectation that it would reduce cataract formation. Melatonin has easy access to the lens, since it is measurable in the fluid of the anterior chamber of the eye,[63] and furthermore, it is reportedly synthesized in lenticular tissue.[64] A commonly used model of cataract formation is to inject newborn rodents with L-buthionine-S, R-sulfoxamine (BSO); BSO is an inhibitor of gamma-glutamylcysteine synthase, the rate limiting enzyme in glutathione biosynthesis. Depletion of glutathione in the lens quickly leads to massive oxidation of lenticular proteins, and other molecules causing the formation of cataracts.[65] Glutathione is an important antioxidant in the lens. To determine whether melatonin could compensate for the reduction of glutathione in BSO-treated rats and reduce cataractogenesis, Abe et al[66] intraperitoneally injected melatonin daily into the newborn, glutathione-depleted rat pups. One hundred percent (18/18) of the rat pups that received BSO only had cataracts when they were 16 days old. Conversely, only 1 of 15 rats that were glutathione-depleted but who had received melatonin had obvious cataracts. All BSO-treated rats, whether they had or had not received melatonin had greatly reduced lenticular glutathione levels although they were partially preserved in the lens of melatonin-treated animals. The authors theorized that melatonin prevented cataracts by functioning as a free radical scavenger in the lens, and due to melatonin's ability to partially preserve levels of another important endogenous antioxidant, glutathione. Using the same animal model, Liet al[67] also documented that melatonin is readily capable of reducing opacification of the lens in glutathione-depleted rats. This group also showed that the level of lipid peroxidation products in the lens of BSO only treated rats was significantly reduced by giving melatonin. This observation is consistent with melatonin and/or its metabolites, quenching free radicals in the lens. Ultraviolet light (UV) exposure is commonly incriminated as a cause of lenticular opacification given that these wavelengths induce free radical generation. Since the damaging effects of UV radiation involves molecular oxidation in the lens, it is not unexpected that melatonin would protect the lens from damage in this case. When tested, both Bardak et al,[68] and Anwar and Mustafa[69] indeed found that melatonin reduced the opacification of UV-exposed lenses. Likewise, melatonin also prevents gamma radiation-induced cataracts.[70] There is a number of essential antioxidants including glutathione, ascorbate, and the antioxidative enzyme, superoxide dismutase, in lenticular tissue. Clearly, melatonin also functions in protecting the lens from oxidative damage; besides functioning as a direct free radical scavenger, melatonin may act synergistically with other antioxidants in preserving lens clarity.[71] The lens is avascular and, therefore, must depend either on melatonin absorbed from the aqueous humor, or after its synthesis locally.[64] Melatonin is also produced in the human ciliary body from where it can be released into the fluid of the anterior chamber. Melatonin receptors

have been identified on the lens surface;[72] these may mediate the effects of melatonin on antioxidative enzyme activities in these tissues.

