Received: 20 June 2016 | Accepted: 5 August 2016

DOI: 10.1111/jpi.12360

REVIEW ARTICLE

WILEY Journal of Pineal Research
Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Melatonin as an antioxidant: under promises but over delivers

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Abstract

Melatonin is uncommonly effective in reducing oxidative stress under a remarkably large number of circumstances. It achieves this action via a variety of means: direct detoxification of reactive oxygen and reactive nitrogen species and indirectly by stimulating antioxidant enzymes while suppressing the activity of pro-oxidant enzymes. In addition to these well-described actions, melatonin also reportedly chelates transition metals, which are involved in the Fenton/Haber–Weiss reactions; in doing so, melatonin reduces the formation of the devastatingly toxic hydroxyl radical resulting in the reduction of oxidative stress. Melatonin's ubiquitous but unequal intracellular distribution, including its high concentrations in mitochondria, likely aid in its capacity to resist oxidative stress and cellular apoptosis. There is credible evidence to suggest that melatonin should be classified as a mitochondria-targeted antioxidant. Melatonin's capacity to prevent oxidative damage and the associated physiological debilitation is well documented in numerous experimental ischemia/reperfusion (hypoxia/reoxygenation) studies especially in the brain (stroke) and in the heart (heart attack). Melatonin, via its antiradical mechanisms, also reduces the toxicity of noxious prescription drugs and of methamphetamine, a drug of abuse. Experimental findings also indicate that melatonin renders treatment-resistant cancers sensitive to various therapeutic agents and may be useful, due to its multiple antioxidant actions, in especially delaying and perhaps treating a variety of age-related diseases and dehumanizing conditions. Melatonin has been effectively used to combat oxidative stress, inflammation and cellular apoptosis and to restore tissue function in a number of human trials; its efficacy supports its more extensive use in a wider variety of human studies. The uncommonly high-safety profile of melatonin also bolsters this conclusion. It is the current feeling of the authors that, in view of the widely diverse beneficial functions that have been reported for melatonin, these may be merely epiphenomena of the more fundamental, yet-to-be identified basic action(s) of this ancient molecule.

KEYWORDS

diseases of aging, drug toxicity, free radicals, ischemia/reperfusion, mitochondria-targeted antioxidant, organ transplantation, statins

1 | INTRODUCTION: MELATONIN AND THE EVOLUTION OF IDEAS

The ubiquitous distribution and functional diversity of melatonin as currently envisioned far exceeds that of original expectations.[1] In particular, due to definitive studies within the last 15 years, melatonin has been linked to a wide range of functions including anti-inflammation, antioxidant, oncostatic, circadian rhythm regulation, etc. Likely prompted by early observations made on pinealectomized animals[2–4] prior to the discovery of melatonin,[5,6] this indoleamine was initially tested as to its effects on reproduction.[7–9] This led to the discovery that the changing photoperiod, with the pineal gland and the melatonin rhythm as intermediates, unequivocally regulates seasonal reproductive capability in photosensitive species.[10–12]

Seasonal breeding is of utmost importance because it ensures delivery of the young during the most propitious season, thereby improving the chances of survival of the young and the continuation of the species. That the introduction of artificial light at night, which is commonplace in the outdoor environment in economically developed regions, negatively impacts this essential annual cycle was recently reported in a publication from Australia. In a 5-year study on the seasonal reproductive behavior of tammar wallabies living in different photoperiodic environments, it became apparent that ambient light pollution disturbs this cycle.[13] The comparisons were made on wallabies living in a forested area without artificial light at night vs wallabies inhabiting a nearby urban area where anthropogenic light was rampant. In the wallabies witnessing light pollution at night, their circulating melatonin levels were suppressed and the birth of the young was delayed, that is, occurred at an unusual time of the year. These results were entirely predictable given that light at night is well known to interfere with the amplitude and the duration of the nocturnal melatonin signal,[14,15] which dictates the annual reproductive cycle of seasonal breeding mammals.[10,16] The finding also illustrates the dangers of exposing seasonally breeding wild animals to light pollution. This probably is becoming more common, since a number of wild species cohabitate very successfully with residents of urban communities and light pollution is becoming more widespread. The reproductive cycle may be only one of several metabolic disturbances the wallabies and other species experience when exposed to artificial light at night. For example, it may be increasing the frequency of some types of cancer and reducing the total antioxidative capacity of vertebrates.

Even before melatonin was identified, light microscopic observations on the pineal gland showed that its morphology depends on the light:dark environment to which the animals are exposed.[17,18] Both of these authors noted that the cytological changes in the pinealocytes during exposure of the animals to short days were consistent with presumed elevated synthetic activity. This portended that the physiology of the pineal gland may be impacted by the photoperiod. Whether these microscopic observations were, however, used as a justification for studies related to the dark-dependent synthesis of melatonin was not explicitly stated in the introduction to the reports where pineal melatonin production was documented to be confined to the dark phase of the light:dark cycle (Fig. 1).[19–21]

Initially, the rate limiting enzyme in melatonin synthesis was deduced to be hydroxyindole-*O*-methyltransferase (HIOMT), the enzyme currently known as *N*-acetylserotonin methyltransferase (ASMT).[22–24] However, after the discovery of the marked circadian rhythm in the activity of *N*-acetyltransferase (NAT), the enzyme that acetylates serotonin to form *N*-acetylserotonin,[25] interest in this step as being the determinant of maximal melatonin production peaked. While this assumption generally persists until today, there are some who have argued otherwise.[26] More recently, we have raised the issue as to whether the sequence of enzymatic events that convert serotonin to melatonin is always correct.[27] In some plant species at least, we feel that serotonin may be first methylated to form 5-methoxytryptamine followed by its acetylation to melatonin. Moreover, in plants, melatonin may not be the terminal product but rather an intermediate that is subsequently hydroxylated to 2-hydroxymelatonin.[28]

The key studies to confirm the essential nature of the sympathetic nerve terminals in the pineal as being important for its biochemistry and physiology[23,29] were driven by the meticulous morphological studies of Kappers;[30] he showed that the pineal received a rich postganglionic sympathetic neural input from perikarya located in the superior cervical ganglia. Subsequently, surgical removal of the ganglia was shown to biochemically and functionally incapacitate the pineal gland.[23,29] The loss of melatonin due to surgical removal of either the pineal gland or superior cervical ganglia eliminates all known functions of the pineal gland. Yet, in a 1965 report, it was claimed that melatonin synthesis (as judged by the elevated HIOMT activity) was actually increased after superior cervical ganglionectomy.[31] This error may have been made since, when the superior cervical ganglia are surgically removed, the degenerating nerve terminals in the pineal gland release stored norepinephrine that causes a transient rise in pineal synthetic activity.[32]

While melatonin, subsequent to its discovery, was never denied as being a pineal secretory product, it was not always promoted as the major pineal secretion. Rather than melatonin, a number of peptides extracted from pineal tissue, most of which were never structurally identified, were advanced as being the responsible agents for mediating the gland's action on the pituitary–gonadal axis.[33–36] So far as the authors know, there is only a single group that still believes there are

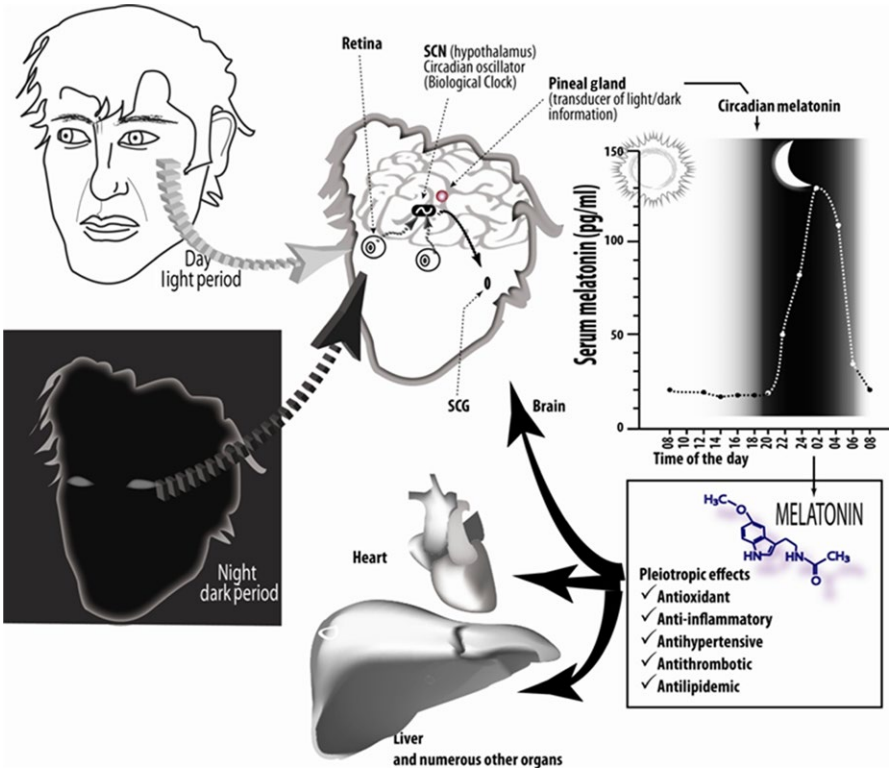

FIGURE 1 The nocturnal rise in the synthesis and release by pineal melatonin drives the circadian rhythms of melatonin in blood and cerebrospinal fluid (CSF). During the day, the central circadian oscillator (the biological clock in the suprachiasmatic nuclei, i.e., the SCN) receives neural messages from highly specialized intrinsically photoreceptive retinal ganglion cells (ipRGC) via the retinohypothalamic pathway in the optic nerves, which prevent the SCN from signaling the pineal gland to gear up melatonin production. When the inhibitory signal from the ipRGC (which are especially sensitive to blue wavelengths of visible light) is lifted at night, the SCN contacts the pineal gland via a multisynaptic pathway in the central and peripheral sympathetic nervous system, which is relayed through the superior cervical ganglia (SCN), to upregulate the melatonin synthetic machinery. The pineal releases melatonin directly into the third ventricular CSF where it generates a larger amplitude melatonin rhythm (not shown in this figure) than exists in the blood; it is the CSF rhythm, in our opinion, which modulates the activity of the SCN rather than the melatonin cycle in the blood. Melatonin released into the blood accesses every cell in the organism and presumably influences the circadian genes in these cells. Additionally, pineal-derived melatonin has numerous functions (some of which are tabulated in the box at the lower right) in multiple organs, where it protects critical molecules from pathophysiology. In addition to melatonin of pineal origin, melatonin produced in many (perhaps all) cells has similar beneficial actions in the cells where it is synthesized (autocrine actions) and in neighboring cells (paracrine actions)

regulatory peptides of any type of pineal origin.[37,38] The release of these peptides from the pineal gland has never been verified, an obvious requirement if they are to have actions throughout the organism. If these mythological peptides do exist, they have never been tested for the diversity of functions exhibited by melatonin, for example, as antioxidants, although in one report the authors claimed that the pineal peptide, epithalamin, provides better protection against free radical damage than does melatonin.[38] This seems doubtful, and this observation requires confirmation in an independent laboratory.

Because melatonin was initially discovered in the pineal gland, it was often surmised that this molecule would be unique to the pineal tissue of vertebrates, given that they are the only species that has this organ. This, however, was not found to be the case. Within less than a decade after the characterization of melatonin, the melatonin-forming enzyme (HIOMT) was also uncovered in the retina by Quay[21,39] and slightly later by Cardinali and Rosner.[40] The presence of melatonin in the vertebrate retina perhaps should not have been a major surprise, as the epithalamus of some nonmammalian vertebrates has a structure reminiscent of the retina of the lateral eyes.[41] This structure has been referred to as the third eye (or parietal eye). In some extinct quadrupeds, the third eye in the epithalamus was believed to be organized like a retina and capable of light perception followed by the transmission of action potentials to the central nervous system; however, this extra eye was probably not capable of forming images. Retinal melatonin of the lateral eyes exhibits a circadian rhythm like that in the pineal gland, but the melatonin-forming cells in the eyes of mammals do not discharge this product into the blood. The retinal melatonin rhythm influences dopamine metabolism and the function of retinal clocks,[42] presumably much like the melatonin cycle in the cerebrospinal fluid (CSF) modulates the superchiasmatic nucleus (SCN).[43–45] The pineal gland of mammals and the

third eye from which it evolved (or related structures) have other common features.[46,47]

In addition to the pineal gland and retinas, numerous vertebrate organs produce melatonin. This is perhaps best exemplified by its production throughout much of the gastrointestinal system,[48,49] where its synthesis is not known to exhibit a daily cycle.[50] The one non-neural organ that may display a 24-hour rhythm of melatonin is the Harderian gland,[51] an intraorbital exocrine gland that is found in only some mammals (but not in the human); the function of these large organs remains a mystery.[52,53] There is a disagreement whether the melatonin cycle in the Harderian glands is disrupted by pinealectomy or changes in response to the light:dark cycle.[54] As with other peripheral organs, the Harderian glands do not contribute melatonin to the systemic circulation.

The concentrations of melatonin in the serum of vertebrates have always been described as being uncommonly low (in the pg/mL range), even during darkness when the values rarely exceed 200 pg/mL. A recent revelation by Dauchy et al.[55] suggests that these values, measured in animals and humans maintained under what is considered low intensity artificial light (which is not similar either in intensity or wavelength to sunlight) during the day and relative darkness at night, may not represent the true melatonin cycle in reference to its nocturnal amplitude. In the study in question, these workers found that when male and female pigmented nude rats were maintained in blue-tinted polycarbonate cages, the nocturnal melatonin peak was up to seven times higher at night compared to that in rats housed in clear polycarbonate cages (Fig. 2); rather than serum melatonin values rising to 150 pg/mL at night, peak concentrations rose to 1000 pg/mL serum in rats kept in blue-tinted cages. Thus, the spectral content of daytime light [in this case light enriched with blue wavelengths (450–495 nmol/L)] profoundly impacted the amplitude of the peak serum melatonin on subsequent nights. Although not examined, this exaggerated rise was likely related to increased synthesis and release of melatonin by the pineal gland and was presumably associated with a proportionally elevated rise of melatonin in other bodily fluids, for example, CSF, and possibly also in somatic cells.

These findings are of particular interest since exposure to blue-enriched light during the day leads to an obvious enhancement of maximal nighttime melatonin concentrations, yet it is also blue light exposure at night that is maximally inhibitory to circulating melatonin concentrations.[56] The latter response is primarily mediated by the intrinsically photosensitive retinal ganglion cells (ipRGC), which contain a unique photopigment, melanopsin, that is especially sensitive to blue light wavelengths.[57,58] Whether blue wavelengths of light detected by ipRGC during the day have a function in relation to the exaggerated melatonin peak on the subsequent nights should be examined.

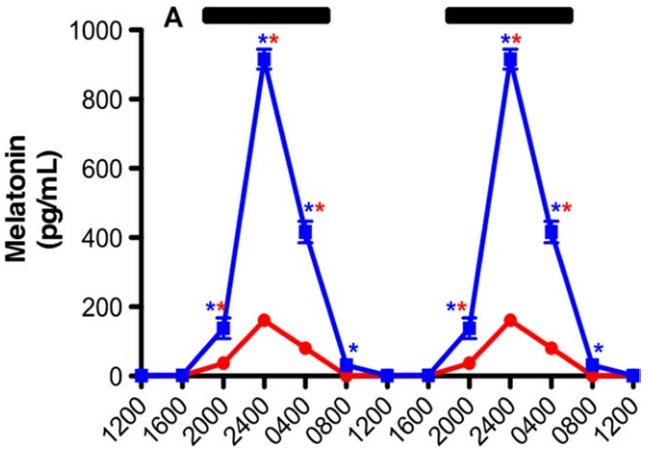

FIGURE 2 Twenty-four hour plasma melatonin rhythms in pigmented nude male rats maintained under a 12:12 light:dark cycle (darkness from 18:00 to 06:00 h) for 6 wk. Animals were kept in either clear polycarbonate cages (red dots and lines) or polycarbonate blue-tinted cages (blue squares and lines). The lighting conditions in the room were identical for both groups of rats (light intensity 300 lux, 125 μW/cm^2) with absolute darkness at night. The average nighttime increase in plasma melatonin in rats kept in clear cages was 9.6-fold over daytime levels while, for animals kept in blue-tinted cages, the average nighttime rise was 55.3-fold. Clearly, rats that experienced light during the day that was enriched with blue wavelengths (450–495 nmol/L) had a much greater nighttime rise in plasma melatonin levels. The supposition is that pineal melatonin synthesis and release is enhanced during darkness if the animals witness blue-enriched light during the day. Less likely would be that the blue light during the day slowed nocturnal hepatic melatonin metabolism. Red and blue * signify significant differences. Data are double-plotted for clarity. Data points are means ±1 SD. From Dauchy et al.[55] with permission

There is another implication of the findings reported by Dauchy et al.[55] This group has a history of publishing eloquent studies on the inhibitory actions of physiological concentrations (1 nmol/L) of melatonin on tumor growth.[59–61] They have also shown that the tumor suppressive actions of melatonin are mediated by the well-characterized MT1 membrane melatonin receptor.[61] The Kd of this receptor is consistent with 1 nmol/L melatonin concentration to which it is routinely exposed.[62] If the nighttime rise in melatonin is substantially greater than originally believed, the newly described melatonin concentration could exceed the Kd of the receptor, possibly leading to its downregulation and rendering it incapable of mediating an inhibitory response in terms of cancer inhibition. That was not the case, however, since the higher melatonin values actually had a greater inhibitory effect on prostate tumor growth.[55] Whether the highly elevated circulating melatonin levels described by Dauchy et al. will be found to influence the function of the MT1 and MT2 membrane receptors awaits further experimentation. Considering the very wide differences in melatonin concentrations in different body fluids (blood levels vs those in the CSF[63] and bile[64]), receptors in different locations are normally exposed to markedly different levels of the indoleamine.

The data related to the markedly elevated nocturnal circulating melatonin concentrations in rats kept under blue-enriched daytime light[55] have implications beyond inhibition of tumor growth. The ability of melatonin to reduce oxidative stress via its free radical scavenging actions is directly related to its concentration. At higher concentrations, there are more molecules of the antioxidant available to quench free radicals thereby lowering oxidative damage and related diseases. Induction of a lifelong melatonin deficit due to pinealectomy leads to an elevation in the amount of oxidative tissue damage in late life relative to the amount of molecular damage seen in pineal-intact rats with a preserved melatonin rhythm.[65] A corollary of this is that the higher concentrations of melatonin, as observed by Dauchy et al.[55] would also be expected to reduce oxidative damage to a greater degree than the commonly accepted lower amplitude rhythm.

Melatonin production is not limited to vertebrates but is also present in all organisms examined including bacteria,[66] unicells,[67] invertebrates,[68,69] and vascular plants.[70–72] None of these species has a pineal gland and some consist of only a single cell. Considering this, melatonin's association with the pineal gland of vertebrates may be only coincidental and perhaps was necessitated by the fact that, in order for the gland to produce melatonin in a circadian manner dependent on the light:dark cycle, it had to be regulated by neural information from organs responsible for light perception, that is, the lateral eyes. Although not examined in most nonvertebrate species, a 24-hour rhythm of melatonin has been described in the dinoflagellate *Gonyaulax polyedra*,[73] and in some plants,[74] at least one of the melatonin's functions is preserved in all species where it has been found, for example, its ability to detoxify free radicals.[75–78]

2 | MELATONIN AS AN ANTIOXIDANT: WAGING WAR ON FREE RADICALS

2.1 | Preparing for battle: melatonin's physiological weapons

The direct free radical scavenging activity of melatonin has been known for almost 25 years.[79] A subsequent report of this process also identified a novel melatonin metabolite, cyclic-3-hydroxymelatonin (c3OHM), which is formed when melatonin scavenges two free radicals; in this report, we also deduced the pathway by which c3OHM is formed.[80] This discovery was followed shortly by a series of studies, conducted both in vitro[81,82] and in vivo,[83–87] which documented the ability of melatonin to quell oxidative damage to molecules, cells and tissues, including human cells.[88,89] Since then, there have been numerous reports confirming the ability of melatonin to directly scavenge oxygen-centered radicals and toxic reactive oxygen species (ROS)[90–95] and to diminish oxidative mutilation to key cellular macromolecules.[96–102] These direct free radical scavenging actions of melatonin and its metabolites have been summarized in a number of reviews,[103–105] so they will not be discussed in detail here.

Other developments in the mid-1990s further advanced melatonin as an effective countermeasure to oxidative insults. Within 2 years after its discovery as a direct free radical scavenger, melatonin was found to stimulate antioxidative enzymes including glutathione peroxidase and glutathione reductase.[106–111] Furthermore, melatonin upregulates the synthesis of glutathione,[112–114] a highly effective intrinsic antioxidant, and synergizes with classic free radical scavengers to improve the reductive potential of tissues and fluids.[115,116] These indirect antioxidant functions of melatonin further leveraged this molecule as being a key endogenous factor in limiting free radical damage. Finally, melatonin was found to neutralize nitrogen-based toxicants, that is, nitric oxide and the peroxynitrite anion, both of which promote nitrosative damage,[117,118] and to suppress the pro-oxidative enzyme, nitric oxide synthase.[119,120] When the total antioxidant capacity of human blood was compared to both day and night endogenous melatonin concentrations, these parameters were found to be positively correlated (Fig. 3).[121] This correlation documents that not only pharmacological levels of melatonin, but likewise physiological concentrations, likely provide protection against damaging free radicals.[122]

As pointed out above, when melatonin functions as a scavenger, one resulting product is the metabolite c3OHM. When tested for its antioxidant capacity, c3OHM proved also to function in radical detoxification[123,124] as do its downstream metabolites, N-acetyl-N-formyl-5-methoxykynuramine (AFMK) and N-acetyl-5-methoxykynuramine (AMK),[125–130] in what has been defined as melatonin's antioxidant cascade.[126] Hence, the first-, second-, and third-generation metabolites of melatonin all have proven to be excellent radical scavengers.[131,132] This cascade predictably allows melatonin to neutralize up to 10 radical products, which contrasts with classic free radical scavengers, which detoxify a single oxidizing molecule. As melatonin is present in plants,[51,52] its function is also that of a radical scavenger in these organisms.[133–137]

Another potentially important action that is often overlooked as being relevant to melatonin's capacity to quench oxidative damage is its ability to bind heavy metals. In 1998, using absorptive voltammetry as a means of assessment, Limson et al.[138] reported that melatonin binds aluminum, cadmium, copper, iron, lead, and zinc not unlike metallothionein. The interaction of melatonin with these metals was found to be concentration dependent. Melatonin chelates both iron (III) and iron (II), which is the form that participates in the Fenton reaction to generate the hydroxyl radical. If the iron is bound to a protein, for example, hemoglobin, melatonin restores the highly covalent iron such as oxyferryl (Fe^{IV}-O)

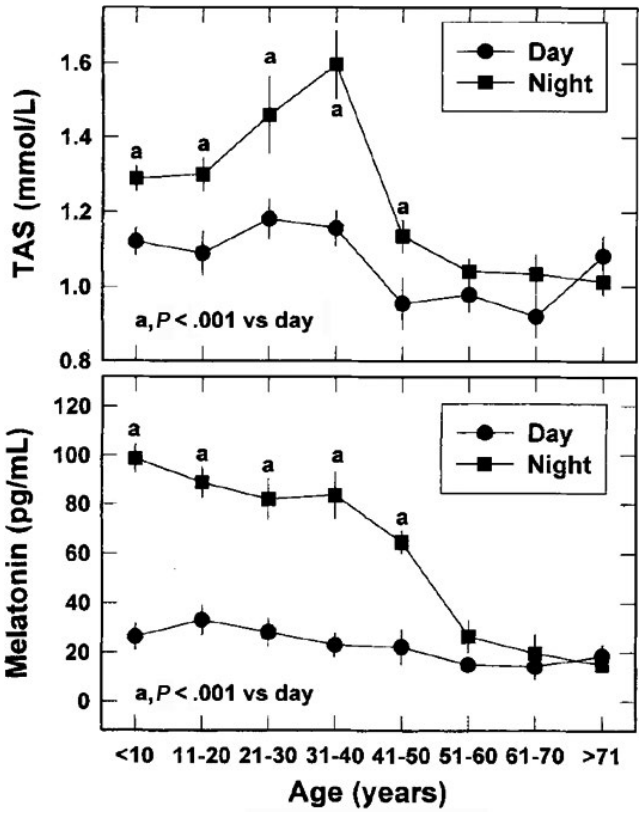

FIGURE 3 The day and nighttime levels of melatonin in the blood correlated with the total antioxidant status (TAS) of this fluid throughout the life of humans. As individuals age, the nighttime melatonin levels wane; likewise, the TSA concentrations drop accordingly. The findings indicate that physiological levels melatonin in the blood enhance the free radical scavenging potential of that medium. From Benot et al.[121] with permission

hemoglobin back to the iron (III) thereby re-establishing the biological activity of the protein. This would be similar to the reducing action of melatonin when it encounters the highly toxic hydroxyl radical. Particularly in the brain, metallothionein plays a less important role regarding its binding of transition metals. Because it is a protein, any bond metallothionein forms with a transition metal would be damaged by the free radical the metal would generate. By comparison, melatonin would neutralize the generated free radical and reduce the damage. This may be especially important in the brain where, as noted, metallothionein has a reduced role in binding metals. We have recently discussed the possibility that the high levels of melatonin in the CSF, relative to the concentration in the blood, may afford the brain extra protection from oxidative stress.[43,63,139] In the brain, melatonin, in addition to its direct scavenging activity and indirect antioxidant actions, may have replaced or supplemented metallothionein as a major binder of transition metals.

Parmar et al.[140] perused these original observations by investigating melatonin's ability to reduce copper-mediated lipid peroxidation in hepatic homogenates. In this study, melatonin may well have reduced lipid damage by directly scavenging radicals sufficiently toxic to initiate lipid peroxidation; additionally, however, electrochemical studies found that melatonin bound both Cu(II) and Cu(I). These actions likely conspired to reduce the oxidation of hepatic lipids. Soon after the report by Parmar et al.,[140] Mayo et al.[141] showed that protein damage resulting from exposure to Cu(II)/H_2O_2 was alleviated by melatonin while Gulcin et al.,[142] in a comparative investigation, found that melatonin had a higher Fe(II) chelating activity of this ion than either α-tocopherol or the synthetic antioxidants, butylated hydroxybutylanisole or butylated hydroxytoluene. Melatonin also markedly reduced the interaction of Al(III), Zn(II), Cu(II), Mn(II), and Fe(II) with amyloid β-peptide.[143]

In the most recent study related to the metal-chelating activity of melatonin, Galano et al.[144] examined the copper sequestering ability of melatonin as well as that of its metabolites c3OHM, AFMK, and AMK. This group pointed out that while copper is essential for optimal cell physiology, when it is in high concentrations, it participates in the Fenton/Haber–Weiss reactions, which generate the hydroxyl radical. Also, a deficiency of copper compromises antioxidant defense processes due to a reduction in the synthesis of the cytosolic antioxidant enzyme, copper superoxide dismutase (CuSOD). Moreover, several neurological diseases including Alzheimer's disease,[145,146] Parkinson's disease,[147,148] Huntington's disease,[149] and hepatolenticular degeneration (Wilson disease)[150,151] are characterized by an overload of copper and/or other metals. Molecular damage associated with some of these conditions is likely a result of the pro-oxidative actions of an excess of copper ions. Considering this, it is important to regulate the levels of copper consistent with cellular needs. When the cooper-chelating ability of melatonin, c3OHM, AFMK, and AMK were compared in the framework of the density function theory, we reported that melatonin as well as its metabolites yielded stable complexes when they bond copper ions (Fig. 4).[144] Two mechanisms were modeled; these were the direct-chelation mechanism (DCM) and the coupled-deprotonation-chelation mechanism (CDCM). Under physiological conditions, it was predicted that the CDCM was the major route of Cu(II) chelation. Melatonin, as well as its metabolites chelated Cu(II) and completely inhibited oxidative stress induced in a Cu(II)/ascorbate mixture. Similarly, melatonin, c3OHM, and AFMK prevented the initial step in the Haber–Weiss reaction consequently reducing the formation of the highly oxidizing hydroxyl radical. On the basis of these findings, Galano et al.[144] proposed that melatonin, besides being the initial molecule in the free radical scavenging casade,[152] is also involved in a metal-chelating cascade as summarized in Fig. 4. A review related to the metal-catalyzed molecular damage that occurs in organisms where the ability of melatonin to chelate

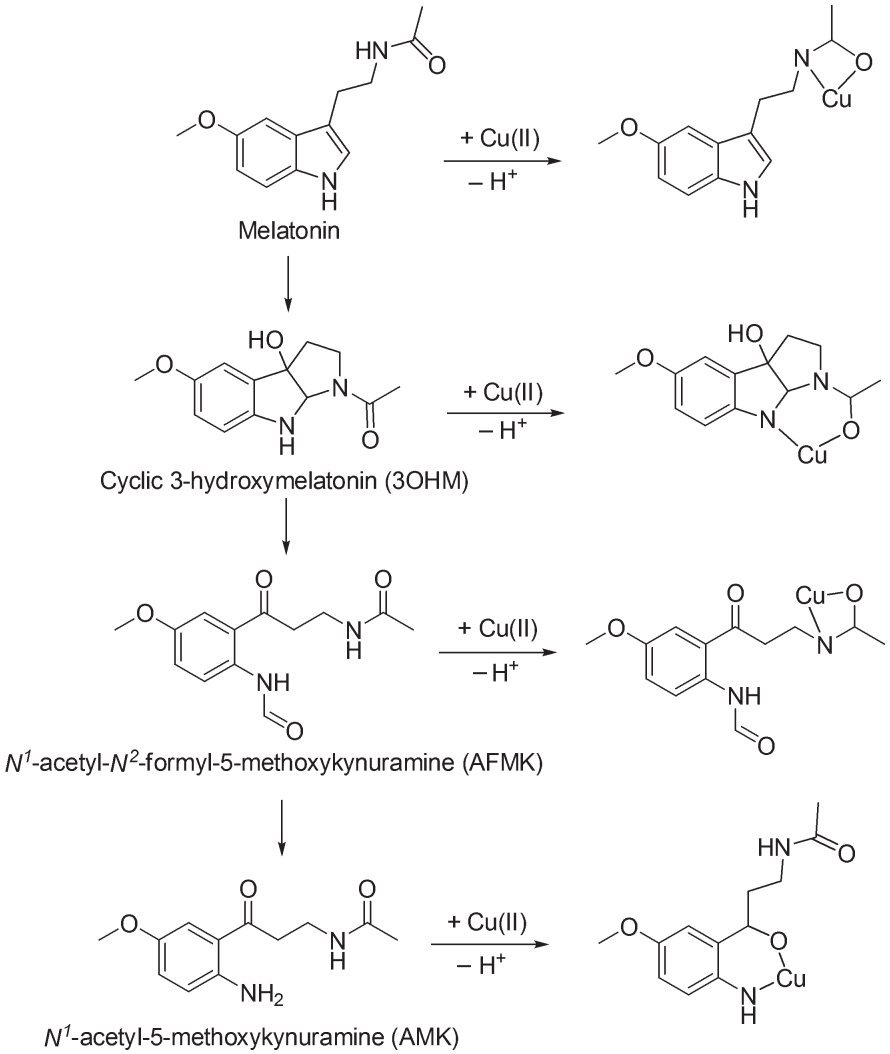

FIGURE 4 The free radical scavenging cascade (vertical) and the metal-chelating cascade (horizontal) of melatonin and its metabolites. The chelation structures shown on the right side of the figure are those that are predicted to be the most abundant. The most likely mechanism for the formation of the predicted complexes is a coupled-deprotonation-chelation mechanism (CDCM). From Galano et al.[144] with permission

these damaging ions may be consequential was recently published.[153]

In addition to the means already discussed, a variety of other factors probably aid melatonin in reducing the total oxidative burden of an organism. As summarized in Fig. 5, melatonin not only neutralizes a host of toxic reactive molecules, but also modulates the activities of a wide variety of enzymes that determine the quantity of ROS/RNS produced. Moreover, there are physiological and metabolic factors that probably contribute to the high efficacy of melatonin, as well as its metabolites, in reducing oxidative damage. For example, melatonin reportedly limits electron leakage from the mitochondrial respiratory chain leading to fewer oxygen molecules being reduced to the superoxide anion radical; this process is referred to as radical avoidance.[154] Melatonin's anti-inflammatory actions indirectly reduce free radical damage given that the inflammatory response typically is accompanied by free radical generation[155] while the ability of melatonin to strengthen circadian rhythms also aids in fewer oxidative processes since chronodisruption enhances the production of oxidizing molecules.[156]

2.2 | Melatonin, a mitochondria-targeted antioxidant

Mitochondria are specifically designed for certain critical functions including the generation of ATP; in normal aerobic cells, oxidative phosphorylation accounts for the efficient production of 95% of the total ATP generated. While performing this essential task, mitochondria also are a major site for the production of oxygen-based toxic species, that is, ROS,[157,158] the majority of which must be detoxified before they irreparably damage these organelles and severely compromise ATP production. Indeed, a major theory of aging, that is, the mitochondrial theory, incriminates damage to these organelles as being responsible for the aging of cells, of organs and of organisms.[159–161] As oxidative damage of mitochondria is central to a number of serious pathologies and to aging, conventional antioxidants should be useful in forestalling these diseases or delaying degenerative processes associated with advanced age. Yet, the evidence is remarkably sparse regarding the successful application of regularly used antioxidants to influence the progression of the diseases or aging.[162–166]

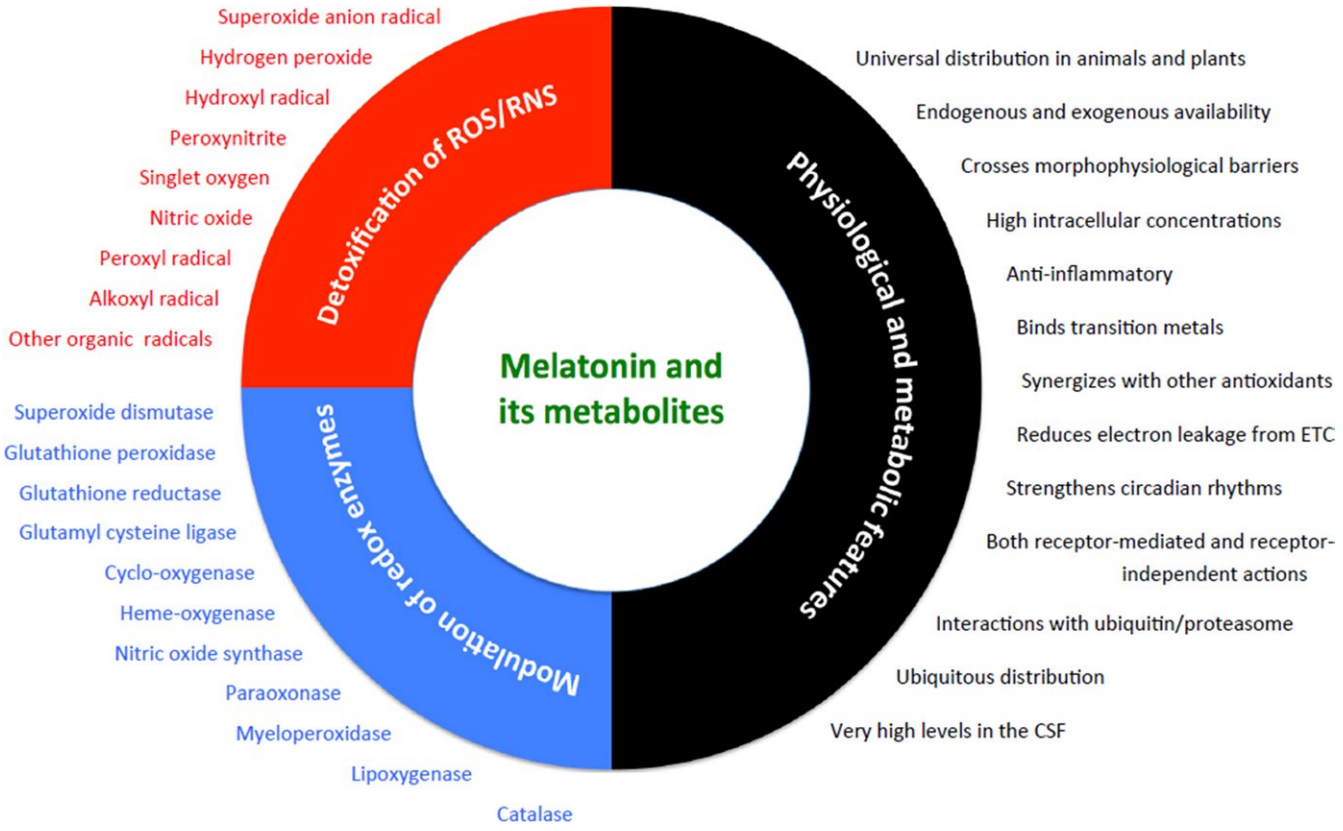

FIGURE 5 This figure summarizes the multiple actions of melatonin in reducing oxidative stress. The red area indicates the reactive oxygen (ROS) and reactive nitrogen species (RNS) that have been shown to be neutralized by melatonin and metabolites that are formed during its antioxidant cascade. The blue area identifies enzymes that impact the redox state of the cell because they either cause the generation of radicals or metabolized them to inactive products. The former are upregulated while the latter are downregulated by melatonin and/or its metabolites. Glutamyl cysteine ligase induces the formation of glutathione, an important intracellular antioxidant. The black areas list features that aid melatonin in terms of its ability to quench free radicals and reduce oxidative damage

One reason for the failure of conventional antioxidants to ameliorate the severity of ROS-related diseases may be a result of their inability to concentrate in mitochondria where free radical production is maximal. Thus, it seemed like a worthwhile strategy would be to develop mitochondria-targeted antioxidants; this has been done and they were shown effective in reducing mitochondrial damage and the resulting apoptosis.[167–169] As an example, to achieve a high concentration in mitochondria, the ubiquinone moiety of endogenous co-enzyme Q10 was conjugated with the lipophilic triphenyl phosphonium cation (TPP).[170] Combining TPP with Q10 allowed the resulting molecule, called MitoQ, to rapidly cross the cell and mitochondrial membranes and to accumulate in concentrations up to several hundred-fold greater in the mitochondrial matrix than that of the unconjugated antioxidant (Fig. 6). Tocopherol (vitamin E) also has been conjugated with TPP with a similar degree of success in terms of targeting it to the mitochondrial matrix; this complex is identified as MitoE.[170] Both MitoQ and MitoE achieve improved protection of mitochondria against free radical damage over that provided by the unconjugated forms of the antioxidants.[168,171–174] Both MitoQ and MitoE are recycled in the mitochondrial matrix thereby increasing their efficacy in minimizing local molecular damage.[175]

Lowes et al.[176] compared the relative efficacies of the two mitochondria-targeted antioxidants, MitoQ and MitoE, with melatonin in reducing inflammation and oxidative damage. A worst case scenario was used to create the molecular carnage. Adult male rats were given both bacteria lipopolysaccharide (LPS) and peptidoglycan (PepG) via a tail vein infusion to induce massive oxidative damage. Shortly, thereafter, the animals received, via the same route, either MitoQ, MitoE or melatonin. Five hours later, plasma and tissue samples were collected. The authors described broadly equivalent protective actions of the three antioxidants relative to their improvement in maintaining mitochondrial respiration, reducing oxidative damage and depressing pro-inflammatory cytokine levels. Additionally, each of the antioxidants had roughly similar protective effects in preserving biochemical parameters of organ physiology as plasma levels of alanine transaminase and creatinine did not differ statistically among the three antioxidant-treated groups. The data relative to the hepatic protein carbonyls and oxidized lipids are summarized in Fig. 7. From the data in this figure, it seems apparent that