Brain injury. As an example of the protective effects of melatonin in the central nervous system (CNS), summarized here are the results of several recent reports that document the preservation of functional neural tissue, after it had been subjected to trauma. Genovese et al[73] used dexamethasone and melatonin, either in combination or alone, to limit tissue damage resulting from experimental spinal cord injury (SCI) produced by subjecting the mouse, surgically-exposed spinal cord (T6 and T7) to extradural compression using an aneurysm clip with a closing force of 24 g. Virtually every endpoint evaluated, exhibited improvement when the animals were treated with combined glucocorticoid and melatonin; these benefits were significantly greater than in mice treated with dexamethasone or melatonin only. The parameters measured included myloperoxidase activity, nitrotyrosine levels, inducible nitric oxide synthase activity, Bax and Bcl-2 expression, apoptosis, neutrophil infiltration, and routine morphology. Combining melatonin with dexamethasone also allowed Genovese et al[73] to significantly reduce the amount of glucocortiod given, without lowering the efficacy of the combined treatment. An elevated activity of calpain, a Ca2+ dependent neutral protease, is involved in the pathogenesis of tissue damage following SCI. To test the efficacy of melatonin in limiting calpain activation, Samantaray et al,[74] pummeled the exposed spinal cord of rats with a 5 g weight dropped from the height of 8 cm. The damaged cords of the melatonin treated rats, compared to those of diliuent-injected controls, exhibited less calpain activation, reduced astrocytosis, lower axonal damage (as indicated by lower levels of de-phosphorylated protein) and fewer apoptotic cells. Since the tissue destruction resulting from SCI is a multifactorial process, a molecule with multiple actions would be best suited for optimal benefit in the injured cord. Samantaray et al[74] feel that melatonin, because of its antioxidant and anti-inflammatory actions, may well be a useful treatment to minimize damage to the spinal cord following trauma. The contusion of SCI in rats induced a persistent 4-8 fold up regulation of the water channel, aquaporin-1 (AQP-1) in ependymal cells, astrocytes and neurons. This increase continued at the site of injury, in this study at T10,[75] for up to 11 months. Moreover, delayed AQP-1 increases were found in the cervical and lumbar cord indicating that AQP-1 up regulation spreads over time. It is believed that the persistent AQP-1 rise is due to chronic hypoxia following cord injury. When melatonin was used as a treatment in SCI rats, it significantly attenuated the up regulation of AQP-1 and, furthermore, it made the cutaneous area affected by the lesion much less painful as indicated by a significant reduction in mechanical allodynia. Spinal cord injury is only one of the numerous conditions/diseases of the CNS where free radicals take their toll in destroying neurons and glia. The actual ability of melatonin to incapacitate free radicals within living brain cells can be visualized with fluorescence microscopy, and the use of appropriate probes. Jou et al[76,77] have elegantly documented melatonin's ability to neutralize free radicals in mitochondria of glia and to prevent these cells from undergoing apoptosis. The mitochondria are a major site of free radical generation within cells. As with glial cells, neuronal apoptosis is likewise prevented by melatonin when oxidative stress is the initiator of the process.

In addition to the high efficacy in protecting against neural tissue loss, and functional deterioration in SCI, melatonin has also been successfully used to combat damage to the brain under a variety of situations and diseases, where other antioxidants have proven less effective. Thus, in conditions of craniocerebral trauma including closed head injury,[78] during ischemia/reperfusion (stroke),[79-82] in models of Parkinson's disease,[83-85] and Alzheimer's disease,[49,86,87] melatonin has been documented as a beneficial agent in limiting tissue destruction, and preserving neurobehavioral functions. Clearly, within the CNS melatonin's benefits seem to be numerous with the primary protective effects seemingly being due to the potent antioxidative actions of melatonin and its metabolites.[57,88,89]

Both Parkinsonism and Alzheimer's diseases have been linked to free radical-mediated degeneration of critical neurons in the CNS. Moreover, in every experimental model of these diseases where melatonin has been used to forestall neuronal loss and preserve function, the indoleamine has proven to be effective.[86,87,90,91] Likewise, in clinical studies that are yet limited in terms of numbers of patients and numbers of publications, melatonin has shown to be beneficial in improving neurophysiological behavior, and possibly slowing the progression of both Parkinsonism and Alzheimer's disease.[92-94] Collectively, the reports suggest melatonin may be useful in the treatment of this highly debilitating neurodegenerative disease.