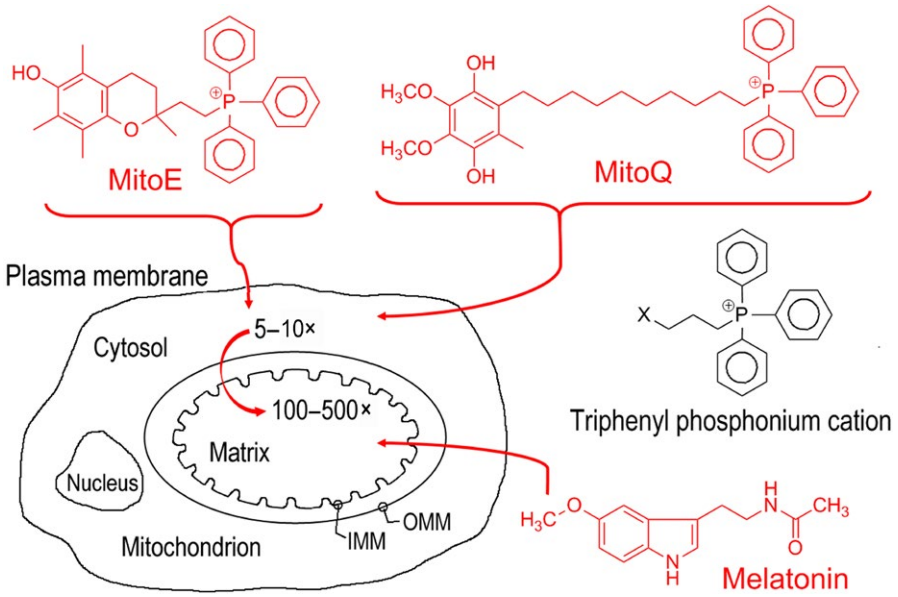

FIGURE 6 The structures of MitoE and MitoQ, mitochondria-targeted antioxidants. Melatonin is an endogenously produced molecule that, based on its relative ability to protect against inflammation and oxidative stress when compared to MitoQ and MitoE, may be capable of accumulating in the mitochondria. MitoQ and MitoE are synthetic mitochondria-targeted antioxidants. They are produced when the ubiquinone moiety of Q_{10} and tocopherol, respectively, is conjugated with triphenyl phosphonium cation. MitoE and MitoQ accumulate in high concentrations in the mitochondria. Melatonin is as effective and in some cases more effective than MitoE and MitoQ in reducing oxidative damage and inflammation (See Fig. 7)

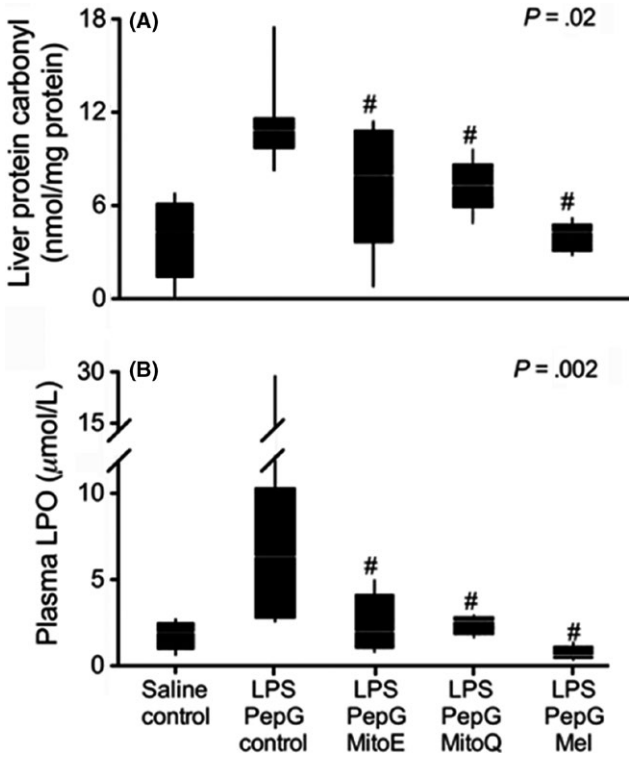

FIGURE 7 Concentration of hepatic protein carbonyls (A) and plasma lipid hydroperoxides (B) in placebo-treated control rats and in animals given toxic bacterial lipopolysaccharide (LPS) and peptidoglycan (PepG) to induce oxidative damage. Some LPS + PepG-treated rats were also infused with the synthetic mitochondria targeted antioxidants, MitoE or MitoQ, or with the endogenously produced antioxidant melatonin. Each of these antioxidants significantly reduced oxidatively damaged hepatic proteins and plasma lipids, with melatonin seemingly being the most effective. #*P*-values are relative to the LPS + PepG control group. Redrawn and with approval from Lowes et al.[176]

the most effective antioxidant related to these parameters was melatonin given the lower mean values of damage molecules and their more uniform inhibition in the animals of this group.

The combination of LPS + PepG is a very aggressive challenge to the defensive makeup of mammals and in this study melatonin handled the attack as well as or better than the synthetic mitochondria-targeted antioxidants.[176] A major implication of these findings is that melatonin should be classified as an endogenous mitochondria-targeted antioxidant (Fig. 6). This would be consistent with the much higher melatonin levels in hepatic mitochondria than in the plasma as reported[177,178] and with the proposal that mitochondria might be the site of intracellular melatonin synthesis.[179] Positioning itself in mitochondria may be a critical aspect of melatonin's potent antioxidant activity. Maintaining the reductive potential of these organelles is important as mitochondria are often the major site of massive free radical generation. In view of the Surviving Sepsis Campaign, a program to identify agents that can counteract the rampant damage that occurs during sepsis and septic shock,[180] melatonin may prove to be a critical component of a treatment paradigm. Of the three antioxidants used, at the conclusion of their report, Lowes et al.[176] state that melatonin may be the most accessible agent to resist the molecular damage and mortality that occurs in septic humans.

2.3 | Melatonin as an antioxidant: evidence from ischemia-reperfusion studies

There are a large number of published reports confirming that melatonin overcomes oxidative destruction of key molecules and death of cells in tissues that are temporarily deprived of oxygenated blood and then reperfused with blood rich in oxygen. During both hypoxia (ischemia) and reoxygenation (reperfusion), cataclysmic levels of ROS/RNS are generated that molest essential molecules leading to the accumulation of molecular debris that compromises both the function and the survival of cells.[181,182] Melatonin's relentless quest to curb

such damage stems in part from its antioxidant potential has been documented during ischemia/reperfusion (IR) of many organs (Fig. 8).

While any IR event is always serious, when it involves the brain (stroke) or the heart (heart attack), it is especially critical and often life threatening. In those individuals who do survive a stroke or a heart attack, the neurobehavioral or physiological consequences are often debilitating and persistent and compromise life quality. Identifying molecules that can prevent or significantly reduce the damage caused by episodes of IR are a major interest of the scientific community.[183,184]

Table 1 summarizes a few of the numerous studies in which melatonin has been effectively used to combat IR damage in the brain and in the heart. As seen in the table, the most common rodent model used to temporarily interrupt the blood supply to a focal area of the brain is middle cerebral artery occlusion (MCAO) with the usual doses of melatonin used to counter the associated neural damage being 4–10 mg/kg body weight (BW). This model is of interest since it is representative of the localized stroke that humans often experience.

The most recent and certainly the most captivating study, although not registered with ClinicalTrials.gov, related to the use of melatonin to overcome the perturbed heart function associated with transitory ischemia and reperfusion is that of the Dwaich et al.[202] When they gave either 10 or 20 mg of melatonin orally for 5 days before coronary bypass surgery to male and female patients (15 individuals per treatment group), the physiological dividend reaped from this treatment was substantial. Twenty-four hours following surgery, there was a significant increase in the cardiac ejection fraction (measured using echocardiography) accompanied with a reduction in heart rate (relative to 15 surgical patients not treated with melatonin). Moreover, there were significant reductions in plasma cardiac troponin 1, interleukin-1β, inducible nitric oxide synthase (iNOS), and caspase 3 due to melatonin treatment. The improvements were greater in the patients who were given 20 mg of melatonin compared with those given 10 mg of the indole; thus, the effects were dose dependent. The results of Dwiach et al.[202] showed that melatonin treatment attenuated myocardial injury (as measured by the ejection fraction and troponin 1), limited the inflammatory response (IL-1β), decreased oxidative stress (iNOS), and arrested the degree of apoptosis (caspase 3). As the responses measured varied with the dose of melatonin given, higher doses of the indole or its administration via another route (e.g., infusion during surgery) may further improve cardiac parameters. Hopefully, such studies are being pursued.

A wide variety of endpoints ranging from infarct volume to molecular markers of cellular damage have been measured in the IR studies to prove the value of melatonin in

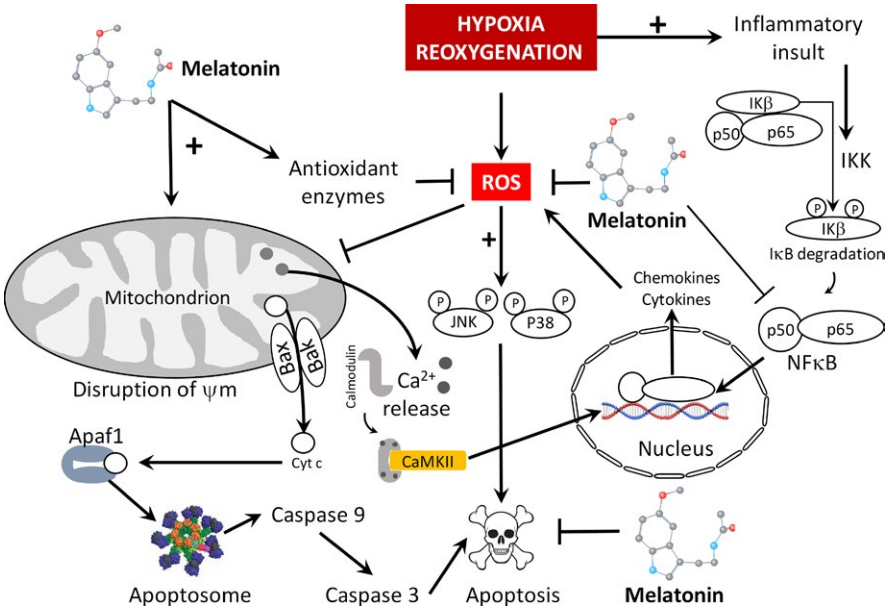

FIGURE 8 A diagrammatic representation of some of the consequences of hypoxia/reoxygenation (ischemia/reperfusion) as they occur in the brain during a stroke or in the heart during a heart attack. Similar changes occur in any organ that experiences hypoxia/reoxygenation. Massive quantities of reactive oxygen (ROS) and reactive nitrogen species are generated during both hypoxia and reperfusion. These toxic agents initiate the release of previously sequestered calcium (Ca^{2+}) into the cytosol and damage mitochondria, which allows the escape of cytochrome c (Cyt c). Released Cyt c activates the apoptotic cascade. Hypoxia/reoxygenation is also associated with an inflammatory response that involves the release of NF-kB and its translocation into the nucleus. This activates the synthesis of chemokines and cytokines which results in the augmentation of ROS production. Melatonin has multiple actions by which it abates the damage inflicted by ROS; these actions include direct free radical scavenging, stimulation of antioxidant enzymes and chelation of transition metals. As a result of these actions, melatonin attenuates cellular apoptosis and tissue loss, thereby partially preserving the function of the damaged organs

TABLE 1 A summary of the results of some of the published reports (there are many more), which illustrate the beneficial effects of melatonin in experimental and clinical ischemia/reperfusion injury (stroke) in the brain and in the heart (heart attack). The majority of studies were performed using rodents as the experimental models

Reference	Species	Type/duration ischemia	Melatonin dose
Brain, animal			
Guerrero et al.[185]	Gerbil	10 min bilateral common carotid clamp	10 mg/kg BW
Kilic et al.[186]	Rat	120 min MCAO	4 mg/kg BW pinealectomy
Kilic et al.[187]	Mouse	90 min MCAO	4 mg/kg BW
Kilic et al.[188]	Mouse	90 min MCAO	4 mg/kg BW
Carloni et al.[189]	Newborn rat	Permanent right common carotid ligation	15 mg/kg BW
Li et al.[190]	Rat	120 min MCAO	5 mg/kg BW
Zheng et al.[191]	Rat	90 min MCAO	5 or 10 mg/kg BW
Paredes et al.[192]	2, 6, 14 mon old rats	Permanent MCAO	10 mg/kg BW
Brain, human			
Fulia et al.[193]	Newborn	During difficult vaginal delivery	80 mg total (first 6 h after birth)
Aly et al.[194]	Newborn	Hypoxic ischemic encephalopathy	50 mg total (5 × 10 mg) + hypothermia
Heart, animal			
Tan et al.[195]	Rat heart ex vivo	10 min ligation of left anterior descending artery	Perfused with 1, 10 or 50 μmol/L
Petrosillo et al.[196]	Rat heart ex vivo	30 min global ischemia	Perfused with 50 μmol/L
Liu et al.[197]	Rat	10 min ligation of left coronary artery	2.5, 5 or 10 mg/kg
Yu et al.[198]	Rat	30 min ligation of left anterior descending coronary artery	10 mg/kg/7d 15 mg/kg
He et al.[199]	Mouse	30 min ligation of left coronary artery	150 mg/kg
Nduhirabandi et al.[200]	Rat heart ex vivo	30 min global ischemia	Perfused with 75 μg/L
Heart, human			
Gogener et al.[201]	Adult	Ischemia during abdominal aortic aneurism repair	Perfused with 50 mg for 2 h; 10 mg/3 d after surgery
Dwaich et al.[202]	Adult	Coronary artery bypass surgery	Oral 10 or 20 mg daily for 5 d

MCAO, middle cerebral artery occlusion.

suppressing brain damage that results from oxygen deprivation followed by oxygen restoration. The majority of the studies concluded that a significant portion of the protective effects of melatonin related either to its direct scavenging actions or to its indirect functions in promoting other free radical neutralizing activities. One report noted that blocking the MT1 and MT2 membrane receptors, which are widely distributed in the brain,[203] did not interfere with melatonin's ability to douse cellular damage.[186] That does not exclude the possibility, however, that the MT3 (quinone reductase, a cytosolic detoxifying enzyme[204]) or nuclear binding sites (ROR, RZR[205]) did not mediate some of the neuroprotective actions of melatonin.

The number of human studies related to hypoxia and melatonin use is obviously limited. Fulia et al.[193] were the first to show that giving 80 mg of melatonin (eight doses of 10 mg each) during the first 6 hours after birth to asphyxiated newborns (due to difficult birth) reduced circulating levels of oxidized lipids and nitrite/nitrate concentrations and decreased mortality (three of 10 asphyxiated newborns not given melatonin died while zero of 10 melatonin-treated asphyxiated infants succumbed). While this is not direct evidence that melatonin protected the brain from the period of hypoxia, this organ is especially sensitive to oxygen deprivation[206] and melatonin readily crosses the blood–brain barrier;[207] so it can be safely assumed that the exogenously administered melatonin relieved the brain of some of the redox imbalance it suffered due to the hypoxia (Fig. 8).

The report by Aly et al.[194] speaks more directly to the neuroprotective actions of melatonin in human neonates. In this prospective study, 5-day hypothermia combined with enteral melatonin treatment reduced numerous oxidative parameters in newborns suffering with hypoxic ischemic encephalopathy (HIE). The neurological endpoints included fewer seizures in the hypothermic melatonin-treated infants and less white matter damage as visualized using magnetic resonance imaging. Finally, the combined treatment was efficacious in terms of improving survival and causing favorable neurodevelopmental outcomes at a month after birth.[194]

The use of melatonin to protect the heart from ST-segment elevation myocardial infarction (STEMI) has been a major interest to the group of Dominguez-Rodriguez et al.[208–210] The safety and efficacy of melatonin as an antioxidant and the participation of free radicals in mediating cardiac damage in

STEMI patients were the basis for the design and rationale of the MARIA trial.[211] This group[212–214] and others[215,216] have published summaries of literature reports that have used melatonin to overcome heart damage from ROS/RNS, whatever the cause.

The neurological damage resulting from a stroke or heart stoppage leaves in its wake a variety of physiological, neurobehavioral, and cognitive residuals that lead to physical and mental debilitation. To limit these devastating conditions, several damaging processes must be targeted including oxidative/nitrosative stress, inflammation, and glutamate excitotoxicity.[217] Each of these processes are modulated by melatonin. As discussed herein, melatonin is among the best molecules available in terms of fighting against the molecular carnage inflicted by oxygen- and nitrogen-based toxic reactants. Furthermore, its anti-inflammatory actions are well described and mechanistically defined,[218,219] and glutamate toxicity, which involves destructive free radicals, is negated by melatonin.[220,221] When measured in experimental studies, the severity of the long-term neurobehavioral deficits associated with stroke have been also shown to be reduced when melatonin was given coincident with the IR episode.[222,223] Many of the molecular details that are involved in melatonin's protective actions during IR have been elucidated in recent reports.[190,224–227]

Herein, emphasis was placed on the neuroprotective and cardioprotective actions of melatonin that result from IR as hypoxia/reoxygenation in these organs often has dire consequences. These are, however, not the only organs where melatonin has preserved the morphological and functional integrity when they are subjected to IR. Published reports have shown that the lung,[228–230] liver,[231–233] kidney,[234] pancreas,[235] intestine,[236] urinary bladder,[237,238] corpus cavernosum,[239] skeletal muscle[240,241] spinal cord,[242,243] and stem cells[244] are also protected by melatonin. It would be expected that if a molecule limits IR damage in one organ, it would do so in all, as was shown to be the case for melatonin. Stem cells in culture sometimes suffer from periods of hypoxia and this also happens when they are implanted. That an endogenously produced, nontoxic molecule protects them from damage and death may prove to be of great importance given that stem cell transplantation is increasing in frequency.

2.4 | Melatonin as an antioxidant: evidence from organ transplantation studies

Organ transplantation is a valuable procedure for individuals suffering with end-stage organ failure. Among a number of factors that compromise success of transplanted organs are immune intolerance and apoptotic/necrotic cell death due to hypoxia/reoxygenation.[245,246] In reference to this latter point, the information related to the efficacy of melatonin in reducing IR-mediated cellular injury is relevant to the transplantation procedure. This molecule could be useful in protecting transplantable organs from hypoxia associated with organ storage and reoxygenation when the tissue is reperfused after transplantation. The utility of melatonin for this purpose was initially recognized by Viarette et al.[247] In this study, the liver was isolated from rats and immersed in either University of Wisconsin (UW) or Celsior storage solutions for 20 hours at 4°C. Thereafter, the hepatic tissues were perfused with Krebs Henseleit bicarbonate (KHB) buffer with or without melatonin (25, 50, 100 or 200 μmol/L). Perfusing the livers with melatonin caused a dose–response rise in bile production (Fig. 9) and in the amount of bilirubin in the bile. All doses of melatonin induced a comparable increase in hepatic ATP levels. Both hepatic and biliary concentrations rose proportional to the melatonin dose. The authors concluded that the addition of melatonin to the perfusion fluid led to more healthy hepatocytes increasing the likelihood that, if transplanted, the liver would have an improved its chance of survival.

In a follow-up study,[248] this group histochemically examined the levels of ROS in situ in cold-preserved livers that were subsequently perfused with warm KHB solution with or without melatonin (100 μmol/L). Cold storage was achieved in either UW or Celsior preservation solution. The presence of melatonin in the reperfusion medium reduced histochemical evidence of ROS production in hepatocytes and also maintained a more normal morphology of the cells.

Many livers destined for transplantation are steatotic; therefore, they more likely to functionally fail when transplanted. Zaouali et al.[249] performed studies similar to those described above to determine whether melatonin would also improve the function of fatty livers. Steatotic and nonsteatotic livers were obtained from obese and lean Zucker rats, respectively, and were stored for 24 hours at 4°C in either UW or Institute Georges Lopez (IGL-1) solution with or without melatonin (100 μmol/L); thereafter, they were subjected to ex vivo normothermic reperfusion (2 hours at 37°C). In both liver types, melatonin lowered the release of transaminases (indicative of fewer damaged hepatocytes), improved bile output, enhanced bromosulfophthalein clearance, and caused a diminution in vascular resistance. These benefits were consistent with the observed reduction in oxidative stress and lowered cytokine release. The implication is that the use of melatonin in organ storage solutions may improve the function of these organs once they are transplanted. Also, the fact that melatonin recouped the function of the steatotic livers suggests moderately damaged livers could potentially be used for successful transplantation if they were treated with melatonin; this is particularly important given the acute shortage of healthy transplantable organs.

The most thorough investigation as the utility of melatonin in organ transplantation was provided by Garcia-Gil et al.[250] who performed pancreas allotransplants in pigs. In

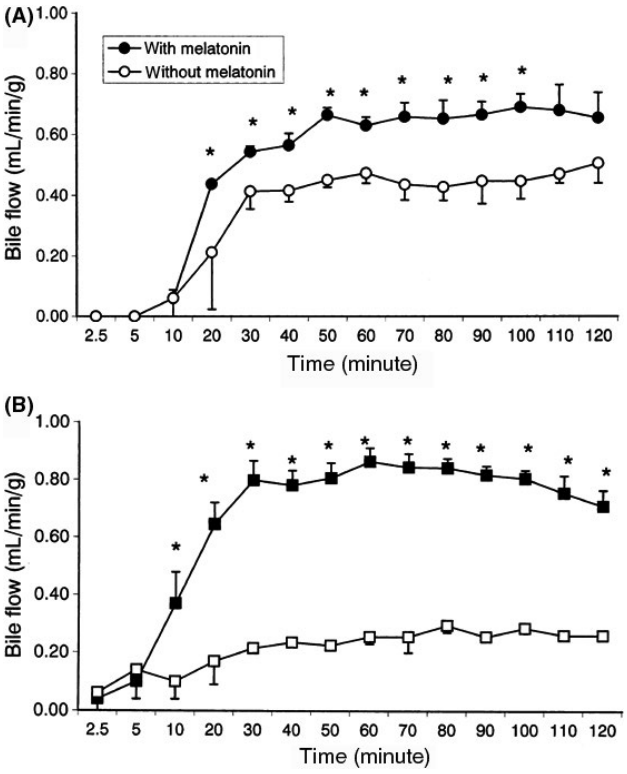

FIGURE 9 Melatonin improves bile production from livers prepared for transplantation. Surgically removed livers were flushed with Wisconsin (A) or Celsior (B) preservation solutions. They were then stored for 20 h at 4°C. Thereafter, they were perfused with Krebs–Henseleit buffer containing melatonin (100 μmol/L) or no melatonin. Melatonin (solid points) significantly (*P<.05) improved bile flow compared to that from livers not perfused with melatonin (hollow points). From Viaretti et al.[247] with permission

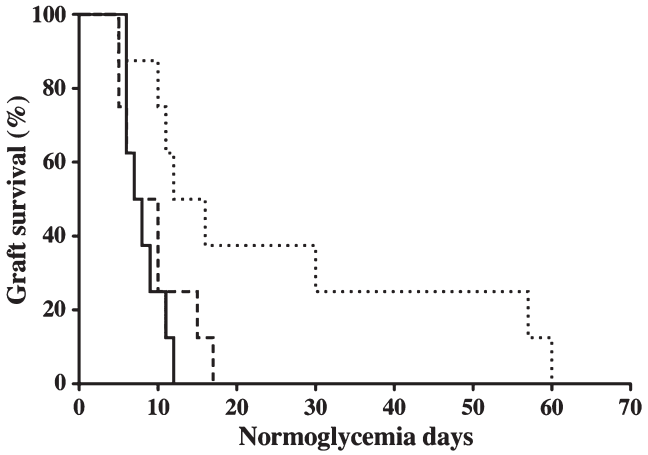

FIGURE 10 Duration of survival of pancreaticoduodenal allografts in pigs. The untreated control pigs rejected the grafts by 12 d postoperatively (solid line). Ascorbic acid (heavy dashed line) did not prolong the autografts beyond those of the control pigs. Melatonin treatment (dotted line) significantly prolonged the survival time of the pancreaticoduodenal grafts. Each group contained eight pigs. From Garcia-Gil[250] with permission

this study, the efficacy of two antioxidants was compared, that is, melatonin and ascorbic acid (AA). The antioxidants were intravenously administered to the donor and recipient pigs during surgery and for 7 days postsurgery; these antioxidants were also added to the UW storage solution before the organs were transplanted. Melatonin proved highly effective in prolonging allograft survival (25 days) relative to the survival of grafts from control (8 days) or AA-treated pigs (9 days) (Fig. 10). Melatonin also had a greater inhibitory effect on indices of lipid peroxidation (malondialdehyde and 4-hydroxyalkenal) in pancreatic tissues. Moreover, melatonin reduced serum pig's major acute-phase protein/inter-α-trypsin inhibitor heavy chain 4 (PMAP/ITIH4) in the early post-transplantation period. By all indices, the benefits of melatonin exceed those of AA and suggest tests of this important molecule in additional transplantation studies, including in clinical trials. The findings related to the likely utility of melatonin in organ transplantation have recently been reviewed,[251,252] and, in a separate report, melatonin was also suggested for use in ovary transplantation.[253]

2.5 | Melatonin as an antioxidant: evidence from toxic drug studies

Drugs for the treatment of diseases are approved on the basis of their cost/benefit ratio. Often drugs have a significant physiological downside, but when the benefits are presumed to outweigh the damage they inflict, they are sanctioned. Some of the side effects of these drugs progress to the point where the damage becomes life threatening. In many cases, the damage that drugs cause are a consequence of molecular processes within cells that culminate in free radical generation leading to oxidative stress and cellular malfunction. Because of this, over a decade ago, we introduced the idea that toxic drugs should be taken in combination with melatonin, so the associated free radical damage could be mitigated.[254,255] Melatonin has not been found to interfere with the efficacy of prescription drugs and, in those cases, where a drug's use is limited by its toxicity, for example, doxorubicin, if given it in combination with melatonin may allow the use of a larger dose with greater efficacy.[255]

Cholesterol-lowering statins are some of the most widely prescribed drugs in the world, and their side effects are well documented. Well-known adverse effects of statin use include myalgia and myopathy; occasionally these progress to rhabdomyolysis,[256] a serious consequence that can lead to incapacitation and death. Moreover, rhabdomyolysis can cause acute renal failure, electrolyte disturbances, disseminated intravascular coagulation and other negative effects.[257] Other potential negative consequences of regular statin use include elevation in the levels of serum aminotransferase,[258] cognitive impairment,[259] and what has been referred to as new-onset diabetes mellitus, while rare, older patients may be at greater risk for the latter complication.[260]

Each of the side effects of statin use likely involves free radical production, and, mechanistically, this is especially the case with the most serious complication, rhabdomyolysis. While the causes of this degenerative muscle condition are complex, a final common pathway involves large increases in free ionized Ca^{2+} in the sarcoplasm and mitochondria of muscle cells.[261] The rise in free Ca^{2+} leads to downstream events that culminate in mitochondrial damage, reduced ATP production, and generation of free radicals, which cause further damage and malfunction.[262] At the level of the kidney, myoglobin released from the damaged sarcomeres induces oxidative damage and dysfunction of renal mitochondria leading to acute renal failure.[263]

As repeatedly stated herein, melatonin is a potent protector against oxidative stress, a major contributory factor to the side effects of statins. Moreover, melatonin regulates free Ca^{2+} movement intracellularly.[264–266] To potentially improve the utility and safety of statins, these authors urged the performance of both experimental studies and clinical trials to determine whether melatonin has the ability to forestall the toxicity of these very widely used, cholesterol-lowering drugs.

There are only a few studies where melatonin and statin drugs have been examined in the same report. Atorvastatin, in addition to lowering cholesterol, has protective actions on endothelial cells, which retard the development of arthrosclerosis. This statin promotes the expression of endothelial nitric oxide synthase (eNOS) resulting in vasodilation. As melatonin also has beneficial actions at the level of the endothelium, Dayaub et al.[267] tested the synergistic effects of melatonin and atorvastatin on human umbilical vein endothelial cells (HUVEC) incubated with bacterial LPS. The combination of drugs induced higher eNOS protein expression than they did individually. Melatonin, but not the statin, exhibited the predictable antioxidant actions; however, when the drugs were combined, the protection against LPS was further improved. These findings are consistent with melatonin's ability to provide beneficial effects to atorvastatin while reducing oxidative stress associated with the inflammation advanced by LPS.[267]

Statins reportedly have additional benefits including oncostatic actions and antifibrillating and defibrillating potential. Melatonin was found to exaggerate the cancer-inhibiting actions of pitavastatin[268] and pravastatin[269] against breast cancer in experimental studies. Melatonin also has antiarrhythmic potential equivalent to that of atorvastatin in an isolated heart model.[270] These findings support a closer examination of melatonin as an adjunct treatment with statins.

Methamphetamine is a common drug of abuse. This toxin, in addition to destroying the gingiva and periodontium in the oral cavity,[271] has even more serious effects in the central nervous system.[272,273] There is general agreement that the toxicity of methamphetamine involves oxidative stress.[274] This likely prompted the group led by Govitrapong to test whether melatonin could ameliorate the effects of this drug on the brain.[275] In in vitro studies, they have shown that melatonin reduces methamphetamine-elicited autophagy,[276] inflammation,[277] and hippocampal progenitor cell death[278] and conserves blood–brain barrier integrity of brain microvascular endothelial cells.[279] They and others also have conducted in vivo studies and report that melatonin prevented the changes in neuronal nestin, doublecortin, and beta-III tubulin in mice treated with methamphetamine (Fig. 11).[280] The toxic drug also suppressed neuronal nitrogen-activate protein kinase and altered the expression of the N-methyl-D-aspartate receptor subunits NR2A and NR2B; each of these effects was attenuated when the mice were given melatonin. Using mice and a liposomal melatonin preparation, Nguyen et al.[281] found that one of the major targets by which melatonin reduces methamphetamine-related neuronal damage is due to the inhibition of the PKC-δ gene. This could account for the ability of melatonin to protect against mitochondrial dysfunction, apoptosis, and dopaminergic degeneration which occurs when mice are treated with methamphetamine.

2.6 | Melatonin as an antioxidant: food for thought

In the United States and many other countries, alcohol consumption and cigarette smoking are permitted. These habits reduce the quality of life of hundreds of thousands of humans annually. They contribute greatly to healthcare costs, which are already strained and their use causes the premature death of numerous humans. Yet, their use is endorsed. In contrast, getting support for an endogenously produced molecule such as melatonin, which experimentally at least, reduces the toxicity of alcohol consumption (Fig. 12)[282,283] and cigarette smoke,[284,285] has not been easy.

One criticism that is often levied against melatonin is that the potential negative consequences of its chronic use are not known. As noted above, melatonin is a component of the metabolic machinery perhaps of every organism, extinct and living, including organisms from bacteria to humans and plants; it predictably evolved a couple billion years ago.[286] Humans and all other species have managed to survive even though melatonin is continually endogenously produced throughout the life time of these species; thus, at least at physiological concentrations, melatonin has been "tested" in the long term. Pharmacological levels could, of course, have negative effects that have not yet revealed themselves. The vast majority of the published data, however, document that melatonin has a high-safety profile and many publications have verified its beneficial actions. Regarding tests to define the consequences of its long-term use, it should be noted there are numerous highly toxic drugs approved for use in humans. Moreover, in at least some cases, melatonin reduces the toxicity of these

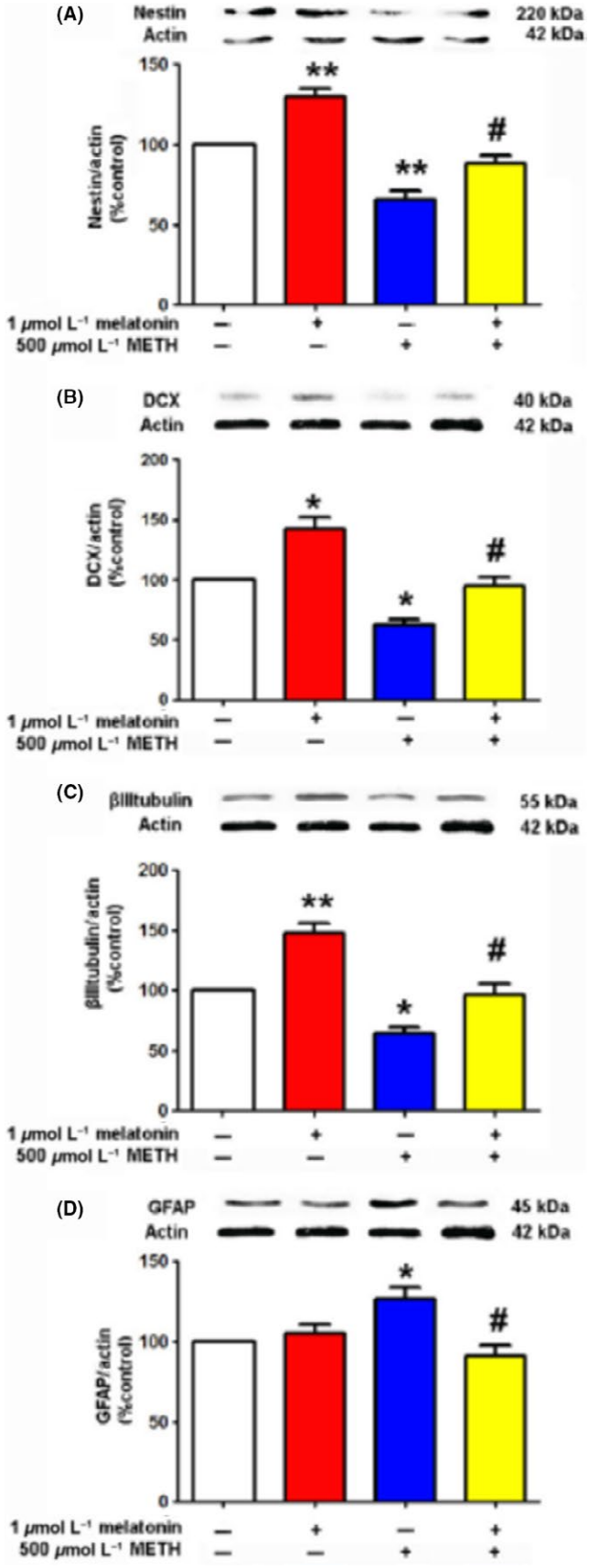

FIGURE 11 Effects of methamphetamine (METH) (500 μmol/L) without/with melatonin (100 μmol/L) on (A) nestin, (B) doublecortin (DCX), (C) BIII tubulin, and (D) glial fibrillary acid protein in neuronal phenotypes in culture. * and ** indicate $P<.05$ or $P<.01$, respectively, compared to controls; # indicates $P<.05$ compared to the METH group. From Ekthuwapranee et al.[278] with permission

pharmacological agents in normal cells[254,287,288] while enhancing the cancer-killing actions (also, see below) of conventional chemotherapeutic agents.[254,289–291] Yet, melatonin has not been sanctioned for use with these drugs or chemotherapies when they are given.

Glioblastoma, a common and deadly brain cancer that rapidly invades the surrounding tissue, is often refractory to conventional therapies that are used to kill them. The resistant glioblastoma cells do not respond well to TRAIL, the death receptor ligand, which promotes apoptosis signaling cascades.[292] When TRAIL was combined with melatonin for the treatment of A172 and U87 human glioblastoma cells, however, apoptotic cell death was greatly exaggerated over that caused by TRAIL alone (Fig. 13).[13] Based in their results, the authors proposed that the observed effect was related to a modulation of protein kinase c which reduced Akt activation resulting in a rise in death receptor 5 (DR5) levels; concurrently, the combination treatment reduced concentrations of the anti-apoptotic proteins, Bcl-2 and survivin. These observations are consistent with the repeated confirmation that melatonin enhances apoptotic cell death in many cancer types while reducing apoptosis in normal cells.[293] Because of these differential responses, the effects of melatonin on apoptosis are defined as being context specific.

The finding of Martin et al.[292] using glioblastoma cells are not an isolated observation. The same group reported that Ewing sarcoma, the second most common bone cancer, was also more profoundly killed when melatonin was in the mix.[294] Thus, Ewing cancer cells exhibit a greatly exaggerated apoptotic response when vincristine or ifosfamide treatment is combined with melatonin. Again, the major action seems to involve the extrinsic apoptotic pathway with marked increases in caspases 3, 8, and 9 and Bid when the treatments are combined. Also, in these cells, there was a substantial rise in free radical production that likely aided in apoptosis induction. The pro-oxidant action of melatonin is common in cancer cells, while in normal cells, the indoleamine is a powerful antioxidant.[104] This, again, points out the context specificity of melatonin's actions.

Cultured human breast cancer cells otherwise moderately sensitive to ionizing radiation were increasing susceptible to radiotherapy when they were treated for a week with physiological concentrations of melatonin.[295,296] Molecular studies of these cells indicated that the elevated sensitivity of the

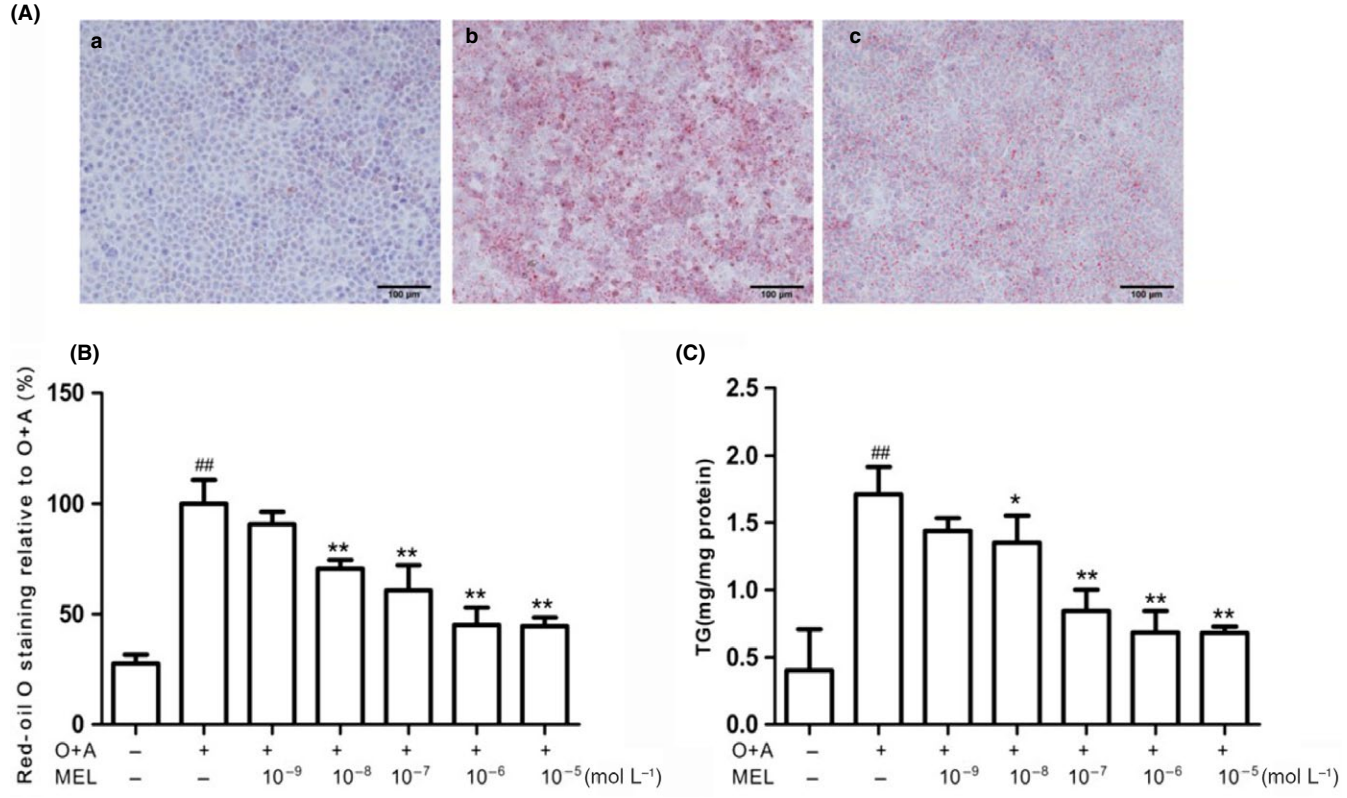

FIGURE 12 (A) Histological evidence showing that melatonin (10^{-6} mol/L) reduced lipid accumulation (evaluated by oil red O staining) in alcohol plus oleic acid-treated HepG2 hepatocytes only (b); (a) are untreated control cells. (B) Dose–response inhibition of lipid accumulation by melatonin. (C) Dose–response inhibition of triglycerides in hepatocytes. O, oleic acid; A, alcohol. ##P<.01 verses untreated controls; **P<.01 verses untreated control cells; *P<.05 and P<.01 alcohol-treated cells (second histogram from the left in both B and C). From Rui et al.[283] with permission

cancer cells involved a host of intracellular processes concerned with the regulation of proteins related to double-strand DNA breaks and to estrogen biosynthesis. Similar studies in human lung adenocarcinoma cells (SK-LV-1) showed that melatonin also increased their sensitivity to the chemotherapy, cisplatin.[297] In this case, the reduced cell proliferation was mediated by cell cycle arrest in the S phase.