Protection against ionizing radiation. Ionizing radiation mutilates molecules, and kill cells because it generates oxygen-based radicals and related toxic agents. While free radicals are continuously produced within cells because they use oxygen as a basis of energy production within mitochondria;[95] when tissues are exposed to ionizing radiation these reactants are produced in clusters, leading to extensive molecular damage.[96] Shortly after the discovery of melatonin as a hydroxyl radical scavenger,[45] the indole was soon

tested for its ability to overcome the cellular destruction normally inflicted by high energy radiation. These studies were particularly important since up to 70% of the damage induced by ionizing radiation is believed to be attributable to the hydroxyl radical. When Tan et al[45] exposed rats to whole body ionizing radiation they measured increased levels of cyclic 3-hydroxymelatonin in their urine; this cyclic derivative is formed when melatonin scavenges 2 hydroxyl radicals. Although in this study the degree of molecular damage was not measured, a protective effect of the indoleamine could be inferred from the elevated levels of urinary cyclic 3-hydroxymelatonin. The following year, Blinkenstaff et al[97] exposed rats to a lethal dose of ionizing radiation (950cGy whole body irradiation), and reported that administering melatonin or its homologues prior to the exposure improved the 12 day survival by 43%. Likewise, Vijayalaxmi et al[98,99] found that melatonin highly significantly improved the 30 day survival of mice subjected to 850cGy ionizing radiation, and furthermore, the indoleamine greatly attenuated DNA damage, as assessed using the micronuclear assay, in bone marrow. This is of great importance since the loss of blood cell progenitors in bone marrow contributes to death of the animals/humans exposed to high radiation doses. In a study in which melatonin was administered to humans followed by the exposure of their leucocytes in a collected blood sample to ionizing radiation, it was again shown that melatonin-enriched blood protected the DNA within cells from forming micronuclei; this is consistent with melatonin protecting the genome from radiation-induced damage.[100]

A reliable means of estimating free radical destruction to DNA is to measure levels of 8-hydroxy-2-deoxyguanosine (8-OHdG). When rats were exposed to whole-body radiation, high levels of 8-OHdG were noted in their livers with the response being highly significantly attenuated in animals given melatonin just prior to the radiation exposure.[101] Moreover, radiation also causes the oxidation of lipids in cellular membranes making them rigid; hepatic membrane rigidity was also reduced in the irradiated rats given melatonin. Amifostine (WR-2721), an amniothiol, is a pharmacological agent that has significant radioprotective activity. This molecule was compared with melatonin with the endpoint being the level of genotoxicity in human lymphocytes during their exposure to ionizing radiation.[102] By evaluating the incidence of micronuclei and sister chromatid exchanges, the authors concluded that amifostine and melatonin provide equivalent protection at the level of DNA against the hazards of high energy radiation. When the 2 agents were combined, the level of protection was even greater. While amifostine and melatonin may be equally effective as radioprotectors, the rather high toxicity of the former compound is not shared by melatonin. The amifostine has a number of toxic side effects including nausea, vomiting, hypotension, and hypocalemia.[103] Thus, while amifostine significantly reduces the functional capacity of an individual, melatonin does not. Also, to be effective, amifostine is given intravenously while melatonin can be taken orally.

Collectively, the findings suggest that melatonin would have greater utility than amifostine in situations where protection from ionizing radiation is desirable. The highly significant protective effects of melatonin have been critically summarized in several reviews.[104-106]

Blood pressure regulation. While there have been a number of drugs developed to reduce blood pressure (BP), the optimal control of hypertension is not widely realized, and high BP remains a major detriment to healthy living. There is evidence that melatonin may be beneficial in reducing the BP, and that it may function as an endogenous anti-hypertensive agent. In rats, surgical removal of the pineal gland, a procedure that eliminates the nighttime rise in blood melatonin levels, leads to a gradual development of hypertension.[107] Conversely, daily administration of exogenous melatonin prevents the augmentation of BP in rats lacking their pineal gland.[108] Finally, melatonin administration to spontaneously hypertensive rats not only reduces their hypertension but also diminishes renal inflammation, oxidative stress parameters, and the expression of nuclear factor-Kappa B in the kidney.[109,110] In humans as well, evidence is accumulating that melatonin levels may be consequential in determining arterial BP. While correlative only, BP typically gradually increases with age, coincident with the loss of the circadian melatonin rhythm.[111] Also, treatment of both healthy women and men with melatonin lowers their systolic, diastolic, and mean arterial blood pressure as well as lowering circulating norepinephrine concentrations.[112,113] Also, there is an obvious correlation between nocturnal blood melatonin concentrations, and daily variations in BP in humans.[114] Patients with no or only a slight drop in nighttime BP, the so-called non-dippers, have an impaired nocturnal melatonin rise relative to dippers, in other words, individuals whose BP decreases at night.[114,115] This is important since non-dippers have a higher risk of cardiovascular morbidity and mortality.[116] The results of the studies where melatonin has been administered are also compatible with the idea that this endogenously-produced indoleamine may normally be involved in BP regulation. In a placebo-controlled double-blind study, nighttime melatonin administration for 3 weeks lowered both nocturnal systolic and diastolic BP in otherwise untreated hypertensive men.[117] Likewise in hypertensive women, melatonin given daily reduced