In vivo, as well, melatonin changes the sensitivity of cancer cells to chemotherapies. Some breast cancers are resistant to the chemotherapeutic agent, doxorubicin. Xiang et al.[60] showed that MCF-7 human breast cancer cells growing in athymic nude rats grew faster when the daily dark period (animals on a 12:12 LD cycle) was contaminated with a light intensity that reduced the nocturnal endogenous melatonin peak (Fig. 14). Conversely, in rats experiencing darkness at night, which allowed the nighttime rise in melatonin, the tumor latency to onset, tumor regression, and reduced tumor metabolism were observed. Moreover, tumors growing in the rats exposed to darkness at night greatly increased their sensitivity to doxorubicin. The authors reported, in a related publication, that metabolically the tumors grown in rats exposed to light pollution at night are markedly different from the metabolism of those in rats exposed to darkness at night. The conclusion is that chronodisruption and melatonin suppression due to light at night accounted for the decreased sensitivity of the tumors to doxorubicin.

The collective data on the association of melatonin with cancer indicate that while melatonin itself has intrinsic cytotoxic actions in cancer cells,[59,61,298–300] it also sensitizes some cancers to conventional therapies and it reduces the toxicity of chemotherapies in normal cells, that is, it reduces the side effects of these drugs. This latter action would allow the chemotherapy to be given at higher doses, which would likely increase its cancer-killing activity. Overall, this information should be of interest to clinical oncologists; it is the hope of the authors that this information does not merely languish in the published literature. In view of the published data related to melatonin's ability to change the sensitivity of cancer cells to therapeutic agents, it is interesting to imagine that bacteria that become insensitive to drugs would perhaps exhibit renewed sensitivity if they were exposed to melatonin.

As already noted, a major consideration for the approval of any drug is its cost-to-benefit ratio. If the benefits derived from the use of even a highly damaging drug are determined to outweigh the physiological impairment it causes, its use may be approved. Using the same formula to evaluate melatonin, the data are overwhelmingly in favor of its benefits far

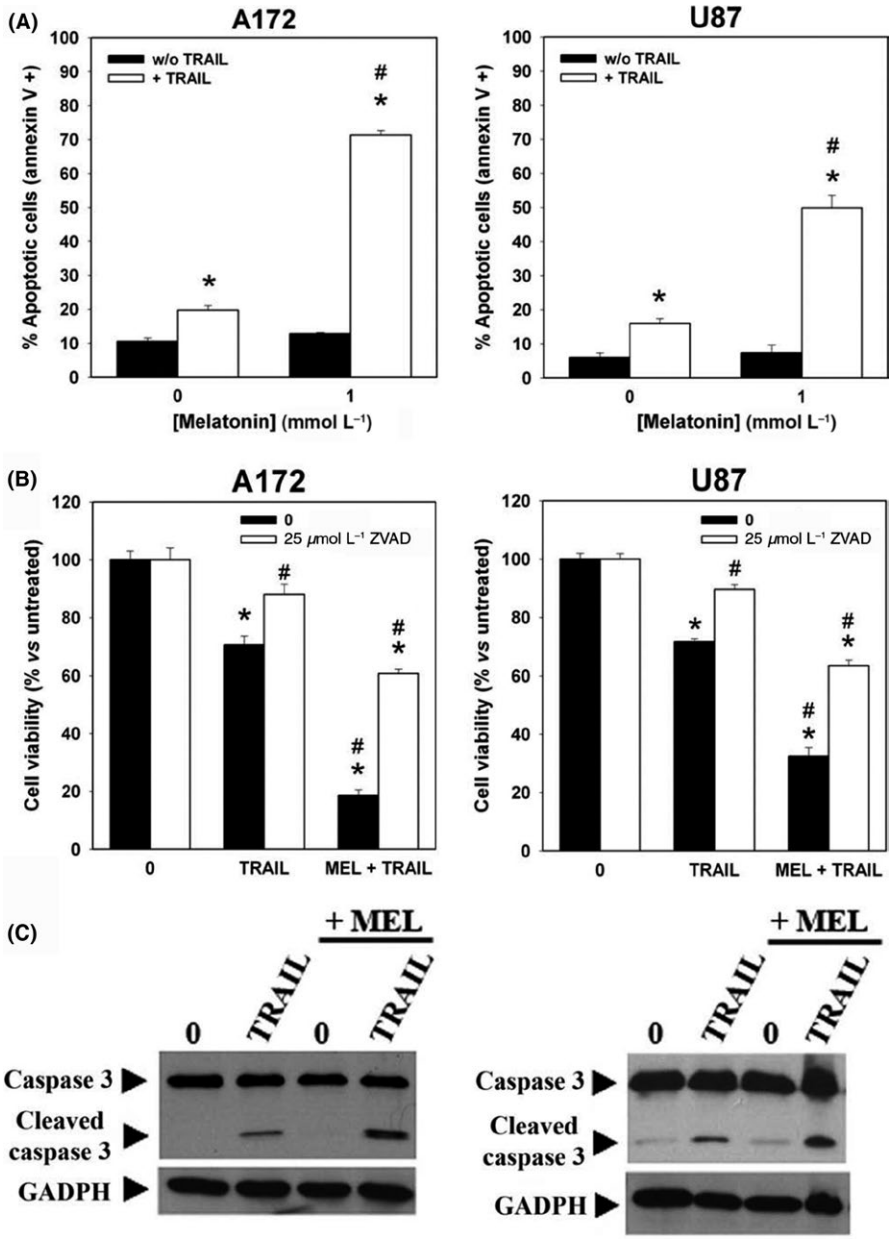

FIGURE 13 Melatonin sensitizes two human glioma cell lines, that is, A172 and U87, to TRAIL-mediated apoptosis. (A) Melatonin (1 mmol/L for 24 h) greatly increased apoptosis in both glioma cell types treated with TRAIL (100 ng/mL) added after melatonin. Apoptosis was assessed using the annexin V-binding assay. **P*<.05 verses untreated controls; #*P*<.05 verses TRAIL alone. (B) the pan-caspase inhibitor, ZVAD-fmk, reduced the apoptotic effects of combined melatonin/TRAIL treatment in both glioma cell types. ZVAD was added 4 h before melatonin. Cell viability was determined using the MTT assay. **P*<.05 verses untreated controls; #*P*<.05 verses TRAIL alone. (C) Western blots of caspase cleavage after combined melatonin/TRAIL treatment. From Martin et al.[292] with permission

exceeding the potential negative side effects, which under the worst case scenarios, seem minimal.

Melatonin has been available to the public for about 20 years, and, based on published sales figures, it may be taken regularly by tens of thousands of individuals. There are few reports of serious side effects due to its regular use and, if highly damaging, individuals would be "dropping like flies." If industry had a patentable molecule as efficacious as melatonin, it likely would have been tested and approved for large-scale, long-term use years ago. There are a number of patented melatonin analogs that are already sanctioned as drugs which, as they do not exist in nature, are always given in pharmacological doses. It seems reasonable to assume that the likelihood of them having toxicity in the long term would be greater than that for melatonin. There should be long duration trials of melatonin against serious diseases (where few treatments are available) where it has been shown beneficial in limited clinical studies or where the experimental evidence is compelling. Some examples include melatonin's ability to forestall Alzheimer's disease,[301–303] Parkinson's disease,[304–306] multiple sclerosis,[307,308] osteoporosis,[309–311] diabetes and metabolic syndrome,[312–315] sepsis,[316–318] cancer,[319–321] tropical diseases,[322–325] snake and nematocyst venom toxicity,[326–329] etc. In some cases, rather than a treatment for these conditions, melatonin should be more strongly considered in terms of its preventative actions as prevention always trumps treatment and is usually less expensive and certainly less debilitating.

Finally, during the Ebola epidemic in West Africa in 2014, two groups independently proposed the use of melatonin to

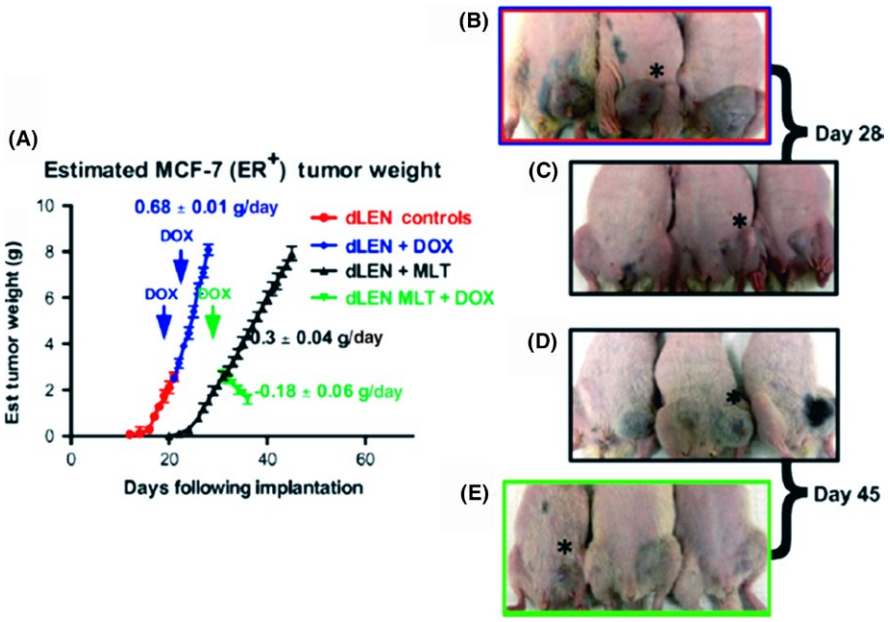

FIGURE 14 Effect of doxorubicin (DOX) on the growth and regression of MCF-7 (ER α+) breast tumor xenografts growing in a athymic nude female rats exposed to a light:dark cycle of 12:12 with the dark period contaminated with dim light exposure at night (dLEN) or dLEN supplemented with melatonin during the dim light period. (A) Estimated tumor weight (based on tumor measurements) in rats exposed to a dLEN lighting schedule and left untreated (red triangles) or DOX (blue diamonds) or exposed to dLEN and supplement with melatonin (black triangles) or dLEN and melatonin plus DOX (inverted green triangles). Photographs of tumors in rats maintained in either LD 12:12 + dLEN (B) or LD12:12 + dLEN but supplemented with melatonin (C). Panels (D) and (E) show tumors in the animals after 45 d after tumor implantation in animals kept in dLEN + melatonin (D) or dLEN + diluent (D). * identifies the location of the tumors. From Xiang et al.[60] with permission

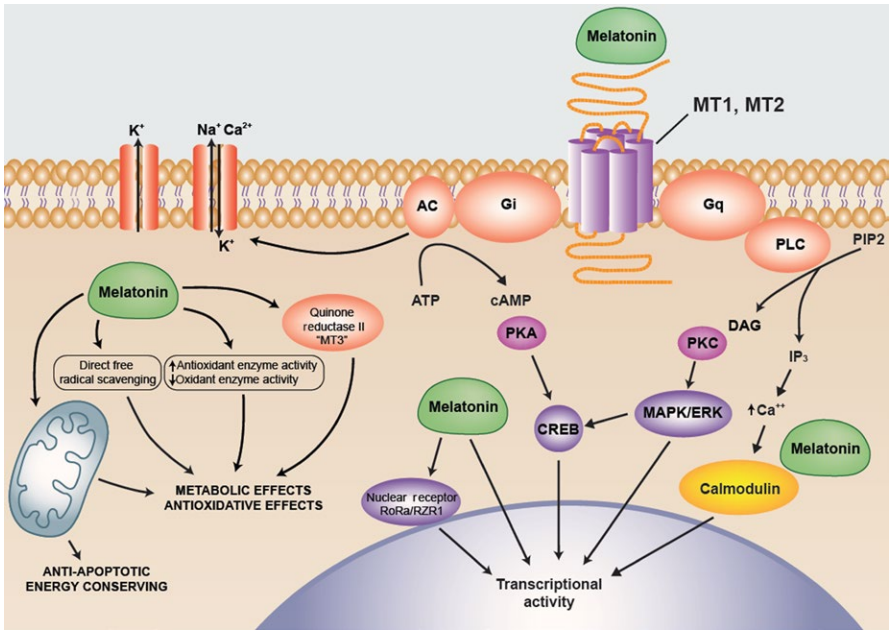

FIGURE 15 Some of the multiple molecular actions of melatonin, which account for its efficacy in reducing oxidative damage. Melatonin directly scavenges (illustrated on the left) ROS/RNS via receptor-independent actions thereby reducing mitochondrial damage and the apoptotic cascade. Melatonin may also act on cytosolic quinone reductase (MT3) to eliminate free radicals and reduce oxidative damage. The receptor-mediated actions are summarized on the right. Melatonin acts via membrane receptors (MT1/MT2) to stimulate a cascade of events which increase transcriptional activity; this leads to an upregulation of antioxidant enzymes and a downregulation of pro-oxidant enzymes as well as a reduction in toxic cytokine synthesis. Melatonin also binds to calmodulin to modulate nitric oxide production. Finally, some of these actions may also involve nuclear binding sites (RoR-α and RZR). Figure provided by Dr. Nicola Robertson

slow the progression of this disease so as to improve survival of the affected individuals.[330,331] In our publication, we highlighted the scientific evidence, which prompted our suggestion to use melatonin against this dreaded condition. Ebola virus disease is characterized by severe inflammation, coagulopathy, and endothelial disruption,[332] changes not unlike those caused by LPS-mediated sepsis, which has been successfully treated with melatonin.[333,334] Numerous reports also have documented the anti-inflammatory actions of melatonin.[335–337] Another feature of melatonin is its ability to reduce endothelial damage.[338,339] Whereas the evidence may be somewhat less compelling, melatonin's favorable effects on coagulopathy also have been described.[340] The rationale for the use of melatonin as a potential treatment voiced by Anderson et al.[331] was similar to that proposed by Tan et al.[330] Anderson et al.[331] also noted that melatonin upregulates heme oxygenase, which inhibits the replication of the Ebola virus.

The most recent viral scourge is that of the Zika virus.[341] Based on the antagonistic effects of melatonin on viral infections generally,[342–345] and as, like Ebola, there are few treatment options for Zika, perhaps melatonin should be given consideration to combat this viral infection as well.

3 | EPILOGUE AND PERSPECTIVE

Melatonin has a very large physiological footprint and some of the mechanisms by which this is achieved are

illustrated in Fig. 15. There is likely no organ or cell that is not impacted by this molecule. As summarized in this report, melatonin has a plethora of actions that make it extraordinarily efficacious in reducing the subcellular turmoil induced by oxidative destruction of key cellular elements which, when damaged, compromise the optimal function of cells often resulting in their disintegration via apoptosis or necrosis. Melatonin, in its capacity as an antioxidant, is proposed to have been the original function of this ancient and ubiquitously distributed molecule. Melatonin seems to be a linchpin of the highly complex antioxidative defense system.

In addition to its steadfastness in resisting oxidative stress, melatonin has a very wide number of essential molecular mechanisms (Fig. 15). What is usually measured as a result of melatonin actions, however, may merely be epiphenomena of its yet-to-be identified most fundamental ethos. Because of its highly divergent manifested actions, since its discovery almost six decades ago, melatonin has been designated as a regulator of regulators,[346] a refiner of physiology,[347] a tranquilizing agent,[348] a multitasking molecule[349] nature's most versatile signal,[156] etc. Recently, it was even classified as a biological Higgs boson,[350] a phrase that may actually best characterize this ingenious agent. It is the authors' current opinion that melatonin's basic function has yet to be uncovered or to put it in less formal terms, we are "seeing the smoke but not the fire."

In our estimation, it is unfortunate that melatonin is not more in the forefront of biomedical research. While it has gained some traction at the clinical level, its low toxicity profile and high efficacy in many pathophysiological states should make it a molecule more commonly tested/used in the medical and veterinary arenas. Certainly, one goal of this review was to strongly urge more attention be directed to melatonin in terms of is likely usefulness as a preventative and treatment for human and animal diseases.

REFERENCES

1. Reiter RJ, Tan DX, Galano A. Melatonin: exceeding expectations. *Physiology (Bethesda)*. 2014;29:325–333.
2. Fao C. Hypertrophie des testicules et de la crete après l'extirpation de la glande pineale chez le cuq. *Arch Ital Biol*. 1912;57:233–252.
3. Izawa Y. The effect of pinealectomy at 20 days of age on the growth of the reproductive system of male and female albino rat. *Trans Soc Pathol Jap*. 1926;16:72–78.
4. Kitay JI. Effects of pinealectomy on ovary weight in immature rats. *Endocrinology*. 1954;54:114–116.
5. Lerner AB, Case JD, Takahashi Y, et al. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc*. 1958;80:2587.
6. Lerner AB, Case JD, Heinzelmann RV. Structure of melatonin. *J Am Chem Soc*. 1959;81:6084–6085.
7. Chu EW, Wurtman RJ, Axelrod J. An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. *Endocrinology*. 1964;75:238–242.
8. Hoffman RA, Reiter RJ. Pineal gland: influence on gonads of male hamsters. *Science*. 1965;148:1609–1611.
9. Hoffman RA, Reiter RJ. Influence of compensatory mechanisms and the pineal gland on dark-induced gonadal atrophy in male hamsters. *Nature*. 1695;207:658–659.
10. Reiter RJ. Pineal control of a seasonal reproductive rhythm in male golden hamsters exposed to natural daylight and temperature. *Endocrinology*. 1973;92:423–430.
11. Reiter RJ. Influence of pinealectomy on the breeding capability of hamsters maintained under natural photoperiodic and temperature conditions. *Neuroendocrinology*. 1973/74;13:366–370.
12. Johnston PG, Zucker I. Antigonadal effects of melatonin in white-footed mice (*Peromyscus leucopus*). *Biol Reprod*. 1980;23:1069–1074.
13. Robert KA, Lesku JA, Partecke J, et al. Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. *Proc R Soc B*. 2015;242:20151745.
14. Minneman KP, Lynch H, Wurtman RJ. Relationship between environmental light intensity and retina-mediated suppression of rat pineal N-acetyltransferase. *Life Sci*. 1974;15:1791–1796.
15. Brainard GC, Richardson BA, King TS, et al. The suppression of pineal melatonin content and N-acetyltransferase activity by different light irradiances in the Syrian hamster: a dose-response relationship. *Endocrinology*. 1983;113:293–296.
16. Reiter RJ. Circannual reproductive rhythms in mammals related to photoperiod and pineal function: a review. *Chronobiologia*. 1974;1:365–395.
17. Quay WB. Volumetric and cytologic variation in the pineal body of *Peromyscus leucopus* (Rodentia) with respect to sex, captivity and daylength. *J Morphol*. 1956;98:471–495.
18. Mogler RK-H. Das endokrine system des syrischem Goldhamsters (*Mesocricetus auratus auratus* Waterhouse) unter Berücksichtigung des natürlicher und experimentellen Winterschlaf. *Z Morphol Ökol Tiere*. 1958;47:267–308.
19. Wurtman RJ, Axelrod J, Phillips L. Melatonin synthesis in the pineal gland: control by light. *Science*. 1963;142:1071–1073.
20. Quay WB. Circadian and estrous rhythms in pineal melatonin and 5-hydroxyindole-3-acetic acid. *Proc Soc Exp Biol Med*. 1964;115:710–713.
21. Quay WB. Retinal and pineal hydroxyindole-O-methyltransferase (HIOMT) activity in vertebrates. *Life Sci*. 1965;4:983–991.
22. Wurtman RJ, Axelrod J, Snyder SH, et al. Changes of the enzymatic synthesis of melatonin in the pineal gland during the estrous cycle. *Endocrinology*. 1965;76:798–800.
23. Wurtman RJ, Axelrod J, Fischer JE. Melatonin synthesis in the pineal gland: effect of light mediated by the sympathetic nervous system. *Science*. 1964;143:1328–1330.
24. Axelrod J, Wurtman RJ, Snyder SH. Control of hydroxyindole-O-methyltransferase activity in the rat pineal gland by environmental lightening. *Nature*. 1985;201:1134.
25. Klein DC, Weller JL. Indole metabolism in the pineal gland: a circadian rhythm in N-acetyltransferase. *Science*. 1970;169:1093–1095.
26. Liu T, Borjigin J. N-acetyltransferase is not rate-limiting enzyme of melatonin synthesis at night. *J Pineal Res*. 2005;39:91–96.
27. Tan DX, Hardeland R, Back K, et al. On the significance of an alternate pathway of melatonin synthesis via 5-methoxytryptomine: comparisons across species. *J Pineal Res*. 2016;61:27–40.
28. Byeon Y, Tan DX, Reiter RJ, et al. Predominance of 2-hydroxymelatonin over melatonin in plants. *J Pineal Res*. 2015;59:448–454.
29. Reiter RJ, Hester RJ. Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation of the endocrine systems of hamsters. *Endocrinology*. 1965;79:1168–1170.
30. Kappers JA. The development, topographical relations and innervation of the epiphysis cerebri in the albino rat. *Z Zellforsch*. 1960;52:163–215.
31. Wurtman RJ, Axelrod J. The pineal gland. *Sci Am*. 2013;1965:50–60.
32. Nagle CA, Cardinali DP, Rosner JM. Retinal and pineal hydroxyindole-O-methyltransferase in the rat: changes following cervical sympathectomy, pinealectomy or blinding. *Endocrinology*. 1973;92:1560–1564.