systolic, diastolic, and mean BP without changing heart rate.[118] The indoleamine has a similar inhibitory action on BP in individuals with nocturnal hypertension,[119] and it reduced diastolic pressure in adolescents with Type I diabetes.[120]

Mechanistically, there are several means by which melatonin may function to lower BP/hypertension. Given that reactive oxygen and reactive nitrogen species are instrumental in the development of high BP,[121] the antioxidant actions of melatonin[122] may have aided in reducing the pressure within blood vessels. Melatonin also has an endothelium-dependent relaxation effect by itself, and causes an exaggerated response after acetylcholine administration.[123] In spontaneously hypertensive rats, melatonin improved bradycardia and tachycardia baroreflexes due to phenylephrine and sodium nitroprusside.[109] Finally, it has been postulated that melatonin may epigenetically modify the set point of neurons in the area postrema, resulting in a shift of the set point to a lower operating pressure.[124] Based on these findings and predictions, melatonin's ability to regulate BP may involve both receptor-independent as well as receptor-mediated actions. In addition to the potential mechanisms whereby melatonin reduces BP/hypertension, there may yet be undefined means by which melatonin modulates cardiovascular physiology.[125]

Conclusion. The pineal secretory product melatonin was isolated, and structurally identified approximately 5 decades ago. Since melatonin's discovery, research related to the heterogenous actions of this molecule has gradually untangled its multiple functions; the intensity of research has progressively expanded in the last 20 years. While initially shown to be effective in the treatment of jet lag and sleep disorders, melatonin's functional repertoire has expanded, to the extent that it is now believed to be important in forestalling, or reducing the severity of a number of disease conditions. For example, its utility has been documented in experimental models of cataracts, stroke, Parkinson's disease, Alzheimer's disease, and in reducing molecular damage due to ionizing radiation. A common denominator for these diseases is excessive free radical generation. Free radicals are readily directly scavenged by melatonin, or they are metabolized to innocuous products by enzymes whose activity is stimulated by melatonin. Not only melatonin, but several of its metabolites, are highly effective in detoxifying free radicals and related reactants. Endogenous melatonin levels diminish with increasing age. The loss of this beneficial molecule may plausibly be related to tissue deterioration associated with aging, and the development of a number of diseases commonly found in the elderly. Besides its endogenous production in the pineal gland and possibly other organs as well,[126,127] melatonin is also consumed in the diet given that essentially all plant materials examined have been found to contain this important indoleamine.[128-130] The evidence to date suggests that not only do plants likely synthesize melatonin,[131] but likewise they take it up from the medium in which they are grown.[130,132,133] Melatonin, when consumed in the diet, is absorbed into the blood.[128,134] Studies related to the potential importance of dietary melatonin in protecting against disease processes are now being initiated.

Melatonin has been widely tested in humans and animals. Its acute and chronic toxicity are remarkably low, and it has a very wide safety margin in terms of dose. It usefulness is currently being tested in humans in a number of clinical situations. Based on studies carried out to date, it is highly likely that melatonin will become a safe and expensive treatment, for a number of diseases/conditions in humans.

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