33. Benson B, Matthews MJ, Rodin AF. Presence of a non-melatonin pineal antigonadotropin. *Acta Endocrinol*. 1972;69:257–266.
34. Ebels I, Benson B, Matthews MJ. Localization of a sheep pineal antigonadotropin. *Anal Biochem*. 1973;56:546–565.
35. Ebels I, Moszkowska A, Scemama A. An attempt to separate a sheep pineal extract fraction showing antigonadotropic activity. *J Neurovisc Rel*. 1970;32:1–10.
36. Pevet P, Karasek M. Are the pineal active compounds of mammals proteinaceous in nature? An ultrastructural contribution. *Acta Med Pol*. 1977;18:351–353.
37. Korkushko OV, Khavinson VKH, Shantilo VB, et al. Geroprotective effect of epithalamine (pineal gland peptide preparation) in elderly subjects with accelerated aging. *Bull Exp Biol Med*. 2006;142:356–359.
38. Zamorskii II, Sopova IY, Khavinson VKh. Effects of melatonin and epithalamin on the content of protein and lipid peroxidation products in rat cortex and hippocampus under conditions of acute hypoxia. *Bull Exp Biol Med*. 2012;154:51–53.
39. Quay WB. Indole derivatives of pineal and related neural and retinal tissues. *Pharmacol Rev*. 1965;17:321–345.
40. Cardinali DP, Rosner JM. Retinal localization of the hydroxyindole-O-methyltransferase (HIOMT) in the rat. *Endocrinology*. 1971;89:301–303.
41. Eakin RM, Westfall JA. Fine structure of the retina in the reptilian third eye. *J Biophys Biochem Cytol*. 1959;6:133–134.
42. Besharse JC, McMahon DG. The retina and other ocular clocks. *J Biol Rhythms*. 2016;31:223–243.
43. Reiter RJ, Tan DX, Kim SJ, et al. Delivery of pineal melatonin to the brain and SCN: role of canaliculi, cerebrospinal fluid, tanycytes and Virchow-Robin perivascular spaces. *Brain Struct Funct*. 2014;219:1873–1887.
44. Pevet P. The internal time giving role of melatonin: a key to our health. *Rev Neurol (Paris)*. 2014;170:646–652.
45. Vriend J, Reiter RJ. Melatonin feedback on clock genes: a theory involving the proteasome. *J Pineal Res*. 2015;58:1–11.
46. Wolloscheck T, Kunst S, Kelleher DK, et al. Transcriptional regulation of nucleoredoxin-like genes takes place on a daily basis in the retina and pineal gland of rats. *Vis Neurosci*. 2015;32:E002.
47. Korf HW. Evolution of melatonin producing pinealocytes. *Adv Esp Med Biol*. 1999;460:17–29.
48. Bubenik GA, Brown GM, Groto LJ. Immunohistological localization of melatonin in the rat digestive system. *Experientia*. 1977;33:662–663.
49. Chen CQ, Fichna J, Bashashati M, et al. Distribution, function and physiological role of melatonin in the lower gut. *World J Gastroenterol*. 2011;17:3888–3898.
50. Acuna-Castroviejo D, Escames G, Venegas C, et al. Extrapineal melatonin: sources, regulation and potential functions. *Cell Mol Life Sci*. 2014;79:2997–3028.
51. Reiter RJ, Richardson BA, Matthews SA, et al. Rhythms in immunoreactive melatonin in the retina and Harderian gland of rats: persistence after pinealectomy. *Life Sci*. 1983;32:1229–1236.
52. Hoffman RA, Wertz P, Habeeb P. Harderian glands of golden hamsters: morphological and biochemical responses to thyroid hormones. *J Comp Physiol B*. 1989;159:293–299.
53. Coto-Montes A, Garcia-Macia M, Caballero B, et al. Analysis of constant tissue remodeling in Syrian hamster Harderian gland: intra-tubular and inter-tubular syncytial masses. *J Anat*. 2013;222:558–569.
54. Hoffman RA, Johnson LB, Reiter RJ. Regulation of melatonin in the harderian glands of golden hamsters. *J Pineal Res*. 1989;6:63–71.
55. Dauchy RT, Dauchy EM, Hanifin JP, et al. Effects of spectral transmittance through standard laboratory cages on circadian metabolism and physiology in nude rats. *J Am Assoc Lab Anim Sci*. 2013;52:146–156.
56. Brainard GM, Hanifin JP, Warfield B, et al. Short-wavelength enrichment of polychromatic light enhances human melatonin suppression potency. *J Pineal Res*. 2015;58:352–361.
57. Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. *Nature*. 2002;415:493.
58. Hattar S, Kumar M, Park A, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. 2006;497:326–349.
59. Blask DE, Dauchy RT, Sauer LA. Putting cancer to sleep at night: the neuroendocrine/circadian melatonin signal. *Endocrine*. 2005;27:179–188.
60. Xiang S, Dauchy RT, Hauch A, et al. Doxorubicin resistance in breast cancer is driven by light at night-induced disruption of the circadian melatonin signal. *J Pineal Res*. 2015;59:60–69.
61. Hill SM, Belancio VP, Dauchy RT, et al. Melatonin: an inhibitor of breast cancer. *Endocr Relat Cancer*. 2015;22:R183–R204.
62. Liu J, Clough SJ, Hutchinson AJ, et al. MT1 and MT2 melatonin receptors: a therapeutic perspective. *Annu Rev Pharmacol Toxicol*. 2016;56:361–383.
63. Legros C, Chesneau D, Boutin JA, et al. Melatonin from cerebrospinal fluid but not from blood reaches sheep cerebral tissues under physiological conditions. *J Neuroendocrinol*. 2014;26:151–163.
64. Tan DX, Manchester LC, Reiter RJ, et al. High physiological levels of melatonin in the bile of mammals. *Life Sci*. 1999;65:2523–2529.
65. Reiter RJ, Tan DX, Kim SS, et al. Augmentation of indices of oxidative damage in live-long melatonin deficient rats. *Mech Aging Dev*. 1999;110:157–173.
66. Manchester LC, Poeggeler B, Alvares FL, et al. Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: implications for an ancient antioxidant defense system. *Cell Mol Biol Res*. 1995;41:391–395.
67. Poeggeler B, Hardeland R. Detection and quantification of melatonin in a dinoflagellate, *Gonyaulax polyedra*: solutions to the problem of methoxyindole destruction in non-vertebrate material. *J Pineal Res*. 1994;17:1–10.
68. Arnault F, Vivien-Roels B, Pevet P, et al. Melatonin in the nemertine worm *Lineus lacteus*: identification and daily variations. *Neuro Signals*. 1994;3:296–301.
69. Hardeland R, Poeggeler B. Non-vertebrate melatonin. *J Pineal Res*. 2003;34:233–241.
70. Dubbels R, Reiter RJ, Klenke E, et al. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J Pineal Res*. 1995;18:28–31.
71. Hattori A, Migitaka H, Iigo M, et al. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem Mol Biol Int*. 1995;35:627–634.
72. Arnao MB, Hernandez-Ruiz J. Functions of melatonin in plants: a review. *J Pineal Res*. 2015;59:133–150.
73. Hardeland R, Fuhrberg B, Uria H, et al. Chronobiology of indoleamines in the dinoflagellate *Gonyaulax polyedra*: metabolism and effects related to circadian rhythmicity and photoperiodism. *Braz J Med Biol Res*. 1996;29:119–123.
74. Tan DX, Manchester LC, DiMascio P, et al. Novel rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. *FASEB J*. 2007;21:1724–1729.
75. Bajwa VS, Shukla MR, Sherif SM, et al. Roles of melatonin in alleviating cold stress in *Arabidopsis thaliana*. *J Pineal Res*. 2014;56:238–245.
76. Tal O, Haim A, Harel O, et al. Melatonin as an antioxidant and its semilunar rhythm in green macroalga *Ulva* sp. *J Exp Bot*. 2011;62:1903–1910.
77. Reiter RJ, Tan DX, Zhou Z, et al. Phytomelatonin: assisting plants to survive and thrive. *Molecules*. 2015;20:7396–7437.
78. Tan DX, Manchester LC, Zhou Z, et al. Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism. *Molecules*. 2015;20:18886–18906.
79. Tan DX, Chen LD, Poeggeler B, et al. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr J*. 1993;1:57–60.
80. Tan DX, Manchester LC, Reiter RJ, et al. A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. *Biochem Biophys Res Commun*. 1998;253:614–620.
81. Marshall KA, Reiter RJ, Poeggeler B, et al. Evaluation of the antioxidant activity of melatonin in vitro. *Free Radic Biol Med*. 1995;21:307–315.
82. Sewerynek E, Melchiorri D, Reiter RJ, et al. Melatonin reduces H_2O_2-induced lipid peroxidation in homogenates of different rat brain regions. *J Pineal Res*. 1995;19:51–56.

83. Pierrefiche G, Topall G, Courborin G, et al. Antioxidant activity of melatonin in mice. *Res Commun Chem Pathol Pharmacol*. 1993;80:211–223.
84. Abe M, Reiter RJ, Orhii PB, et al. Inhibitory effect of melatonin on cataract formation in newborn rats: evidence for an antioxidative role for melatonin. *J Pineal Res*. 1994;17:94–100.
85. Daniels WMU, Reiter RJ, Melchiorri D, et al. Melatonin counteracts lipid peroxidation induced by carbon tetrachloride but does not restore glucose-6-phosphatase activity. *J Pineal Res*. 1995;19:1–6.
86. Melchiorri D, Reiter RJ, Attia AM, et al. Potent protective effect of melatonin on in vivo paraquat-induced oxidative damage in rats. *Life Sci*. 1995;56:83–89.
87. Sewerynek E, Melchiorri D, Chen LD, et al. Melatonin reduces both basal and bacterial lipopolysaccharide-induced lipid peroxidation in vitro. *Free Radic Biol Med*. 1995;19:903–909.
88. Vijayalaxmi , Reiter RJ, Meltz ML. Melatonin protects red human blood lymphocytes from radiation induced chromosome damage. *Mutat Res*. 1995;346:23–31.
89. Vijayalaxmi , Reiter RJ, Sewerynek E, et al. Marked reduction of radiation-induced micronuclei in human blood lymphocytes pre-treated with melatonin. *Radiat Res*. 1995;18:104–111.
90. Matuszak Z, Reszka K, Chignell CF. Reaction of melatonin and related indoles with hydroxyl radicals: EPR and spin trapping investigations. *Free Radic Biol Med*. 1997;23:367–372.
91. Stasica P, Ulanski P, Rosiak JM. Melatonin as a hydroxyl radical scavenger. *J Pineal Res*. 1998;25:65–66.
92. Mahal HS, Sharma HS, Mukherjee T. Antioxidant properties of melatonin: a pulse radiolysis study. *Free Radic Biol Med*. 1999;16:557–565.
93. Ebelt H, Peschke D, Brömme HJ, et al. Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. *J Pineal Res*. 2000;28:65–72.
94. Galano A. On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals. *Phys Chem Chem Phys*. 2011;13:7178–7188.
95. Harasimowicz J, Marques KL, Silva AF, et al. Chemiluminometric evaluation of melatonin and selected melatonin precursors' interaction with reactive oxygen and nitrogen species. *Anal Biochem*. 2012;420:1–6.
96. Garcia JJ, Lopez-Pingarron L, Almeida-Souza P, et al. Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: a review. *J Pineal Res*. 2014;56:225–237.
97. Reiter RJ. Functional pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front Neuroendocrinol*. 1995;16:383–415.
98. Zhang L, Wei W, Xu J, et al. Inhibitory effect of melatonin on diquat-induced lipid peroxidation in vivo as assessed by the measurement of F2-isoprostanes. *J Pineal Res*. 2006;40:326–331.
99. Slominski A, Tobin DJ, Zmijewski MA, et al. Melatonin in the skin: synthesis, metabolism and functions. *Trends Endocrinol Metab*. 2008;19:17–26.
100. Da Silva Borges L, Dermargos A, de Silva Junior EP, et al. Melatonin decreases muscular oxidative stress and inflammation induced by strenuous exercise and stimulates growth factor synthesis. *J Pineal Res*. 2015;58:166–172.
101. Zhao L, An R, Yang Y, et al. Melatonin alleviates brain injury in mice subjected to cecal ligation and puncture via attenuating inflammation, apoptosis, and oxidative stress: the role of SIRT1 signaling. *J Pineal Res*. 2015;59:230–239.
102. San-Miguel B, Crespo I, Sanchez DI, et al. Melatonin inhibits autophagy and endoplasmic reticulum stress in mice with carbon tetrachloride-induced fibrosis. *J Pineal Res*. 2015;59:151–162.
103. Hardeland R. Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. *J Pineal Res*. 2013;55:325–356.
104. Zhang HM, Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. *J Pineal Res*. 2014;57:131–146.
105. Manchester LC, Coto-Montes A, Boga JA, et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. *J Pineal Res*. 2015;59:403–419.
106. Barlow LR, Reiter RJ, Abe M, et al. Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int*. 1995;26:497–502.
107. Pablos MI, Agapito MT, Gutierrez R, et al. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks. *J Pineal Res*. 1995;19:111–115.
108. Pablos MI, Chuang JI, Reiter RJ, et al. Time course of melatonin-induced increase in glutathione peroxidase activity in chicks. *Biol Signals*. 1995;4:325–330.
109. Pablos MI, Reiter RJ, Ortiz GG, et al. Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. *Neurochem Int*. 1998;32:69–75.
110. Rodriguez C, Mayo JC, Sainz RM, et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res*. 2004;36:1–9.
111. Fischer TW, Kleszczynski K, Hardkop LH, et al. Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVB-induced depletion, and protects against the formation of DNA damage (8-hydroxy-2′-deoxyguanosine) in ex vivo human skin. *J Pineal Res*. 2013;54:303–312.
112. Urata Y, Honma S, Goto S, et al. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic Biol Med*. 1999;27:839–847.
113. Winiarska K, Drozak J, Wegrzynowiz M, et al. Diabetes-induced changes in glucose synthesis, intracellular glutathione status and hydroxyl free radical generation in rabbit kidney-cortex tubules. *Mol Cell Biochem*. 2004;26:91–98.
114. Winiarska K, Fraczyk T, Malinska D, et al. Melatonin attenuates diabetes-induced oxidative stress in rabbits. *J Pineal Res*. 2006;40:168–176.
115. Poeggeler B, Reiter RJ, Hardeland R, et al. Melatonin, a mediator of electron transfer and repair reactions, acts synergistically with the chain breaking antioxidant ascorbate, trolox, and glutathione. *Neuroendocrinol Lett*. 1995;17:87–92.
116. Gitto E, Tan DX, Reiter RJ, et al. Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. *J Pharm Pharmacol*. 2001;53:1393–1401.
117. Gilad E, Cuzzocrea S, Zingarilli B, et al. Melatonin is a scavenger of peroxynitrite. *Life Sci*. 1997;60:PL169–PL174.
118. Noda Y, Mori A, Liburty R, et al. Melatonin and its precursor scavenge nitric oxide. *J Pineal Res*. 1999;27:159–163.
119. Pozo D, Reiter RJ, Calvo JR, et al. Physiological concentrations of melatonin inhibit nitric oxide synthase in rat cerebellum. *Life Sci*. 1994;55:PL455–PL460.
120. Bettahi I, Pozo D, Osuna C, et al. Melatonin reduces nitric oxide synthase activity in the rat hypothalamus. *J Pineal Res*. 1996;20:205–210.
121. Benot S, Goberna R, Reiter RJ, et al. Physiological levels of melatonin contribute to the antioxidant capacity of human serum. *J Pineal Res*. 1999;27:59–64.
122. Reiter RJ, Tan DX, Maldonado MD. Melatonin as an antioxidant: physiology versus pharmacology. *J Pineal Res*. 2005;39:215–216.
123. Galano A, Tan DX, Reiter RJ. Cyclic 3-hydroxymelatonin, a key metabolite enhancing the peroxyl radical scavenging activity of melatonin. *RSC Adv*. 2014;41:304–316.
124. Tan DX, Hardeland R, Manchester LC, et al. Cyclic 3-hydroxymelatonin (C3OHM), a potent antioxidant, scavenges free radicals and suppresses oxidative reaction. *Curr Med Chem*. 2014;21:1557–1565.
125. Tan DX, Manchester LC, Reiter RJ, et al. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway for melatonin biotransformation. *Free Radic Biol Med*. 2000;29:1177–1185.
126. Tan DX, Reiter RJ, Manchester LC, et al. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr Top Med Chem*. 2002;2:181–187.
127. Ressmeyer AR, Mayo JC, Zelosko V, et al. Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK) scavenging of free radicals and prevention of protein destruction. *Redox Rep*. 2003;8:205–213.

128. Rosen J, Than NN, Koch D, et al. Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. *J Pineal Res*. 2006;41:374–381.
129. Hardeland R, Tan DX, Reiter RJ. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. *J Pineal Res*. 2009;47:109–126.
130. Schaefer M, Hardeland R. The melatonin metabolite N-acetyl-5-methoxykynuramine as a potent singlet oxygen scavenger. *J Pineal Res*. 2009;46:49–52.
131. Galano A, Tan DX, Reiter RJ. On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. *J Pineal Res*. 2013;54:245–257.
132. Reiter RJ, Tan DX, Galano A. Melatonin reduces lipid peroxidation and membrane viscosity. *Front Physiol*. 2014;5:377.
133. Reiter RJ, Tan DX, Burkhardt S, et al. Melatonin in plants. *Nutr Rev*. 2001;59:286–290.
134. Tan DX, Hardeland R, Manchester LC, et al. Functional roles of melatonin in plants and perspectives in nutritional and agricultural science. *J Exp Bot*. 2012;63:577–597.
135. Merry JF, Xu TF, Wang ZZ, et al. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplasts. *J Pineal Res*. 2014;57:200–212.
136. Shi H, Wang W, Tan DX, et al. Comparative physiological and proteomic analyses reveal the actions of melatonin in the reduction of oxidative stress in Bermuda grass (*Cynodon dactylon* (L) Pers.). *J Pineal Res*. 2015;59:120–131.
137. Shi H, Qian Y, Tan DX, et al. Melatonin induces the transcripts of CBF/DREB1s and their involvement in both abiotic and biotic stresses in *Arabidopsis*. *J Pineal Res*. 2015;59:334–342.
138. Limson J, Nyokong T, Daya S. The interaction of melatonin and its precursors with aluminum, cadmium, copper, iron, lead and zinc: an absorptive voltammetric study. *J Pineal Res*. 1998;24:15–21.
139. Tan DX, Manchester LC, Reiter RJ. CSF generation by pineal gland results in a robust melatonin circadian rhythm in the third ventricle as a unique light:dark signal. *Med Hypotheses*. 2016;86:3–9.
140. Parmar P, Limson J, Nyokong T, et al. Melatonin protects against copper-mediated free radical damage. *J Pineal Res*. 2002;32:237–242.
141. Mayo JC, Tan DX, Sainz RM, et al. Protection against oxidative protein damage induced by metal-catalyzed reaction or alkylperoxyl radicals: comparative effects of melatonin and other antioxidants. *Biochim Biophys Acta*. 2003;1620:139–150.
142. Gulcin I, Buyukokuroglu ME, Kufrevioglu OI. Metal chelating and hydrogen peroxide scavenging effects of melatonin. *J Pineal Res*. 2003;34:278–281.
143. Zatta P, Tognon G, Carampi P. Melatonin prevents free radical formation due to the interaction between β-amyloid peptides and metal ions [Al(III), Zn(II), Cu(II), Mn(II), Fe(II)]. *J Pineal Res*. 2003;35:95–103.
144. Galano A, Medina ME, Tan DX, et al. Melatonin and its metabolite as copper chelating agents and their role in inhibiting oxidative stress: a physiochemical analysis. *J Pineal Res*. 2015;58:107–116.
145. Huang X, Moir RD, Tanzi RE, et al. Redox-sensitive metals, oxidative stress and Alzheimer's disease pathology. *Ann NY Acad Sci*. 2004;1012:153–163.
146. Donnelly PS, Xiao Z, Wedd AG. Copper and Alzheimer's disease. *Curr Opin Chem Biol*. 2007;11:128–133.
147. Gaggelli E, Kozlawski H, Valensin D, et al. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). *Chem Rev*. 2006;106:1995–2044.
148. Barnham KJ, Bush AI. Metals in Alzheimer's and Parkinson's diseases. *Curr Opin Chem Biol*. 2008;12:222–230.
149. Fox JH, Kawa JA, Liebermann G, et al. Mechanisms of copper mediated Huntington's disease progression. *PLoS One*. 2007;2:e334.
150. Hayashi H, Yano M, Fujita Y, et al. Compound overload of copper and iron in patients with Wilson's disease. *Med Mol Morphol*. 2006;39:121–126.
151. Aaseth J, Flaten TP, Andersen O. Hereditary iron and copper deposition: diagnostics, pathogenesis and therapeutics. *Scand J Gastroenterol*. 2007;42:673–681.
152. Tan DX, Manchester LC, Terron MP, et al. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and reactive nitrogen species? *J Pineal Res*. 2007;42:28–42.
153. Romero A, Ramos E, de los Rios C, et al. A review of metal-catalyzed molecular damage: prevention by melatonin. *J Pineal Res*. 2014;56:343–370.
154. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine*. 2005;27:119–130.
155. Mauriz JL, Collado PS, Veneroso C, et al. A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. *J Pineal Res*. 2013;54:1–54.
156. Pandi-Perumal SR, Srinivasan V, Maestroni GJ, et al. Melatonin: nature's most versatile biological signal. *FEBS Lett*. 2006;273:2813–2838.
157. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003;552:335–344.
158. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J*. 2009;417:1–13.
159. Miguel J. An integrated theory of aging as the result of mitochondrial DNA mutation in differentiated cells. *Arch Gerontal Geriatr*. 1991;12:99–117.
160. Kalous M, Drahota Z. The role of mitochondria in aging. *Physiol Res*. 1996;45:351–359.
161. Kowald A. The mitochondrial theory of aging. *Biol Signals Recept*. 2001;10:162–175.
162. Goode HF, Cowley HC, Walker BE, et al. Decreased antioxidant status and increased lipid peroxidation in patients and sepsis and secondary organ dysfunction. *Crit Care Med*. 1995;23:646–653.
163. Mistra V. Oxidative stress and role of antioxidant supplementation in critical illness. *Clin Lab*. 2007;53:199–209.
164. Poljsak B. Strategies for reducing or preventing the generation of oxidative stress. *Oxid Med Cell Longev*. 2011;2011:194586.
165. Halliwell B. Vitamin C: antioxidant or pro-oxidant in vivo? *Free Radic Res*. 1996;25:439–454.
166. James AM, Smith RAJ, Murphy MP. Antioxidant and pro-oxidant properties of mitochondrial coenzyme Q. *Arch Biochem Biophys*. 2004;423:47–56.
167. Pan MH, Lai CS, Tsai ML, et al. Molecular mechanisms for anti-aging by natural dietary compounds. *Mol Nutr Food Res*. 2012;56:88–115.
168. Smith RAJ, Porteous CM, Cane AM, et al. Delivery of bioactive molecules to mitochondria in vivo. *Proc Natl Acad Sci USA*. 2003;100:5407–5412.
169. Solescio ME, Prime TA, Logan A, et al. The mitochondria-targeted antioxidant MitoQ reduces aspects of mitochondrial fission in the 6-OHDA cell model of Parkinson's disease. *Biochim Biophys Acta*. 2013;1832:174–182.
170. Galley HF. Bench-to-bedside review: targeting antioxidants to mitochondria in sepsis. *Crit Care*. 2012;14:230–240.
171. Kelso GF, Porteous CM, Coulter CV, et al. Selective targeting of redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *J Biol Chem*. 2001;276:4588–4596.
172. Bedogni B, Pani G, Colavitti R, et al. Redox regulation of cAMP-responsive element-binding protein and induction of manganous superoxide dismutase in nerve growth factor-dependent cell survival. *J Biol Chem*. 2003;278:16510–16519.
173. Dhanasekaran A, Kotamraju S, Kalivendi SV, et al. Supplementation of endothelial cells with mitochondrial-targeted antioxidants inhibit peroxide-induced mitochondrial uptake, oxidative damage and apoptosis. *J Biol Chem*. 2004;279:37575–37587.
174. Mithell T, Rotaru D, Saba H, et al. The mitochondrial-targeted antioxidant mitoquinone protects against cold storage injury of renal tubular cells and rat kidneys. *J Pharmacol Exp Ther*. 2011;336:682–692.
175. Ramis MR, Esteban S, Miralles A, et al. Protective effects of melatonin and mitochondria-target antioxidants against oxidative stress: a review. *Curr Med Chem*. 2015;22:2690–2711.
176. Lowes DA, Webster NR, Murphy MP, et al. Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial

function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *Br J Anesth*. 2013;110:472–480.

177. Venegas C, Garcia GA, Escames G, et al. Extrapineal melatonin: analysis of its subcellular distribution and daily functions. *J Pineal Res*. 2012;52:217–227.
178. He C, Wang J, Zhang Z, et al. Mitochondria synthesize melatonin to ameliorate its function and improve mice oocyte quality under in vitro conditions. *Int J Mol Sci*. 2016;17:E939.
179. Tan DX, Hardeland R, Manchester LC, et al. Changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness. *Biol Rev Comb Philos Soc*. 2010;85:607–623.
180. Marshall JC, Vinent JL, Guyatt G, et al. Outcome measures for clinical research in sepsis: a report of the 2nd Cambridge Colloquium of the International Sepsis Forum. *Crit Care Med*. 2005;33:1708–1716.
181. Carden DL, Granger DN. Pathophysiology of ischemia-reperfusion injury. *J Pathol*. 2000;190:255–266.
182. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species. The evolution of a concept. *Redox Biol*. 2015;6:524–551.
183. Sluijter JP, Cordonelli G, Davidson SM, et al. Novel therapeutic strategies for cardioprotection. *Pharmacol Ther*. 2014;144:60–70.
184. Qu J, Chen W, Hu R, et al. The injury and therapy of reactive oxygen species in intracerebral hemorrhage: looking at mitochondria. *Oxid Med Cell Longev*. 2016;2016:2592935.
185. Guerrero JM, Reiter RJ, Ortiz GG, et al. Melatonin prevents increases in neural nitric oxide and cyclic GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (*Meriones unguiculatus*). *J Pineal Res*. 1997;23:2431.
186. Kilic U, Yilmaz B, Ugur M, et al. Evidence that membrane-bound G Protein-coupled melatonin receptors MT1 and MT2 are not involved in the neuroprotective effects of melatonin in focal brain ischemia. *J Pineal Res*. 2012;52:228–235.
187. Kilic U, Yilmaz B, Reiter RJ, et al. Effects of memantine and melatonin on signal transduction pathways vascular leakage and brain injury after focal cerebral ischemia in mice. *Neuroscience*. 2013;237:268–276.
188. Kilic E, Ozdemir YG, Bolay H, et al. Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J Cereb Blood Flow Metab*. 1999;19:511–516.
189. Carloni S, Albertini MC, Galluzzi I, et al. Melatonin reduces reticulum stress and preserves sirtuin 1 expression in neural cells of newborn rats after hypoxia–ischemia. *J Pineal Res*. 2014;57:192–199.
190. Li H, Wang Y, Feng D, et al. Alterations in the time course of expression of the Nox family of the brain in a rat experimental cerebral ischemia and reperfusion model: effects of melatonin. *J Pineal Res*. 2014;57:110–119.
191. Zheng Y, Hou J, Lin J, et al. Inhibition of autophagy contributes to melatonin-mediated neuroprotection against transient focal cerebral ischemia in rats. *J Pharmacol Sci*. 2014;124:354–384.
192. Paredes SD, Rancan L, Kireev R, et al. Melatonin counteracts at the transcriptional level in inflammatory and apoptotic response secondary to ischemic brain injury by middle cerebral artery blockade in aging rats. *Biomed Open Access*. 2015;4:407–416.
193. Fulia F, Gitto E, Cuzzocrea S, et al. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. *J Pineal Res*. 2001;31:343–349.
194. Aly H, Elmahdy H, El-Dib W, et al. Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. *J Perinatol*. 2015;35:185–191.
195. Tan DX, Manchester LC, Reiter RJ, et al. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. *J Pineal Res*. 1998;25:184–191.
196. Petrosillo G, Colantuono G, Moro N, et al. Melatonin protects against heart ischemia-reperfusion injury by inhibiting mitochondrial permeability transition pore opening. *Am J Physiol Heart Circ Physiol*. 2009;297:H1487–H1493.
197. Liu LF, Qin Q, Qin ZH, et al. Protective effects of melatonin on ischemia-reperfusion induced myocardial damage and hemodynamic recovery in rats. *Eur Rev Med Pharmacol Sci*. 2014;18:3681–3688.
198. Yu L, Sun Y, Cheng L, et al. Melatonin receptor-mediated protection against myocardial ischemia-reperfusion injury: role of SIRT1. *J Pineal Res*. 2014;57:228–238.
199. He B, Zhao Y, Xu L, et al. The nuclear melatonin receptor RORα is a novel endogenous defender against myocardial ischemia/reperfusion injury. *J Pineal Res*. 2014;60:313–326.
200. Nduhirabandi F, Lamont K, Albertyn Z, et al. Role of toll-like receptor 4 in melatonin-induced cardioprotection. *J Pineal Res*. 2016;60:39–47.
201. Gogenur I, Kuchukakin B, Jensen LP, et al. Melatonin reduces cardiac morbidity and markers of myocardial ischemia after elective abdominal aorta aneurism repair: a randomized, placebo-controlled, clinical trial. *J Pineal Res*. 2014;57:10–15.
202. Dwaich KH, Al-Amran FGY, Al-Sheibani BIM, et al. Melatonin effects on myocardial ischemia-reperfusion injury: impact on the outcome in patients undergoing coronary artery bypass grafting surgery. *Int J Cardiol*. 2016;221:977–986.
203. Lacoste B, Angeloni D, Dominguez-Lopez C, et al. Anatomical and cellular localization of melatonin MT1 and MT2 receptors in the adult rat brain. *J Pineal Res*. 2015;58:397–417.
204. Boutin JA. Quinone reductase 2 as a promising target of melatonin therapeutic actions. *Expert Opin Ther Targets*. 2016;20:303–317.
205. Hardeland R. Melatonin, hormone of darkness and more: occurrence, control mechanisms, actions and bioactive substances. *Cell Mol Life Sci*. 2008;65:2001–2018.
206. Cobb CA, Cole MP. Oxidative and nitrosative stress in neurodegeneration. *Neurobiol Dis*. 2015;84:4–21.
207. Miller E, Morel A, Saso L, et al. Melatonin redox activity. Its potential clinical applications in neurodegenerative disorders. *Curr Top Med Chem*. 2015;15:163–169.
208. Dominguez-Rodriguez A, Abreu-Gonzalez P. Melatonin ischemia-reperfusion injury: possible role of melatonin. *World J Cardiol*. 2010;2:233–236.
209. Dominguez-Rodriguez A, Abreu-Gonzalez P, Arrayo-Ucar E, et al. Decreased level of melatonin in serum predicts left ventricular remodeling after acute myocardial infarction. *J Pineal Res*. 2012;53:319–323.
210. Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ. Melatonin and cardioprotection in acute myocardial infarction: a promising cardioprotective agent. *Int J Cardiol*. 2012;158:309–310.
211. Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez MJ, et al. A unicenter, randomized, double-blind, parallel group, placebo-controlled study of melatonin as an adjunct in patients with acute myocardial infarction undergoing primary angioplasty. The Melatonin Adjunct in the acute mycaRdial Infarction treated with Angioplasty (MARIA) trial: study design and rationale. *Contemp Clin Trials*. 2007;28:532–539.
212. Dominguez-Rodriguez A, Abreu-Gonzalez P, Avanzas P. The role of melatonin in acute myocardial infarction. *Front Biosci*. 2012;17:2433–2441.
213. Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ. Cardioprotection and pharmacological therapies in acute myocardial infarction: challenges in the current era. *World J Cardiol*. 2014;6:100–106.
214. Dominguez-Rodriguez A, Abreu-Gonzalez P, Piccalo R, et al. Melatonin is associated with reverse remodeling after cardiac resynchronization therapy in patients with heart failure and ventricular dyssynchrony. *Int J Cardiol*. 2016;221:359–363.
215. Lochner A, Huisamen B, Nduhiratundi F. Cardioprotective effect of melatonin against ischemia/reperfusion damage. *Front Biosci*. 2013;5:305–315.
216. Simko F, Baka T, Paulis L, et al. Elevated heart rate and non-dipping heart rate as potential targets for melatonin: a review. *J Pineal Res*. 2016;61:127–137.
217. Chamorro A, Dimage V, Urra X, et al. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. *Lancet Neurol*. 2016;15:869–881.
218. Radogna F, Diederich M, Ghibelli L. Melatonin: a pleiotropic molecule regulating inflammation. *Biochem Pharmacol*. 2010;80:1844–1852.
219. Carrillo-Vico A, Lardone PJ, Alvarez-Sanchez N, et al. Melatonin: buffering the immune system. *Int J Mol Sci*. 2013;14:8638–8683.
220. Da A, Wallace G 4th, Reiter RJ, et al. Overexpression of melatonin membrane receptors increases calcium-binding proteins and protects VSC4.1

motoneurons from glutamate toxicity through multiple mechanisms. *J Pineal Res*. 2012;54:58–68.
221. Paula-Lima AC, Louzada PR, De Mello FG, et al. Neuroprotection against Aβ and glutamate toxicity by melatonin: are GABA receptors involved? *Neurotox Res*. 2003;5:323–327.
222. Letechipia-Vallijo G, Lopez-Loeza E, Espinoza-Gonzalez V, et al. Long-term morphological and functional evaluation of the neuroprotective effects of past-ischemic treatment with melatonin in rats. *J Pineal Res*. 2007;42:138–146.
223. Wang Z, Liu D, Zhan J, et al. Melatonin improves short and long-term neurobehavioral deficits and attenuates hippocampal impairments after hypoxia in neonatal mice. *Pharmacol Res*. 2013;76:84–97.
224. Yang Y, Duan W, Jin Z, et al. JAK2/STAT3 activation by melatonin attenuates the mitochondrial oxidative damage induced by myocardial ischemia/reperfusion injury. *J Pineal Res*. 2013;55:275–286.
225. Lamont K, Nduhirabandi F, Adam T, et al. Role of melatonin, melatonin receptors and STAT3 in the cardioprotective effect of chronic and moderate consumption of red wine. *Biochem Biophys Res Commun*. 2015;465:719–724.
226. Yang Y, Jiang S, Dang Y, et al. Melatonin prevents cell death and mitochondrial dysfunction via a SIRT1-dependent mechanism during ischemic-stroke in mice. *J Pineal Res*. 2015;58:61–70.
227. Zhou H, Jiang C, Gu L, et al. Influence of melatonin on IL-1 Ra gene and IL-1 expression in rats with liver ischemia reperfusion injury. *Biomed Rep*. 2016;4:667–672.
228. Inci I, Inci D, Dutley A, et al. Melatonin attenuates post transplant lung ischemic-reperfusion injury. *Ann Thorac Surg*. 2002;73:220–225.
229. Yip HK, Chang YC, Wallace CG, et al. Melatonin treatment improves adipose-derived mesenchymal stem cell therapy for acute lung ischemia-reperfusion injury. *J Pineal Res*. 2013;54:207–221.
230. Sewerynek E, Reiter RJ, Melchiorri D, et al. Oxidative damage in the liver induced by ischemia-reperfusion: protection by melatonin. *Hepatogastroenterology*. 1996;43:898–905.
231. Rodriguez-Reynoso S, Leal C, Portella E, et al. Effect of exogenous melatonin on hepatic energy status during ischemia/reperfusion: possible role of tumor necrosis factor-alpha and nitric oxide. *J Surg Res*. 2001;100:141–146.
232. Okatani Y, Wakatsuki A, Reiter RJ, et al. Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. *Eur J Pharmacol*. 2003;34:260–264.
233. Chen HH, Chen YT, Yang CC, et al. Melatonin pretreatment enhances the therapeutic effects of exogenous mitochondria against hepatic ischemia-reperfusion injury in rats through suppression of mitochondrial permeability transition. *J Pineal Res*. 2016;61:52–68.
234. Yip HK, Yang CC, Chen KH, et al. Combined melatonin and exendin-4 therapy preserves renal ultrastructural integrity after ischemia–reperfusion injury in the male rat. *J Pineal Res*. 2015;59:434–447.
235. Jaworek J, Leja-Szak A, Bonier J, et al. Protective effects of melatonin and its precursor L-tryptophan on acute pancreatitis induced by caerulein overstimulation on ischemia/reperfusion. *J Pineal Res*. 2003;34:40–52.
236. Ozarmak VH, Sayan H, Arslan SO, et al. Protective effect on contractile activity and oxidative injury induced by ischemia and reperfusion of rat ileum. *Life Sci*. 2005;76:1575–1588.
237. Sener G, Schirli AO, Paskalogler K, et al. Melatonin treatment protects against ischemia/reperfusion-induced functional and biochemical changes in rat urinary bladder. *J Pineal Res*. 2003;34:226–230.
238. Norniya M, Burmeister DM, Sawada N, et al. Effect of melatonin on chronic bladder-ischaemia-associated changes in rat bladder function. *BJU Int*. 2013;112:221–230.
239. Sener G, Paskologlu K, Schirli AO, et al. The effects of melatonin on ischemia-reperfusion induced changes in rat corpus cavernosum. *J Urol*. 2002;167:2624–2627.
240. Halici M, Narin F, Turk CW, et al. The effect of melatonin plasma oxidant-antioxidant skeletal muscle reperfusion injury in rats. *J Int Med Res*. 2004;32:500–506.
241. Wang WZ, Fang XH, Stephenson LL, et al. Microcirculatory effects of melatonin in rat skeletal muscle after prolonged ischemia. *J Pineal Res*. 2005;39:57–65.
242. Samantaray S, Das A, Thalore NP, et al. Therapeutic potential of melatonin in traumatic nervous system injury. *J Pineal Res*. 2009;47:134–142.
243. Aydemir S, Dogan D, Kocak A, et al. The effect of melatonin on spinal cord after ischemia in rats. *Spinal Cord*. 2016;54:560–563.
244. Chang CL, Sung PH, Sun CK, et al. Protective effect of melatonin-supported adipose-derived mesenchymal stem cells against small bowel ischemia-reperfusion injury in the rat. *J Pineal Res*. 2015;59:206–220.
245. Hernandez D, Muriel A, Abraira V. Current state of clinical and end-points assessment in transplants: key points. *Transplant Rev*. 2016;30:92–99.
246. Casillas-Ramirez A, Mosbak IB, Ramalho F, et al. Past and future approaches to ischemia-reperfusion lesion associated with organ transplantation. *Life Sci*. 2006;79:1881–1894.
247. Viaretti M, Ferrigno A, Bertone R, et al. Exogenous melatonin enhances bile flow and ATP levels after cold storage and reperfusion in rat liver: implications for liver transplantation. *J Pineal Res*. 2005;38:223–230.
248. Freitas I, Bertone V, Guamaschelli L, et al. In situ demonstration of improvement in liver mitochondria function by melatonin after cold ischemia. *In Vivo*. 2006;20:229–237.
249. Zaouali MA, Reiter RJ, Padrissa-Alteo S, et al. Melatonin protects steatotic and nonsteatotic liver grafts against cold ischemia and reperfusion injury. *J Pineal Res*. 2011;50:213–221.
250. Garcia-Gil FA, Albendea CD, Escartin J, et al. Melatonin prolongs graft survival of pancreas allotransplants in pigs. *J Pineal Res*. 2011;51:445–453.
251. Esteban-Zubero E, Garcia-Gil FA, Lopez-Pingarron L, et al. Potential benefits of melatonin in organ transplantation. *J Endocrinol*. 2016;229:R129–R146.
252. Esteban-Zubero E, Garcia-Gil FA, Lopez-Pingarron L, et al. Melatonin role in preventing steatohepatitis and improving liver transplantation results. *Cell Mol Life Sci*. 2016;73:2911–2927.
253. Shiroma ME, Botlho NM, Damous LL, et al. Melatonin influence in ovary transplantation: systemic review. *J Ovarian Res*. 2016;9:33.
254. Reiter RJ, Tan DX, Sainz RM, et al. Melatonin: reducing the toxicity and increasing the efficacy of drugs. *J Pharm Pharmacol*. 2002;54:1299–1321.
255. Reiter RJ, Tan DX, Sainz RM, et al. Melatonin protects the heart against both ischemia/reperfusion and chemotherapeutic drugs. *Cardiovasc Drugs Ther*. 2002;16:5–6.
256. Rosenson RS, Baker SK, Jacobson TA, et al. The National Lipid Association's Muscle Safety Expert P. An assessment by the Statin Muscle Safety Task Force: 2014 update. *J Clin Lipidol*. 2014;8(Suppl):S58–S71.
257. Zhang MH. Rhabdomyolysis and its pathogenesis. *World J Emerg Med*. 2012;3:11–15.
258. Bays H, Cohen DE, Chalasani N, et al. The National Lipid Association Task Force F. An assessment by the Statin Liver Safety Task Force: 2014 update. *J Clin Lipidol*. 2014;8(Suppl):S47–S57.
259. Ott BR, Daielto LA, Dahabreh IJ, et al. Do statins impair cognition? A system review and meta-analysis of randomized controlled trials. *J Gen Intern Med*. 2015;30:348–358.
260. Navarese EP, Buffon A, Andreotti F, et al. Meta-analysis of impact of different types of statins on new-onset diabetes mellitus. *Am J Cardiol*. 2013;11:1123–1130.
261. Vandolder R, Sener MS, Erek E, et al. Rhabdomyolysis. *J Am Soc Nephrol*. 2000;11:1553–1556.
262. Campanella M, Pinton P, Riggito R. Mitochondrial Ca^{2+} homeostasis in health and diseases. *Biol Res*. 2004;37:653–660.
263. Ego YP, Anastasia AC, Irina BP, et al. Myoglobin causes oxidative stress, increases of NO production and dysfunction of kidney's mitochondria. *Biochim Biophys Acta*. 2009;1792:796–803.
264. Fernandez A, Ordonez R, Reiter RJ, et al. Melatonin and endoplasmic reticulum stress: relation to autophagy and apoptosis. *J Pineal Res*. 2015;59:292–307.
265. Xu S, Pi H, Zhang L, et al. Melatonin prevents abnormal mitochondrial dynamics resulting from the neurotoxicity of cadmium by blocking

calcium-dependent translocation of Drp 1 to the mitochondria. *J Pineal Res*. 2016;60:291–302.

266. Suwanjang W, Abramov AV, Charngkaew K, et al. Melatonin prevents calcium overload, mitochondrial damage and cell death due to toxically high doses of dexamethasone-induced oxidative stress in human neuroblastoma SH-SY5Y cells. *Neurochem Int*. 2016;97:34–41.
267. Dayaub JC, Ortiz F, Lopez LC, et al. Synergism between melatonin and atorvastatin against endothelial cell damage induced by lipopolysaccharide. *J Pineal Res*. 2011;51:324–330.
268. Kubatka P, Bojkova B, Kassayova M, et al. Combination of pitavastatin and melatonin shows partial antineoplastic effects in a rat breast carcinoma model. *Acta Histochem*. 2014;116:1454–1461.
269. Orendas P, Kubatka P, Bojkova B, et al. Melatonin potentiates the antitumor effect of pravastatin in rat mammary gland carcinoma model. *Int J Exp Pathol*. 2014;95:401–410.
270. Benova T, Knezl V, Viczenczova C, et al. Acute anti-fibrillating and defibrillating potential of atorvastatin melatonin, eicosapentaenoic acid and docosahexaenoic acid demonstrated in isolated heart model. *J Physiol Pharmacol*. 2015;66:83–89.
271. Wang P, Chen X, Zhang L, et al. Comprehensive dental treatment for "meth mouth": a case report and a literature review. *J Formosa Med Assoc*. 2014;113:867–871.
272. Alemikhah M, Faridhosseini F, Kordi H, et al. Comparative study of the activity of brain behavioral systems in methamphetamine and opiate dependents. *Int J High Risk Behav Addict*. 2016;5:e25075.
273. Jablonski SA, Williams MP, Vorhees CV. Mechanisms involved in the neurotoxic and cognitive effects of developmental methamphetamine exposure. *Birth Defects Res C Embryo Today*. 2016;108:131–141.
274. McDonnell-Dowling K, Kelly JP. The role of oxidative stress in methamphetamine-induced toxicity and sources of variation in the design of animal studies. *Curr Neuropharmacol*. 2016; in press.
275. Wongprayoon P, Govitrapong P. Melatonin attenuates methamphetamine-induce neurotoxicity. *Curr Pharm Des*. 2016;22:1022–1032.
276. Napparat C, Porter JE, Ebadi M, et al. The mechanism for the neuroprotective effect of melatonin against methamphetamine-induced autophagy. *J Pineal Res*. 2010;49:382–389.
277. Pempoonputtana K, Govitrapong P. The anti-inflammatory effect of melatonin on methamphetamine-induced proinflammatory mediators in human neuroblastoma dopamine SH-SY5Y cell lives. *Neurotox Res*. 2013;23:189–199.
278. Ekthuwapranee K, Sotthibundhu A, Govitrapong P. Melatonin attenuates methamphetamine-induced inhibition of proliferation of adult rat hippocampal progenitor cells in vitro. *J Pineal Res*. 2015;58:418–428.
279. Junnongprakhon P, Govitrapong P, Torharus C, et al. Melatonin promotes blood-brain barrier integrity in methamphetamine-induced inflammation in primary rat brain microvascular endothelial cells. *Brain Res*. 2016;1646:182–192.
280. Singhakumar R, Boontem P, Ekthuwapranee K, et al. Melatonin attenuates methamphetamine-induced inhibition of neurogenesis in the adult mouse hippocampus: an in vivo study. *Neurosci Lett*. 2015;606:209–214.
281. Nguyen XK, Lee J, Shin EJ, et al. Liposomal melatonin rescues methamphetamine-elicited mitochondrial burdens, proapoptosis, and dopaminergic degeneration through inhibition PKCδ gene. *J Pineal Res*. 2015;58:86–106.
282. Hu S, Yin S, Jiang X, et al. Melatonin protects against liver injury by attenuating oxidative stress, inflammatory response and apoptosis. *Eur J Pharmacol*. 2009;616:287–292.
283. Rui BB, Chen H, Jang L, et al. Melatonin upregulates the activity of AMPK and attenuates lipid accumulation in alcohol-induced rats. *Alcohol Alcohol*. 2016;51:11–19.
284. Shin IS, Shin NR, Park JW, et al. Melatonin attenuates neutrophil inflammation and mucus secretion in cigarette smoke-induced chronic pulmonary diseases via the suppression of Erk-Sp1 signaling. *J Pineal Res*. 2015;58:50–60.
285. Wang Z, Ni L, Wang J, et al. The protective effect of melatonin on smoke-induced vascular injury in rats and humans: a randomized controlled trial. *J Pineal Res*. 2016;60:217–227.
286. Tan DX, Manchester LC, Liu X, et al. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evaluation in eukaryotes. *J Pineal Res*. 2013;54:127–138.
287. Govender J, Loos B, Morais E, et al. Mitochondrial catastrophe during doxorubicin-induced cardiotoxicity: a review of the protective role of melatonin. *J Pineal Res*. 2014;57:367–380.
288. Mohrzadi S, Komrava SK, Dormanesh B, et al. Melatonin synergistically enhances protective action of atorvastatin against gentamicin-induced nephrotoxicity in rat kidney. *Can J Physiol Pharmacol*. 2016;94:265–271.
289. Kosar PA, Naziroglu M, Ovey IS, et al. Synergic effects of doxorubicin and melatonin on apoptosis and mitochondrial oxidative stress in MCF-7 breast cancer cells: involvement in TRPV1 channels. *J Membr Biol*. 2016;249:129–140.
290. Pariente R, Pariente JA, Rodriguez AB, et al. Melatonin sensitizes human cervical cancer HeLu cells to cis-platin-induced cytotoxicity and apoptosis: effects on oxidative stress and DNA fragmentation. *J Pineal Res*. 2016;60:55–64.
291. Woo SM, Min KJ, Kwon TK. Melatonin mediated Bin up-regulation and cyclooxygenase-2 (COX-2) down-regulation enhances tunicamycin-induced apoptosis in MDA-MB-231 cells. *J Pineal Res*. 2015;58:310–320.
292. Martin V, Garcia-Santos G, Rodriguez-Blanco J, et al. Melatonin sensitizes human malignant glioma cells against TRAIL-induced cell death. *Cancer Lett*. 2010;227:216–233.
293. Bizzarri M, Proietti S, Cucina A, et al. Molecular mechanisms of the pro-apoptotic actions of melatonin in cancer: a review. *Expert Opin Ther Targets*. 2013;17:1483–1496.
294. Casado-Zapico S, Rodriguez-Blanco J, Garcia-Santos G, et al. Synergistic antitumor effect of melatonin with several chemotherapeutic drugs on human Ewing sarcoma cancer cells: potentiation of the extrinsic apoptotic pathway. *J Pineal Res*. 2010;48:72–80.
295. Alonso-Gonzalez C, Gonzalez C, Martinez-Campa C, et al. Melatonin sensitizes human breast cancer cells to ionizing radiation by downregulating proteins involved in double-strand DNA repair. *J Pineal Res*. 2015;58:189–197.
296. Alonso-Gonzalez C, Gonzalez A, Martinez-Campa C, et al. Melatonin enhancement of the radiosensitivity of breast cancer cells is associated with the modulation of proteins involved in estrogen biosynthesis. *Cancer Lett*. 2016;370:145–152.
297. Plaimee P, Weerapreeyakul N, Barusrux S, et al. Melatonin potentiates cisplatin-induced apoptosis and cell cycle arrest in human lung adenocarcinoma cells. *Cell Prolif*. 2015;48:67–77.
298. Reiter RJ. Mechanisms of cancer inhibition by melatonin. *J Pineal Res*. 2004;37:213–214.
299. De Blask, Dauchy RT, Dauchy EM, et al. Light exposure at night disrupts host/cancer circadian regulatory dynamics: impact on the Warburg effect, lipid signaling and tumor growth prevention. *PLoS One*. 2014;9:e102776.
300. Cutando A, Aneiros-Fernandez J, Aneiros-Cachaza J, et al. Melatonin and cancer: current knowledge and its application to oral cavity tumor. *J Oral Pathol Med*. 2011;40:593–597.
301. Cardinali DP, Furio AM, Brusco LI. Clinical aspects of melatonin intervention in Alzheimer's disease progression. *Curr Neuropharmacol*. 2010;8:218–227.
302. Rosales-Corral SA, Acuna-Castroviejo D, Coto-Montes A, et al. Alzheimer's disease: pathological mechanisms and the beneficial rate of melatonin. *J Pineal Res*. 2012;52:167–202.
303. Panmanee J, Nopparat C, Chavanich N, et al. Melatonin regulates the transcription of bAPP-cleaving secretases mediated through melatonin receptors in human neuroblastoma SH-SY5Y cells. *J Pineal Res*. 2015;59:308–320.
304. Mayo JC, Sainz RM, Tan DX, et al. Melatonin and Parkinson's disease. *Endocrine*. 2005;27:169–178.

305. Palimeni G, Esporito E, Bevelacqua V, et al. Role of melatonin supplementation in neurodegenerative disorders. *Front Biosci*. 2014;19:429–446.
306. Naskar A, Prabhakar V, Singh R, et al. Melatonin enhances L-DOPA therapeutic effects, helps to reduce its dose, and protects dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice. *J Pineal Res*. 2015;58:262–274.
307. Miller E, Morel A, Saluk J. Melatonin redox activity: its potential clinical application in neurodegenerative disorders. *Curr Top Med Chem*. 2014;15:163–169.
308. Lopez-Gonzalez A, Alvarez-Sanchez N, Lardone PJ, et al. Melatonin treatment improves primary progressive multiple sclerosis: a case report. *J Pineal Res*. 2015;58:173–177.
309. Maria S, Witt-Enderby PA. Melatonin effects on bone: potential use for the prevention and treatment of osteopenia, osteoporosis, and periodontal and for use in bone-grafting procedures. *J Pineal Res*. 2014;56:115–125.
310. Tresguerres IF, Tamini F, Elmar H, et al. Melatonin dietary supplement as an anti-aging therapy for age-related bone loss. *Rejuvenation Res*. 2014;17:341–346.
311. Amstrup AK, Sikjaer T, Heichendorff L, et al. Melatonin improves bone mineral density at the femoral neck in postmenopausal women with osteopenia: a randomized control trial. *J Pineal Res*. 2015;59:221–229.
312. Navaro-Alarcon M, Ruiz-Ojeda FJ, Blanca-Herrera RM, et al. Melatonin and metabolic regulation: a review. *Food Funct*. 2014;5:2806–2832.
313. Reiter RJ, Tan DX, Korkmaz A, et al. Obesity and metabolic syndrome: association with chronodisruption, sleep deprivation and melatonin. *Ann Med*. 2012;44:564–577.
314. Cipolla-Neto J, Amaral FG, Afeche SC, et al. Melatonin, energy metabolism, and obesity: a review. *J Pineal Res*. 2014;56:374–381.
315. Peschke E, Bähr I, Muhlbauer E. Environmental and clinical aspects of melatonin and clock genes in diabetes. *J Pineal Res*. 2015;59:1–23.
316. Gitto E, Karbownik M, Reiter RJ, et al. Role of melatonin treatment in pediatric newborns. *Pediatr Res*. 2001;50:756–760.
317. Ortiz F, Garcia JA, Acuna-Castroviejo D, et al. The beneficial effects of melatonin against heart mitochondrial impairment during sepsis: inhibition of iNOS and preservation of eNOS. *J Pineal Res*. 2014;56:71–81.
318. Galley HF, Lowes DA, Allen I, et al. Melatonin as a potential therapy for sepsis: a phase I dose escalation study and an ex vivo whole blood model under conditions of sepsis. *J Pineal Res*. 2014;56:427–438.
319. Wei JY, Li WM, Zhou LL, et al. Melatonin induces apoptosis of colorectal cancer cells through HDAC4 nuclear impact mediated by CaMKII activation. *J Pineal Res*. 2015;58:429–438.
320. Blask DE, Hill SM, Dauchy RT, et al. Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. *J Pineal Res*. 2011;51:559–569.
321. Ma Z, Yang Y, Fan C, et al. Melatonin as a potential anticarcinogen for non-small cell lung cancer. *Oncotarget*. 2016; in press.
322. Elmahallawy EK, Jimenez-Arauda A, Martinez AS, et al. Activity of melatonin against *Leishmania infantum* promastigotes by mitochondrial dependent pathway. *Chem Biol Interact*. 2014;220:84–93.
323. Laranjara-Silva MF, Zampieri RA, Muxel SM, et al. Melatonin attenuates *Leishmania* (L) *amazonensis* infection by modulating arginine metabolism. *J Pineal Res*. 2015;59:478–487.
324. Brazao V, Santello FH, Del Vecchio Filipin M, et al. Immunological actions of melatonin and zinc during chronic *Trypanosoma cruzi* infection. *J Pineal Res*. 2015;58:210–218.
325. Oliveira LG, Filipin M del V, Santello FH, et al. Protective actions of melatonin against heart damage during chronic Chagas disease. *Acta Trop*. 2013;128:652–658.
326. Katkar GD, Sundaram MS, Hemshekhar M, et al. Melatonin alleviates *Echis carinatus* venom-induced toxicities by modulating inflammatory mediators and oxidative stress. *J Pineal Res*. 2014;56:295–312.
327. Sharma RD, Katkar GD, Sundaram MS, et al. Oxidative stress-induced methemoglobinemia is a silent killer during snake bite: a novel and strategic neutralization by melatonin. *J Pineal Res*. 2015;59:240–254.
328. Abdel-Moneim AE, Ortiz F, Leonardo-Mendonca RC, et al. Protective effects of melatonin against oxidative damage induced by Egyptian cobra (*Naja haje*) crude venom in rats. *Acta Trop*. 2015;143:58–65.
329. Marino A, Di Paola R, Crisafulli C, et al. Protective effect of melatonin against the inflammatory response elicited by crude venom from isolated nematocyst of *Pelagia noctiluca* (*Cnidaria, Scyphozoa*). *J Pineal Res*. 2009;47:56–69.
330. Tan DX, Korkmaz A, Reiter RJ, et al. Ebola virus disease: potential use of melatonin as a treatment. *J Pineal Res*. 2014;57:357–366.
331. Anderson G, Maes M, Markus RP, et al. Ebola virus: melatonin as a readily available treatment option. *J Med Virol*. 2015;87:537–543.
332. Messaoud I, Amarasinghe GK, Basler CF. Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus. *Nat Rev Microbiol*. 2015;13:663–676.
333. Srivivasan V, Mohamed M, Kato H. Melatonin in bacterial and viral infections with a focus on sepsis: a review. *Recent Pat Endocr Metab Immune Drug Discov*. 2012;6:30–39.
334. Esteban-Zubero E, Alatorre-Jimenez MA, Lopez-Pingarron L, et al. Melatonin's role in preventing toxin-related and sepsis-mediated hepatic damage: a review. *Pharmacol Res*. 2016;105:108–120.
335. Carrillo-Vico A, Reiter RJ, Lardone PJ, et al. The modulatory role of melatonin on immune responsiveness. *Curr Opin Investig Drugs*. 2006;7:423–452.
336. Hardeland R, Cardinali DP, Brown GM, et al. Melatonin and brain inflammaging. *Prog Neurobiol*. 2015;127–128:46–63.
337. Sanchez A, Colpena AC, Clares B. Evaluating the oxidative stress in inflammation: role of melatonin. *Int J Mol Sci*. 2015;16:16981–17004.
338. Kaur C, Ling EA. Blood brain barrier function in hypoxic-ischemic conditions. *Curr Neurovasc Res*. 2008;5:71–81.
339. Rodella LF, Favero G, Foglio E, et al. Vascular endothelial cells and dysfunctions: role of melatonin. *Front Biosci*. 2013;5:119–129.
340. Wirtz PH, Spillman M, Bartschi C, et al. Oral melatonin reduces blood coagulation activity: a placebo-controlled study in healthy young men. *J Pineal Res*. 2008;44:127–133.
341. Saiz JC, Vazquez-Calvo A, Blasquez AB, et al. Zika virus: the latest newcomer. *Front Microbiol*. 2016;7:496.
342. Boga JA, Coto-Montes A, Rosales-Corral SA, et al. Beneficial actions of melatonin in the management of viral infections: a new use for this "molecular handyman". *Rev Med Virol*. 2012;22:323–338.
343. Laliena A, San Miguel B, Crespo I, et al. Melatonin attenuates inflammation and promotes regeneration in rabbits with fulminant hepatitis of viral origin. *J Pineal Res*. 2012;53:270–276.
344. Tunon MJ, San Miguel B, Crespo I, et al. Melatonin treatment reduces endoplasmic reticulum stress and modulates the unfolded protein response in rabbits with lethal fulminant hepatitis of viral origin. *J Pineal Res*. 2013;55:221–228.
345. Velma JR, Bonilla E, Chocin-Bonilla L, et al. Effect of melatonin on oxidative stress, and resistance to bacterial, parasitic and viral infection. *Acta Trop*. 2014;137:31–38.
346. Reiter RJ. The pineal gland: a regulator of regulators. *Prog Psychobiol Physiol Psychol*. 1980;9:323–356.
347. Ebadi M, Samejima M, Pfeiffer RF. Pineal gland in synchronizing and refining physiological events. *N Physiol Sci*. 1993;8:30–33.
348. Romijn HJ. The pineal, a tranquilizing organ. *Life Sci*. 1978;23:2257–2273.
349. Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. *Prog Brain Res*. 2010;181:127–151.
350. Dragojevic-Dikic S, Jovanovic AM, Dikic S, et al. Melatonin: a "Higgs boson" in human reproduction. *Gynecol Endocrinol*. 2015;31:92–101.